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

**FINAL REPORT**

**TO THE THREATENED SPECIES AND COMMUNITIES UNIT**

**BIODIVERSITY GROUP**

**ENVIRONMENT AUSTRALIA**

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

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| Project Co-ordinator: | D.J. Coates |
| Editor: | D.I.L. Murray |

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# SCOPE ITEM 1

# MAINTENANCE OF STRONG LINKS BETWEEN THE *PHYTOPHTHORA* AND *DIPLODINA* CANKER PROJECT AND THE NATIONAL THREAT ABATEMENT PLAN FOR DIEBACK

## OUTCOMES

- Formation of a Steering Group (five members) comprising personnel from Environment Australia (EA) and from the Department of Conservation and Land Management (CALM), Perth. The EA representative and two of the four CALM members were directly involved in drafting the National Threat Abatement Plan for Dieback caused by *Phytophthora cinnamomi* (NTAP).

- During the tenure of the project, formal meetings of the EA-CALM Steering Group were held on three occasions. Informal interactions occurred on a regular basis over the same period.

- The Steering Group formally negotiated and ratified the scope items for the *Phytophthora* and *Diplodina* canker project.

- The Steering Group scrutinised all of the proposed components of the *Phytophthora* and *Diplodina* canker project and reviewed them in terms of merit and conformity with priorities and directions emerging from the parallel NTAP drafting process.

- The project outcomes will be incorporated by CALM members of the Steering Group into strategies and key actions for inclusion in the NTAP.

- Assisted by their understanding of both the *Phytophthora* and *Diplodina* canker project, and the requirements that were highlighted during drafting of the NTAP, the Steering Group negotiated (with EA) an independent though related program of collaborative information and technology exchange between the State and Territory organisations engaged in research on *Phytophthora.*

## SCOPE ITEM 2

# A COORDINATED PROGRAM OF PHOSPHONATE APPLICATION FOR THE PROTECTION OF NATIVE PLANT COMMUNITIES IN SOUTH-WEST WESTERN AUSTRALIA

***Russell Smith, Malcom Grant and Ellen Hickman***

## 1 INTRODUCTION

In spring, 1996 and autumn, 1997, two major aerial applications of phosphonate[1] were carried out in the Department of Conservation and Land Management's (CALM) Albany District (Barrett *et al.*, 1997). A total of 285ha of native vegetation were sprayed, including 225ha at Bell Track in the Fitzgerald River National Park and 60ha in the Stirling Range National Park. These operational spray applications were based on the results of three years' research into the use of phosphonate to protect native flora against dieback disease caused by *Phytophthora* in the Albany District (Komorek *et al.*, 1997).

The planning of further strategic applications of phosphonate, to protect endangered flora, commenced in spring of 1997. During field surveys undertaken in summer, 1997/98, Albany District staff identified a number of additional areas in the Stirling Ranges where endangered flora were at risk from *Phytophthora*. Other sites supporting threatened species were identified at Cape Arid (Esperance District) and Busselton (South West Capes District).

The final list of sites to receive aerial application of phosphonate in 1998 was drawn up after consultation with CALM's District staff, Wildlife Branch and Science and Information Division. A list of the sites sprayed in 1998 is provided in Appendix 1 together with the proposed program for 1999. All sprayed areas support one or more species of Declared Rare Flora (DRF). Selection of sites for spraying was based on satisfaction of the major criteria listed below:

- presence of species classified as critically endangered, endangered or vulnerable DRF, or Priority 1 or 2;
- presence of DRF populations recognised as susceptible to direct or indirect effects of *Phytophthora* species; and
- existence of threat to conservation value due to the presence or imminent arrival of dieback disease associated with infection by *Phytophthora*.

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

The work outlined in this report was undertaken to address Scope Item No. 2 for the *Phytophthora* and *Diplodina* Canker project (1997/98) and this constitutes the objective stated below.

# 2 OBJECTIVE

The major objective of this work was to implement an extended and coordinated program of actions for the use of phosphonate in the protection of native species of critically endangered, endangered or vulnerable DRF, and Priority 1 or 2 species, from the direct or indirect effects of *Phytophthora* spp.

# 3 METHODS

The Project comprised four main phases:

- field surveys to locate target species;
- marking target sites;
- spraying operations; and
- monitoring.

## 3.1 FIELD SURVEYS

The locations of target plant species at Cape Arid (Esperance District) and South West Capes were well known, thus further surveying of those areas was not required. However, extensive field surveys were necessary for selection of appropriate sites in the Stirling Ranges. This usually involved lengthy treks (2-3 hours) into the peaks of the ranges before commencing the search for threatened flora. The adopted strategy was to locate patches of uninfected thicket, at least 1ha in area, where one or more target species were present.

## 3.2 SITE MARKING

To ensure that target areas were readily visible from the air, the corners of each site were marked conspicuously. In the Stirling Ranges, orange or yellow flags (0.2 x 0.6m) on 4m-tall poles were driven into the ground at the corners of each site. Because of damage due to frequent strong winds, flags had to be re-erected prior to each aerial application of phosphonate. Helium-filled balloons were used to mark sites in South West Capes district, but these proved to be less visible from the air and will be replaced by flags in future.

## 3.3 SPRAYING OPERATIONS

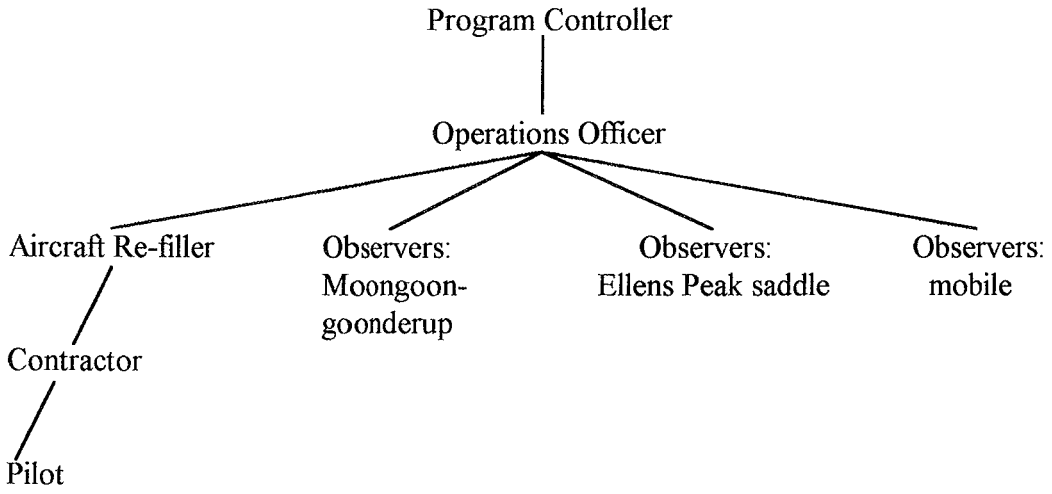
### 3.3.1 The Stirling Ranges

At Mondurup Peak, a large infection on the northern slope of the mountain was sprayed with phosphonate in an attempt to arrest its spread. Mondurup supports the only montane thicket (= Mixed Stirling Range Thicket; see Anon., 1997) in the Stirling Range that is not infected by *Phytophthora*.

On Moongoongoonderup, Ellens Peak, Pyungoorup and Bakers Knob, small islands of healthy montane thicket were present and spraying was carried out to halt or delay incursions of *Phytophthora* from surrounding areas. Similarly, spraying was conducted at the South Bluff Track where an area of jarrah mallee heath including *Dryandra anotona* (DRF) is surrounded by dieback-infected vegetation.

Experience gained during the 1997 program has indicated, that when aerial spraying is conducted in the Stirling Ranges, it is imperative that the pilot receives up-to-date reports on wind conditions in the mountains prior to departure from the airstrip, and thereafter until delivery of the spray. In the absence of remote weather stations, this requires personnel to be deployed on the peaks with equipment for measuring wind speed and for communication with the pilot.

This year, two observers camped on the Ellen's Peak saddle and two on Moongoongoonderup. They used hand-held anemometers to gauge wind speed and communicated by VHF radio with the aircraft re-filler and operations officer. UHF transmitters were used to communicate directly with the aircraft pilot if wind strength had altered significantly since departure. The personnel and communications structure used for the aerial spraying operation is outlined below.




Overall control and day-to-day management of spraying operations were the responsibilities of the program controller and operations officer, respectively. The controller made strategic decisions in relation to resources allocation. Decisions such as that to carry out spraying at a given location or time were left to the operations officer, and were based on wind conditions reported by observers. At Mondurup, the mobile observer team set up flags and monitored wind conditions .

In the Stirling Ranges, wind conditions at altitudes of 700-1100m were critical for spraying operations. For mountain sites, winds of less than 5km hour $^{-1}$ were ideal. It was important that wind speed remained fairly constant and that unexpected up-drafts on the faces of the peaks did not occur. There was often little or no breeze at the

airstrip near Bluff Knoll when 30-60km hour $^{-1}$ winds were blowing on the peaks. The typical barometric map for optimum spraying conditions comprised a large, high-pressure system anchored in the Great Australian Bight.

### 3.3.2 South West Capes and Esperance Districts

Three sites were sprayed in the South West Capes District. These all contained both southern ironstone heathland, which is listed as a threatened environmental community by CALM, and the jarrah-marri forest ecotone. At each site, the declared rare species *Dryandra nivea* ssp. *uliginosa, D. squarrosa* ssp. *argillaceae* or *Petrophile latericola* are affected by *Phytophthora* root-rot disease.

At Cape Arid National Park in the Esperance District, a large infection of *Phytophthora cinnamomi* is located in *Banksia speciosa* woodland. At Lucky Bay, a small remnant of shrubland containing *Lambertia echinata* ssp. *echinata* (DRF) is present in a *Phytophthora*-infected, disused gravel pit which is undergoing rehabilitation. Here, the strategy is to prolong the life of remaining adult plants and protect cuttings of this species which are to be translocated in winter.

By comparison with the Stirling Ranges, phosphonate spraying operations were much less complicated in the South West Capes and Esperance Districts. Both areas were basically flat and readily accessible by road. Wind conditions were a less critical factor, and the requirement for dry conditions to prevail for about a day after spraying was generally the only limitation.

### 3.3.3 Application of Phosphonate

Phosphonate was applied as an emulsion of Foli-R-Fos 400 (40%) supplemented with 2% Synertrol, a surfactant consisting mainly of canola oil. Between 30th March and 3rd April, 1998, the montane community sites in the Stirling Ranges were sprayed with phosphonate at the rate of 15$\ell$ ha$^{-1}$ to provide delivery of the active ingredient (phosphite) at 6kg ha$^{-1}$. Lowland sites were sprayed with twice that amount of phosphonate in the same period. Another spray treatment, delivering phosphite at 4kg ha$^{-1}$ or 6kg ha$^{-1}$, was applied between 4th and 8th May at the South Bluff Track and Mondurup sites. High winds at altitudes above 600m prevented re-spraying of Moongoongoonderur, Pyungoorup, Bakers Knob and the three sites on Ellens Peak. Sites in the South West Capes and Esperance Districts were sprayed with phosphonate (to deliver phosphite at12kg ha$^{-1}$) on 17th April or 1st May, respectively. Re-spraying of sites in Esperance district is planned for 15th-19th June.

The aircraft were Cessna Agwagons (188B) fitted with Micronair Rotary Atomiser spray systems or CP Nozzles (Esperance). Spray swaths were approximately 15m wide. The planes were generally flown at 5-10m above ground while spraying was carried out. The aircraft was fitted with a GPS navigation system which allowed the pilot to display previously sprayed areas on a screen in the cockpit and to record the position of site corner flags. Lights mounted in front of the pilot indicated the correct flight path and when to activate the spray nozzles.

### 3.4 MONITORING

Monitoring is to be carried out at most sites to determine the effectiveness of treatments. This is usually accomplished by emplacement and subsequent inspection of infection boundary markers at sites where a clearly defined dieback front is present, and/or by counting individuals of DRF and other susceptible species within quadrats located in sprayed areas. The infection boundary markers consist of steel droppers embedded in the ground at intervals to provide a clear indication of the extent of infection in autumn, 1998. At some sites where *Phytophthora* was widespread, susceptible DRF were tagged and mapped to provide a record of which plants were alive prior to spraying.

## 4 RESULTS

The monitoring program which commences in December, 1998, will facilitate determination of the effectiveness of applied treatments. So far, some localised leaf burning has been noted at the South Bluff Track and Mondurup sites four weeks after application of the first spray. This was mainly confined to mallee eucalypts. Past experience has shown that the effect is transitory and no long term damage is sustained. In order to prevent recurrence of leaf burning at these sites, application rates for the second treatment was reduced (see Section 3.3.3).

## 5 OUTCOMES

- Our knowledge on the distribution of rare flora in the Stirling Ranges has increased substantially as a result of the field survey component of this project. It is now apparent that very little of the original montane thicket still remains in the eastern Stirling Ranges. The remnants identified and sprayed during the current work appear to be all that is left of a hitherto widespread community that includes a large proportion of endemic taxa, many of which are classified as endangered.

- It is expected that the program of actions initiated in this project will greatly assist the conservation of native populations of DRF threatened by the presence or impending arrival of dieback disease caused by *Phytophthora*.

## 6 REFERENCES

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## Appendix 1. Phosphite application targets for autumn 1998

| Site Name | District | Area (ha) | Vegetation | Species | Application Method | Date of First Application | Application Rate (a.i.) | Date of Second Application | Application Rate | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| Will 01 (Williamson Rd) | S.W. Capes | 4.2 | Southern ironstone shrubland | *Hakea* aff. *varia* (yellow)<br>*Isopogon scaber*<br>*Petrophile latericola* | Fixed-wing aircraft | 17th April 1998 | 12 kg $ha^{-1}$ | not sprayed | | |
| Smith 01 (Smith Rd, Treeton Block) | S.W. Capes | 11.0 | Southern ironstone shrubland | *Dryandra nivea ssp. Uliginosa*<br>*D. squarrosa ssp. Argillaceae*<br>*Hakea* aff. *varia* (yellow) | Fixed-wing aircraft | 17th April 1998 | 12 kg $ha^{-1}$ | not sprayed | | |
| Yiron 01 (Gale & Treeton-Jindong Rds) | S.W. Capes | 6.4 | Southern ironstone shrubland | *Dryandra nivea ssp. Uliginosa*<br>*D. squarrosa ssp. Argillaceae*<br>*Hakea* aff. *varia* (yellow) | Fixed-wing aircraft | 17th April 1998 | 12 kg $ha^{-1}$ | not sprayed | | |
| Quindalup Siding | S.W. Capes | 0.01 | Marri forest | *Caladenia busselliana* | Backpack mister | 14th May 1998 | 0.04% | not sprayed | | |
| Ellens Peak (Western Saddle) (SRNP) | Albany | 1.0 | Eastern Stirling Range montane community | *Andersonia axilliflora*<br>*Sphenotoma drummondii* | Fixed-wing aircraft | 2nd April 1998 | 6 kg $ha^{-1}$ | not sprayed | | |
| Ellens Peak (SSE spur) (SRNP) | Albany | 7.5 | Eastern Stirling Range montane community | *Banksia brownii*<br>*Lambertia fairallii* | Fixed-wing aircraft | 2nd April 1998 | 6 kg $ha^{-1}$ | not sprayed | | |
| Pyungoorup (SRNP) | Albany | 3.5 | Eastern Stirling Range montane community | *Dryandra montana*<br>*Andersonia axilliflora*<br>*Banksia brownii* | Fixed-wing aircraft | 2nd April 1998 | 6 kg $ha^{-1}$ | not sprayed | | |
| Bakers Knob (SRNP) | Albany | 3.0 | Eastern Stirling Range montane community | *Darwinia collina*<br>*Sphenotoma drummondii* | Fixed-wing aircraft | 30th March 1998 | 6 kg $ha^{-1}$ | not sprayed | | |

## Appendix 1. (Cont.) Phosphite application targets for autumn 1998

| Site Name | District | Area (ha) | Vegetation | Species | Application method | Date of First Application | Application Rate (ai) | Date of Second Application | Application Rate | Comments |
|---|---|---|---|---|---|---|---|---|---|---|
| Moongoon-goonderup (1) (SRNP) | Albany | 4.0 | Eastern Stirling Range montane community | *Banksia brownii*<br>*Andersonia axilliflora*<br>*Darwinia squarrosa* | Fixed-wing aircraft | 2nd April 1998 | 6 kg ha$^{-1}$ | not sprayed | | |
| Moongoon-goonderup (2) (SRNP) | Albany | 4.0 | Eastern Stirling Range montane community | *Banksia brownii* | Fixed-wing aircraft | 2nd April 1998 | 6 kg ha$^{-1}$ | not sprayed | | |
| South Bluff Track (SRNP) | Albany | 25.0 | Jarrah-*Lambertia erectifolia* shrubland | *Dryandra anotona* | Fixed-wing aircraft | 30th March 1998 | 12 kg ha$^{-1}$ | 4th/6th May 1998 | 4 kg ha$^{-1}$ & 6.4 kg ha$^{-1}$ | Reduced rate for second spraying because of LLB. See map. |
| Mondurup (SRNP) | Albany | 35.0 | Western Stirlings mallee | | Fixed-wing aircraft | 1st April 1998 | 12 kg ha$^{-1}$ | 6th May 1998 | 4 kg ha$^{-1}$ & 6 kg ha$^{-1}$ | Reduced rate for second application because of LLB; heavier rate on lower slope. |
| Cape Arid | Esperance | 55.0 | | *Adenanthos ellipticus* | Fixed-wing aircraft | 1st May 1998 | 12 kg ha$^{-1}$ | 15th June 1998 | 12 kg ha$^{-1}$ | |
| Lucky Bay | Esperance | 2 | Open heathland with *Eucalyptus doratoxylon* | *Lambertia echinata* ssp. *echinata* | Fixed-wing aircraft | 1st May 1998 | 6 kg ha$^{-1}$ | 15th June 1998 | 6 kg ha$^{-1}$ | Translocation site |
| Main Roads Dept. Reserve | Perth | | *Banksia* woodland | *Caladenia huegeli* | Trunk injection/Back pack mister | 24th April 1998 | | | | |
| Valley of the Giants | Walpole | 4 trees | Tingle forest | *Eucalytus jacksonii* | Trunk injection | 13th May 1998 | 0.05% | | | |

## Appendix 1. (Cont.)

| Site Name | District | Area (ha) | Vegetation | Species |
|---|---|---|---|---|
| Sharpe Block | Walpole | | | *Astartea* sp. Mt Johnston |
| Poison Hill | Walpole | | Shrubland -outcrop | *Banksia verticillata* |
| Nicol Road | Walpole | < 1 ha | | *Lambertia rariflora* subsp. *lutea* |
| Valley of the Giants | Walpole | | Karri forest | |
| Twin Swamps NR | Perth | | *Banksia* woodland | *Stylidium longitubum* |
| Ken Hurst Park | Perth | | *Banksia* woodland | *Caladenia huegelii* |
| Main Roads Deptartment Reserve | Perth | | | *Caladenia huegelii* |
| Popanyinning | Wheatbelt | | *Banksia* woodland | *Banksia cuneata* |
| Murtin Block | Pemberton | 1-2 ha | Jarrah-Marri forest | *Caladenia winfieldii* |

## Appendix 1. (Cont.)

## Proposed 1999 program

| Site Name | District | Area (ha) | Vegetation | Species | Comments |
|---|---|---|---|---|---|
| Point Anne (junction Perabulup Drive) | Albany | ???? | | *Banksia baxteri* | *P. megasperma* (Trial site?) |
| Yule Brook/Rixton Reserves | Perth | ????? | Herb rich shrublands in claypans | *Calytrix breviseta* ssp. *Breviseta* | CALM/UWA/State Planning Commission - difficult site in suburbs |
| Bull06 Gt. Northern Hwy., Bullsbrook | Perth | ???? | Herb rich saline shrublands in claypans | | |
| Boulder Hill | Albany | | | *Andersonia* sp. Two Peoples Bay | High impact abt 80% dead |
| Goodga River | Albany | | | *Andersonia* sp. Two Peoples Bay | High impact abt 80% dead |
| South Sister NR | Albany | | Open mallee shrubland | *Banksia brownii* | High impact, remnant of uninfected remains |
| Vancouver Peninsula | Albany | | Coastal shrubland on granite | *Banksia brownii, Isopogon uncinatus* | Moderate impact – 20% dead |
| Waychinicup NP | Albany | | | *Banksia brownii* | High impact – 50% dead |
| Talyaberlup (SNRP) | Albany | | Mallee heathland | *Darwinia wittwerorum* | Moderate impact |
| Mt. Success(SNRP) | Albany | | | *Dryandra anatona, Lambertia fairallii* | Moderate impact |
| Coyanarup (SRNP) | Albany | | | *Dryandra montana, Sphenotoma drummondii* | ? |
| Kyanorup Eminence | Albany | | | *Dryandra montana* | ? |
| North Isongerup Peak | Albany | | Jarrah-*Banksia* mallee heathland | *Verticordia carinata* | ? *P. cinnnamomi* present |
| Mt. James Track | Albany | | | *Verticordia carinata* | ? *P. cinnnamomi* present |

## SCOPE ITEM 3

# DEVELOPMENT OF PROTOCOLS FOR PRIORITY SETTING IN MANAGEMENT SYSTEMS ESSENTIAL TO THE PROTECTION OF NATIVE BIOTA THREATENED BY *PHYTOPHTHORA*

*P. Gioia and F.D. Podger*

## 1 INTRODUCTION

This report describes an approach to the development of protocols for management of *Phytophthora*-sensitive vegetation, with particular emphasis directed towards the evaluation of any threat of extinction posed by the pathogen to taxa of native plants.

Details of other approaches to the management of disease caused by *Phytophthora* in native vegetation were provided in earlier progress reports. Wills & Chapman (1993) proposed the development of Geographic Information Systems (GIS)-based tools for predicting the distribution of *Phytophthora*. The shortcomings of this strategy were highlighted by Gioia *et al.* (1997) who suggested an alternative, expert systems approach that did not attempt to model the physical behaviour of *Phytophthora* in the natural environment. The current work further utilises the expert systems approach to provide a methodology for the development of simple protocols that can be readily assimilated and used by planning or operational staff for managing threats of extinction to native flora.

Gioia *et al.* (1997) discussed the potential role of GIS in assisting land managers to prioritise deployment of resources for management of problems arising from the presence of *Phytophthora cinnamomi* in native plant communities. More specifically, examples were generated to illustrate how managers might set priorities for application of phosphonate (fungicide) to threatened plant populations based on the proximity of confirmed *Phytophthora* infestations.

Although rare or threatened taxa are of obvious concern, and have been ranked accordingly for the allocation of available resources, less attention has been paid to the potentially widespread destruction of some common species. An example is *Banksia grandis*, a species known to be highly susceptible to *P. cinnamomi*. While widely distributed in the jarrah forest, *B. grandis* is almost completely restricted to high rainfall zones where conditions are relatively favourable for establishment of *P. cinnamomi*, at least when soil moisture and temperature levels are conducive to production of infective spores.

Currently, there is no cost-effective treatment for the control of *Phytophthora* on a broad scale. In a hypothetical worst case scenario, the eventual spread of the pathogen might be expected to occur throughout its preferred environment. If so, a widespread species such as *B. grandis* could be under threat of extinction.

Therefore, land managers must consider not only taxa with restricted populations, but also comparatively common species in vulnerable situations such as that illustrated by the worst case scenario referred to above. Clearly, a protocol designed to evaluate threats of extinction would assist managers to determine if complete loss of a species might be expected, or whether some reduction in its distribution was a more likely and less significant outcome. A rational allocation of control resources would thus be facilitated.

The methodology for developing such a protocol is described here. The protocol was based on expert knowledge in combination with GIS decision support tools. This involved a series of detailed interviews with an acknowledged expert on the pathology and management of *Phytophthora* and the disease it causes. GIS technology was used to assemble various data layers pertinent to the vulnerability of sensitive taxa and to visualise combinations of these layers so that a logical set of criteria could be applied to assess the threat of extinction for individual taxa. Through simple visualisation and exploration of relevant GIS layers, expert knowledge was formulated into a series of steps for assessing the threat of extinction.

# 2 OBJECTIVES

The purpose of this work was to develop methodology for the generation of a protocol to assess threats of extinction posed by *Phytophthora* to native plant taxa and, thereby, to assist priority setting in management systems essential to the protection of indigenous biota under existing or imminent threat from the pathogens.

# 3 METHODS

Three genera that included *Phytophthora*-sensitive species were selected initially to provide test cases for developing a protocol for assessing threats of extinction to native taxa. These were *Banksia*, *Darwinia* and *Xanthorrhoea*. However, work on the last two of those genera was discontinued at an early stage when it became apparent that adequate datasets were unavailable. Moreover, it was considered that scenarios associated with selected *Banksia* spp. would be sufficient to develop a comprehensive protocol relevant to most situations.

Data were initially acquired from the Department of Conservation and Land Management (CALM) Herbarium specimen record database (WAHERB). Because each record corresponded to a voucher, uncertainty regarding identification of species could be easily dealt with. On the other hand, records from specimen databases are typically opportunistic in nature and might be expected to overlook important populations.

A number of historical records were also available. These afforded a wider context for assessing the natural occurrence of a species, but it was recognised that they might provide misleading information on the size or distribution of populations since many of the old records preceded land clearance for agriculture. This was very important in the context of threat assessment, as the occurrence of even one population in a non-vulnerable situation would increase the prospects of survival for a particular species.

Thus, the Banksia Atlas (Taylor & Hopper, 1988) was used to provide comprehensive information on the distribution of *Banksia* populations in Western Australia although it was recognised that, due to the non-vouchered nature of the data, there would be a level of uncertainty associated with plant identifications. In the case of important populations, this would require field verification.

Development of the protocol involved an expert systems approach entailing collaboration between an expert in the use of computer systems, particularly GIS, and an expert on both the pathology of *Phytophthora* and the management of its impact on native flora. This formalised, within a computer-based system, knowledge on the management of *Phytophthora* derived from many years of laboratory and field-based experience. The advantage of the strategy is that it employs that knowledge to maximum effect, with direct impact on management of disease, without resorting to the complex process of modelling behaviour of *Phytophthora* in the field.

Formulation of the protocol required a capability to easily visualise distribution maps for existing positive or negative isolations of *Phytophthora* as well as any arbitrary species of *Banksia*. Simple, uncluttered maps that display vouchered (WAHERB) and unvouchered records (Banksia Atlas; Taylor & Hopper, 1988) are needed to allow examination of both recent and historical data. Sufficient cadastral overlays should be available to facilitate knowledge of local situations.

The primary tool for visualising distribution maps was ArcView™ V3.0a (ESRI, 1997). Scripts were written in the programming language for ArcView, Avenue™, to import data from remote sources, calculate species population numbers and rapidly display records for any given species of *Banksia*.

*Banksia* distribution data were imported from WAHERB and from the Banksia Atlas (Taylor & Hopper, 1988). Isolation records for *Phytophthora* spp. were sourced from the CALM Vegetation Health Service database and the Northern Sandplains Dieback Working Party (Shearer & Dillon, 1996; Stukely *et al.*, 1997).

A range of maps were visualised for each *Banksia* sp. Factors that could be used to assess the overall threat of extinction for a given species were selected from the maps and formatted as a protocol for use at both operational and resource planning levels.

# 4 RESULTS AND DISCUSSION

Assessment of the threat of extinction to a particular species requires knowledge of the factors that broadly affect the likelihood of plant mortality due to *Phytophthora*. These can be grouped into factors affecting the ability of *Phytophthora* to survive in a given

physical environment, and the ability of a plant to resist the destructive effects of pathogens either through natural immunity or through disease escape mechanisms.

## 4.1 PHYSICAL FACTORS AFFECTING PATHOGEN ACTIVITY

The ability of *Phytophthora* to become active or reproduce is affected by a number of interacting factors in the physical environment. For example, a significant determinant in the survival and propagation of *P. cinnamomi* is the availability of adequate soil moisture. This in turn will obviously be influenced by other factors, some of which are season, rainfall and sub-surface drainage. Soil temperature is also of critical importance to production of infective zoospores by *P. cinnamomi*. A number of factors were selected to assist the development of a protocol for assessing threats of extinction using the expert systems approach. No attempt was made to document or analyse the complex interactions between factors. Rather, knowledge and experience was employed to assess how a given factor might influence the management of a particular species.

### 4.1.1 Rainfall

Distribution records of samples tested for *P. cinnamomi* were examined. Figure 1 shows positive and negative records for the pathogen, together with annual rainfall isohyets. All positive isolations and a selection of negative samples are displayed. Selected negative samples in areas receiving less than 600mm rainfall are shown to demonstrate that absence of positive isolations in relatively dry areas is unlikely to be a result of inadequate sampling. The dense cluster of positive isolations in the 1200mm rainfall band corresponds to jarrah forest and it reflects the intensive collection effort associated with mandatory pre-logging dieback assessments.

Figure 2 shows the distributions of positive isolations for a range of *Phytophthora* spp. including *P. citricola*, *P. cryptogea*, *P. drechsleri*, *P. megasperma* and *P. nicotianae*. These distributions, together with Figure 1, confirm existing knowledge that no positive isolations of *P. cinnamomi*, or the other *Phytophthora* spp. cited above, have ever been obtained in areas receiving less than 400mm average annual rainfall.

### 4.1.2 Climatic Zones

The climatic zones of Western Australia include the Mediterranean zone in the south-west land division (SWLD), the semi-arid and arid zones, and the sub-tropical zone of the Kimberleys in northern Australia. Although there is sufficient rainfall to support the existence of *Phytophthora* in the sub-tropical zone it has not been recorded there, possibly due to an unfavourable combination of rainfall, seasonality and temperature.

### 4.1.3 Soil Characteristics

Experience has shown that, in soils possessing similar properties to the Spearwood Dune system, there is less mortality of plants attributable to *Phytophthora* than would be expected in many other types of soil. The mechanism whereby these calcareous soils reduce plant mortality is not fully understood, but it is thought that their drainage

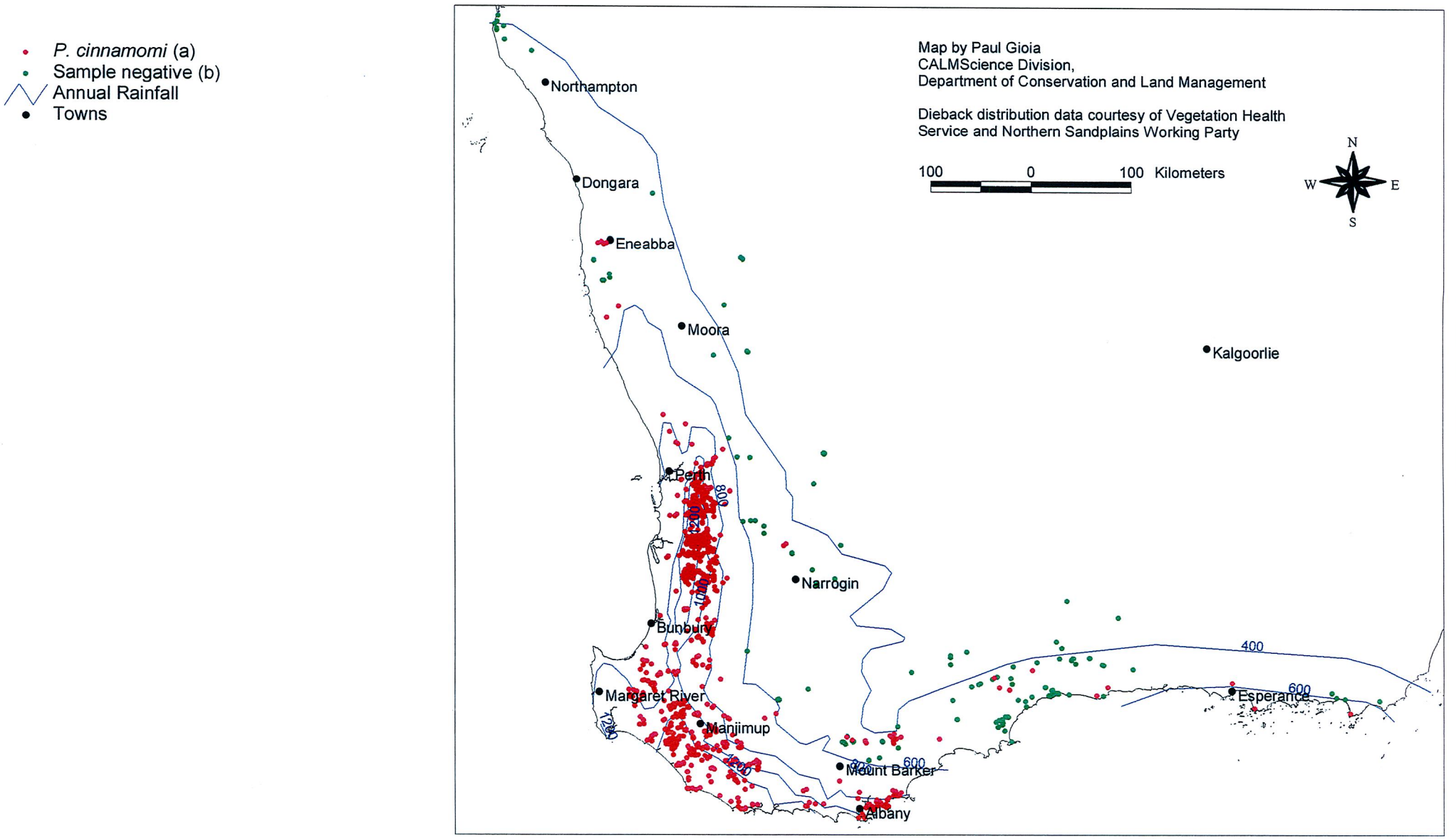



Figure 1. Distribution of records of isolations of a) *P. cinnamomi* and b) selected locations beyond the 600mm isohyet where no evidence of *P. cinnamomi* was found

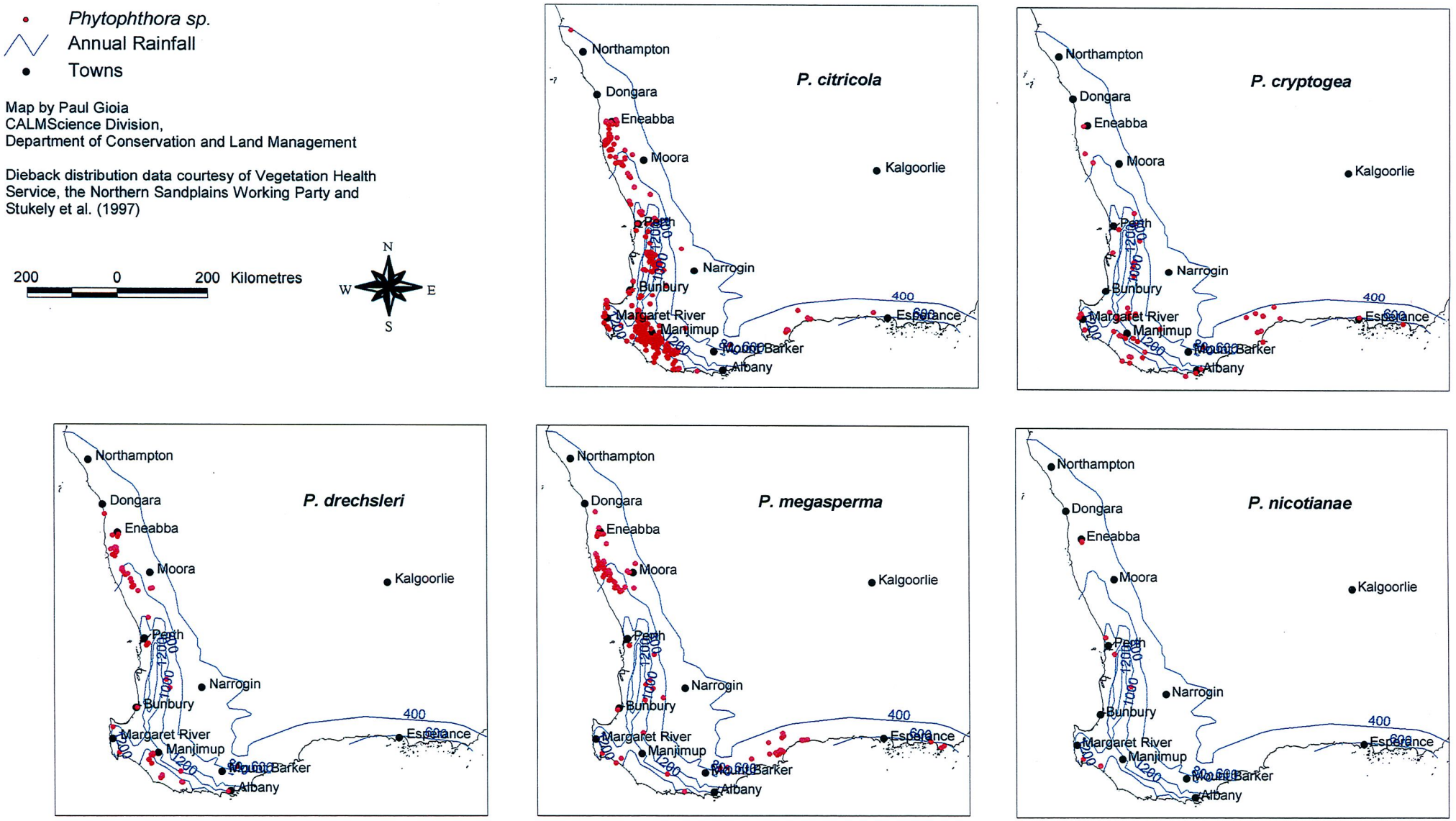



Figure 2. Distribution of records of *Phytophthora* spp. (other than *P. cinnamomi* ) in relation to rainfall zones

characteristics may play a role in preventing a build-up of soil moisture, thus inhibiting activity of the pathogen.

### 4.1.4 Topographic Position

Depending on the overall amount of precipitation, and seasonal differences in soil moisture, occupation of a high topographic position may afford some protection against *Phytophthora*. In areas receiving only 400–600mm annual rainfall, a position on ridges or hilltops might be associated with soil moisture levels that are usually too low to support the activity of *Phytophthora*. Conversely, a low topographic position in a predominantly dry area may harbour relatively high soil moisture levels, thus providing a suitable micro-environment for *Phytophthora*. Topographic position is unlikely to influence the spread of *Phytophthora* in areas receiving more than 800mm annual rainfall.

## 4.2 HOST VULNERABILITY TO *PHYTOPHTHORA*

The concept of vulnerability is valuable in determining the degree to which an inherently susceptible plant species may be threatened with extinction by *Phytophthora*. In the present context, vulnerability of a plant can be defined in terms of the extent to which the environment it is growing in favours survival and pathogenic activity of *Phytophthora*. Thus, a summation of the environmental factors discussed above provides an indication of vulnerability.

For example, if a susceptible plant population occupies an area receiving less than 400mm annual rainfall, it would be regarded as having extremely low vulnerability to *Phytophthora*. Conversely, a population of the same species growing within the 800mm isohyet might be regarded as extremely vulnerable unless it occurred in a protective zone such as the Spearwood Dune System. The vulnerability of a population in the 400–600mm isohyets would be influenced by its topographic position.

## 4.3 HOST SUSCEPTIBILITY TO *PHYTOPHTHORA*

Also important in assessing a threat of extinction is the notion of susceptibility, i.e., the degree to which the inherent biological characteristics of a species render it sensitive to the pathogenic activity of *Phytophthora*. However, a species considered to be highly susceptible to *Phytophthora* may not be critically threatened with extinction if a number of individual populations occupy situations of low vulnerability.

There are differing views on the concept of susceptibility. If it is considered to reflect the degree to which a plant suffers physical damage, certain monocotyledons might be regarded as highly susceptible to *Phytophthora*. However, due to their ability to rapidly replace damaged roots, few symptoms are observed, and these plants are regarded as resistant, even though acting as hosts for the pathogen (Phillips & Weste, 1984). Nevertheless, very few plant species can be viewed as truly resistant to *Phytophthora* (Tippett *et al.*, 1985). In this report, the term susceptibility is used in the context of assessing threats of extinction. Species or populations that are unlikely to die as a result of infection with *Phytophthora* are regarded as non-susceptible.

## 4.4 DISEASE ESCAPE

Although a species may be deemed highly susceptible to *Phytophthora*, its regenerative capability might afford a mechanism for survival. After an infection front has passed through a plant community, the number of individuals of a highly susceptible species is often drastically reduced. As a consequence, the number of hosts available to the pathogen is correspondingly diminished and its inoculum reservoir decreases accordingly. If the time required for surviving individuals to flower is sufficiently short, seed setting may occur before the pathogen inoculum base has reattained a critical level. Therefore, when applying the protocol for assessing threat of extinction, species within a worst case scenario should also be assessed for possible survival through disease escape.

## 4.5 PROTOCOL FRAMEWORK

Based on the vulnerability of individual populations of species, and the notions of susceptibility and disease escape, an initial framework for assessing threats of extinction can be specified. This should not be seen as definitive for any specific situation, but rather as a model on which to base an operational framework. It is also notable that the framework is predicated on a worst case scenario – that the spread of *Phytophthora* is inevitable throughout all niches in which it can survive. This is an unfortunate, but entirely possible scenario that should be recognised and incorporated in management planning.

The various factors described above can be framed as a series of questions to be asked of a given species. Each question acts as a filter that will influence the final determination of threat of extinction. These questions are summarised below, with a view to rating the threat of extinction from *Phytophthora* for a given species:

1. Is the species considered highly susceptible to *Phytophthora*?
   **Yes**: Go to 2
   **No** : No threat of extinction
2. Does the species have a mechanism such as disease escape to ensure survivors?
   **Yes**: No threat of extinction
   **No** : Go to 3
3. Are any or all populations of the species located in a rainfall zone receiving less than 400mm annual precipitation?
   **Yes**: No threat of extinction
   **No** : Go to 4
4. Are all known populations of the species restricted to a subtropical zone?
   **Yes**: No threat of extinction
   **No** : Go to 5
5. Do any populations occur on soils similar to the Spearwood Dune system?
   **Yes**: Verify the existence of populations in the field and their actual occurrence within the protective soil zone. If populations are extant, then species may not be threatened with extinction so long as their component populations are

managed defensively to prevent inadvertent spread of the pathogen.
**No** : Go to 6

6. Do any populations occur within the 400–600mm annual rainfall zone?
**Yes**: Assess the topographic position of each of these populations by reference to existing records. If necessary, verify the presence of populations in the field and assess their vulnerability. The species may not be threatened with extinction providing that some populations do not occupy vulnerable situations, and that these are managed defensively to prevent inadvertent spread of the pathogen.

**No** : Go to 7

7. Do all populations occur in areas receiving more than 600mm annual rainfall?
**Yes**: If there are no extant populations in any of the protective situations described above, then this species is threatened with extinction because of the inevitable spread of *Phytophthora* throughout localities receiving rainfall in excess of 600mm. Extant populations should be managed using intensive methods such as fencing and, if necessary, phosphonate application.

## 4.6 CASE STUDIES WITH *BANKSIA* SPECIES

The distribution of all available *Banksia* records for south Western Australia is shown in Figure 3, with rare or priority species differentiated from other taxa by a contrasting symbol. The distribution is overlain with mean annual rainfall isohyets and with isolation records for *P. cinnamomi.* The genus *Banksia* is widespread throughout the SWLD, with most records occurring in areas receiving more than 400mm annual rainfall. There are a number of clusters of rare or priority taxa. Of particular note are those in the Stirling Range National Park and on the south coast, directly east of Albany, where relatively heavy infestations of *P. cinnamomi* are present.

Threats of extinction to species of *Banksia* were assessed for as many situations as possible, using the draft framework outlined in Section 4.5. A sufficient number of taxa were examined to cover the broad spectrum of situations that populations of any given species might experience.

The distributions of four selected species are displayed in separate maps (Figures 4, 5, 6 and 7) each of which includes records from both WAHERB and the Banksia Atlas (Taylor & Hopper, 1988). The maps all comprise annual rainfall isohyets and a crude representation of the extent of the Spearwood Dune system. The numbers of records from each source of *Banksia* data is displayed for four rainfall zones. The *Banksia* spp. selected for examination (see below) were all considered to be susceptible to *Phytophthora*.

### 4.6.1 *Banksia audax*

*B. audax* is restricted to a region extending from the Goldfields, west of Kalgoorlie, to the Great Southern wheatbelt east of Narrogin (Figure 4). All recorded populations lie within a zone receiving less than 400mm annual rainfall. Therefore, this species is under no threat of extinction from *Phytophthora*.

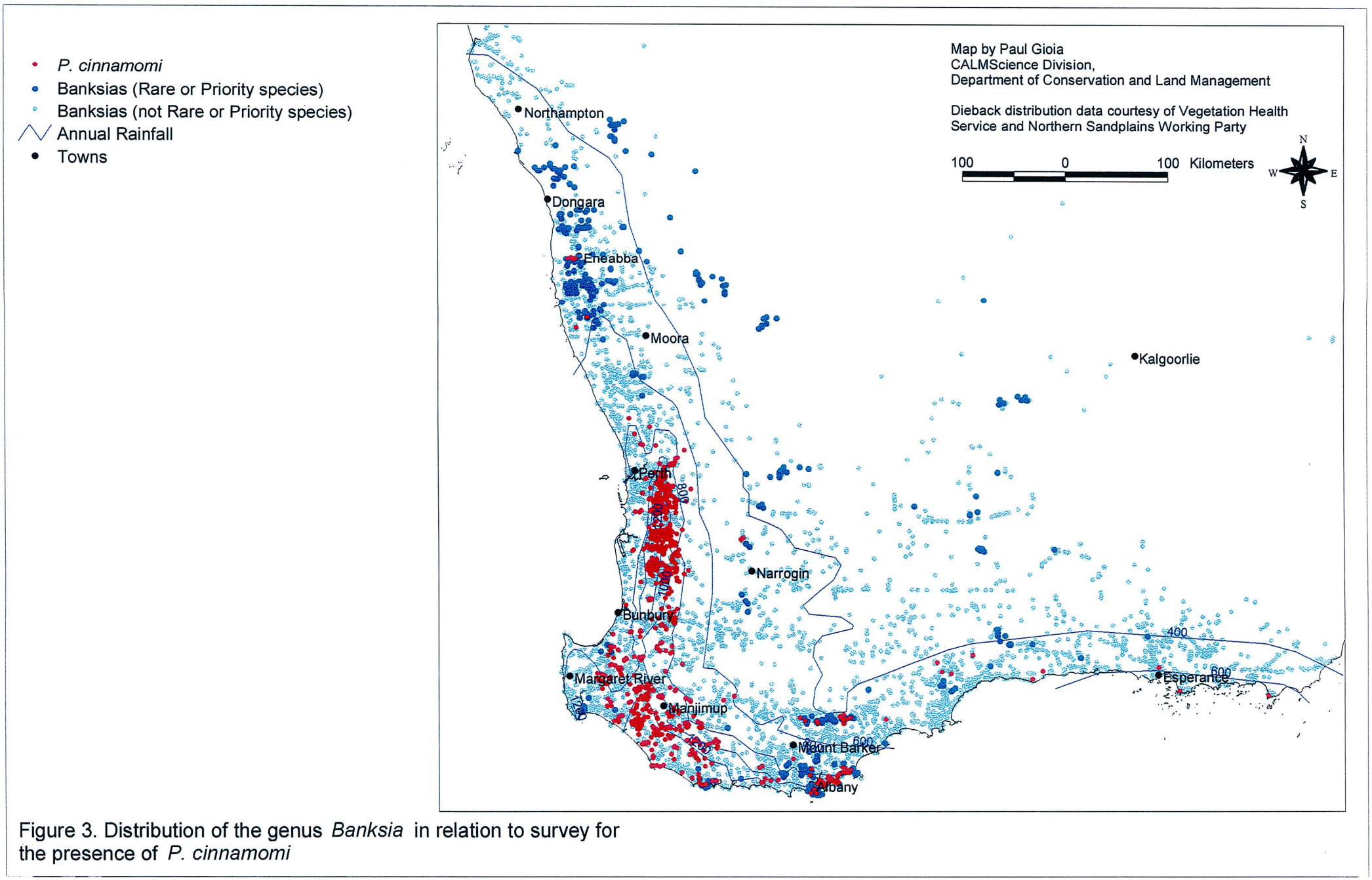



Figure 3. Distribution of the genus *Banksia* in relation to survey for the presence of *P. cinnamomi*

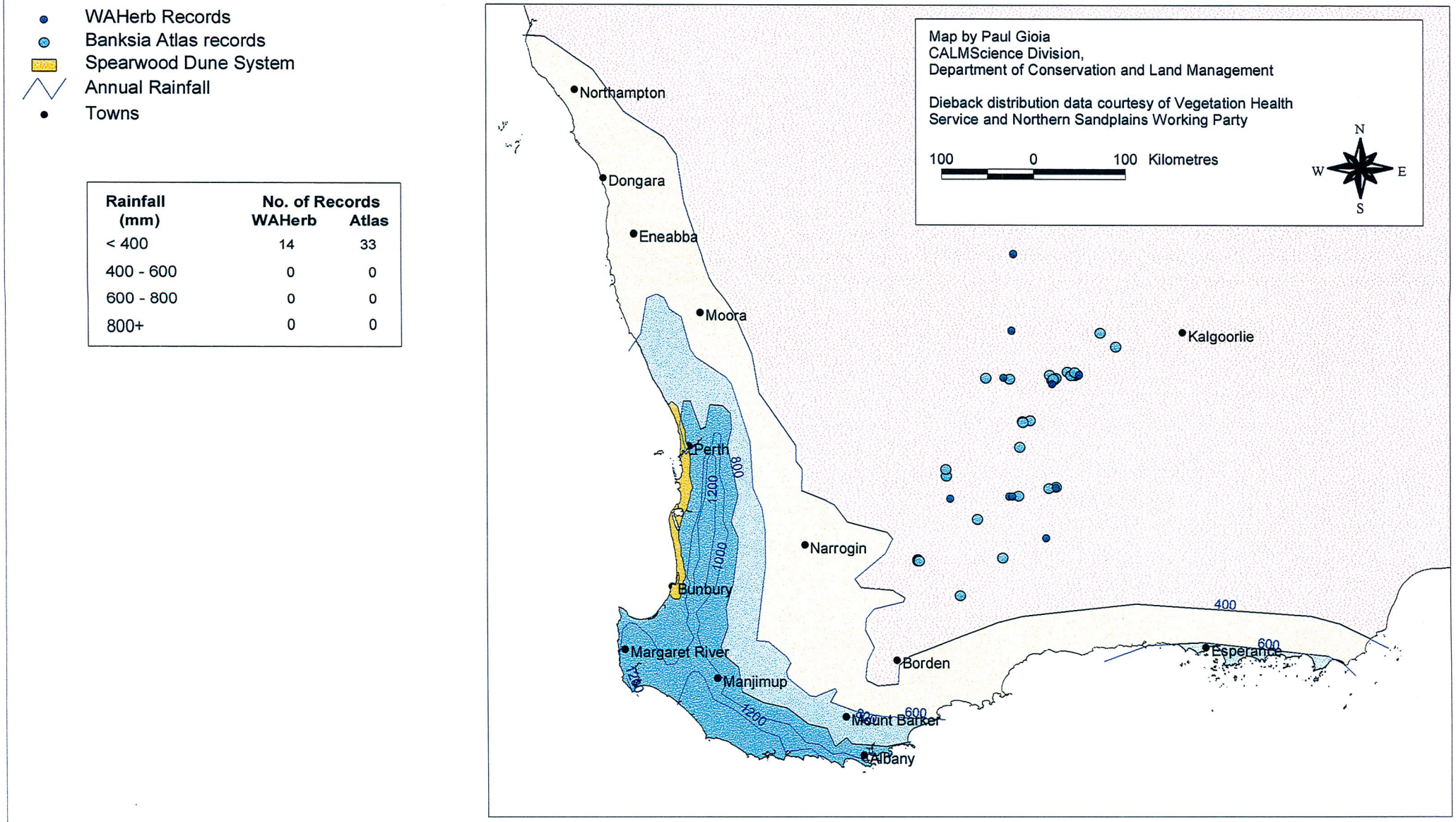



Figure 4. Distribution of records of *Banksia audax* in relation to rainfall zones and the Spearwood dune system

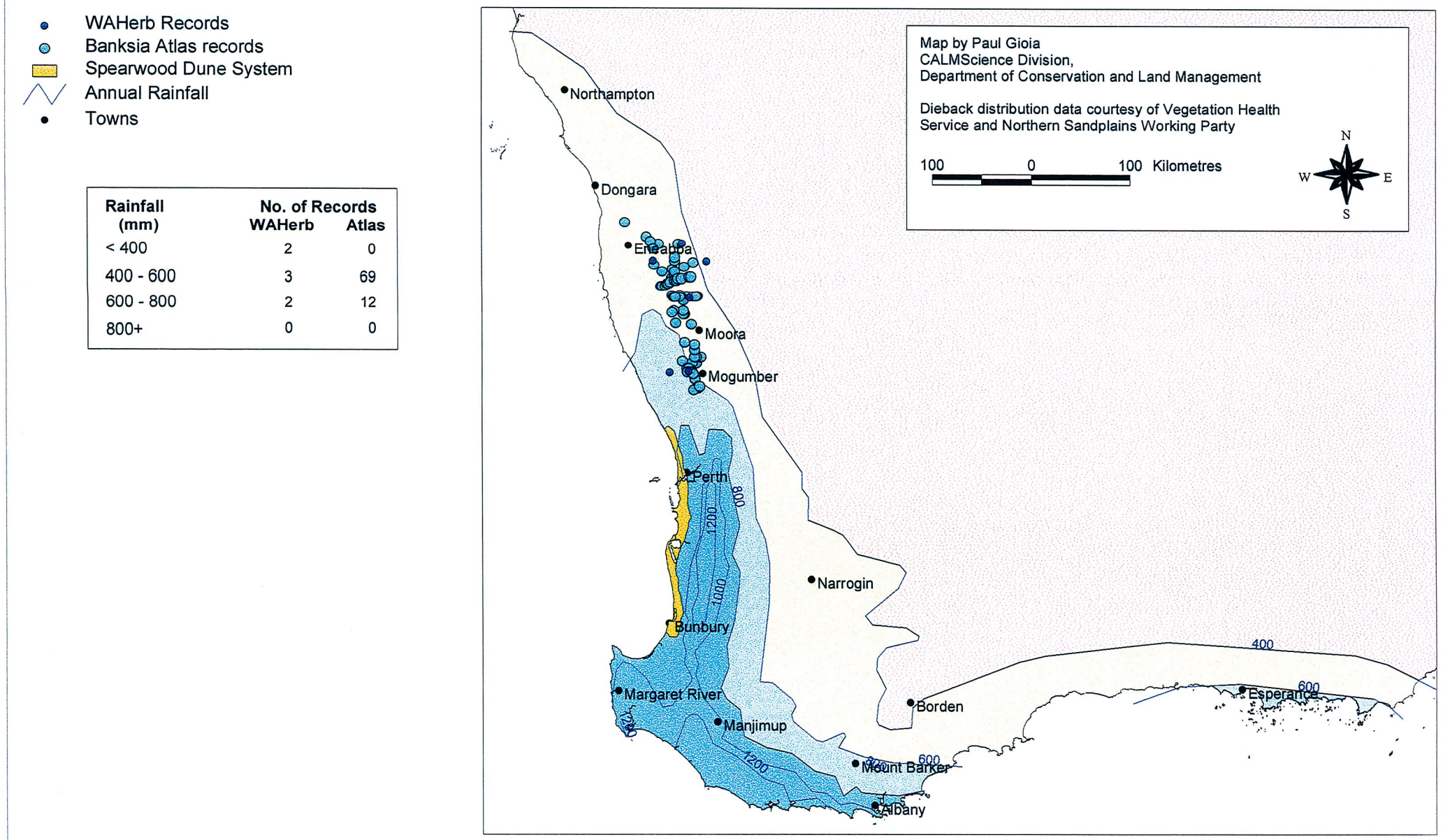
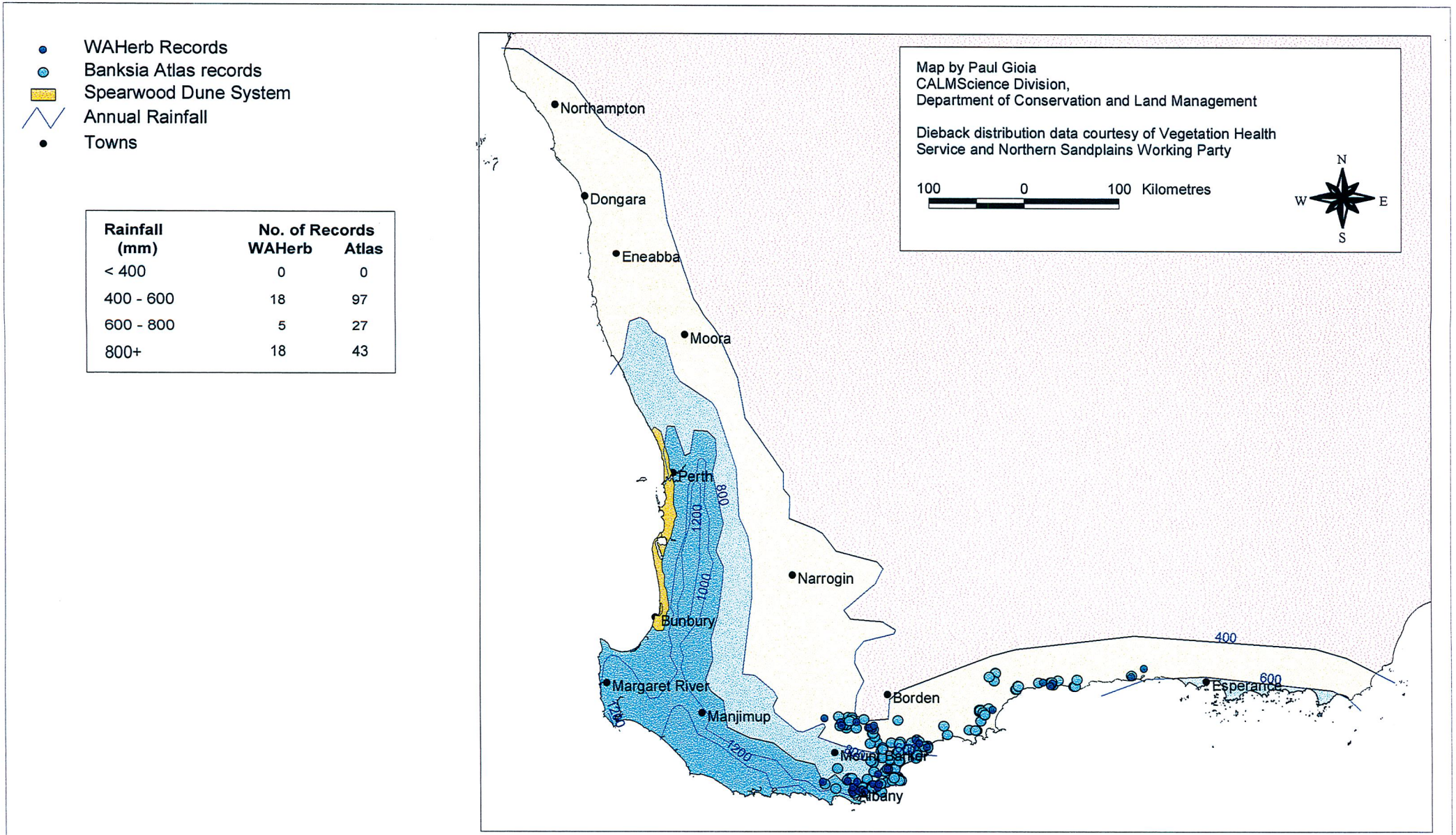



Figure 5. Distribution of records of *Banksia burdettii* in relation to rainfall zones and the Spearwood dune system




| Rainfall (mm) | No. of Records WAHerb | Atlas |
|---|---|---|
| < 400 | 0 | 0 |
| 400 - 600 | 18 | 97 |
| 600 - 800 | 5 | 27 |
| 800+ | 18 | 43 |

Figure 6. Distribution of records of *Banksia coccinea* in relation to rainfall zones and the Spearwood dune system

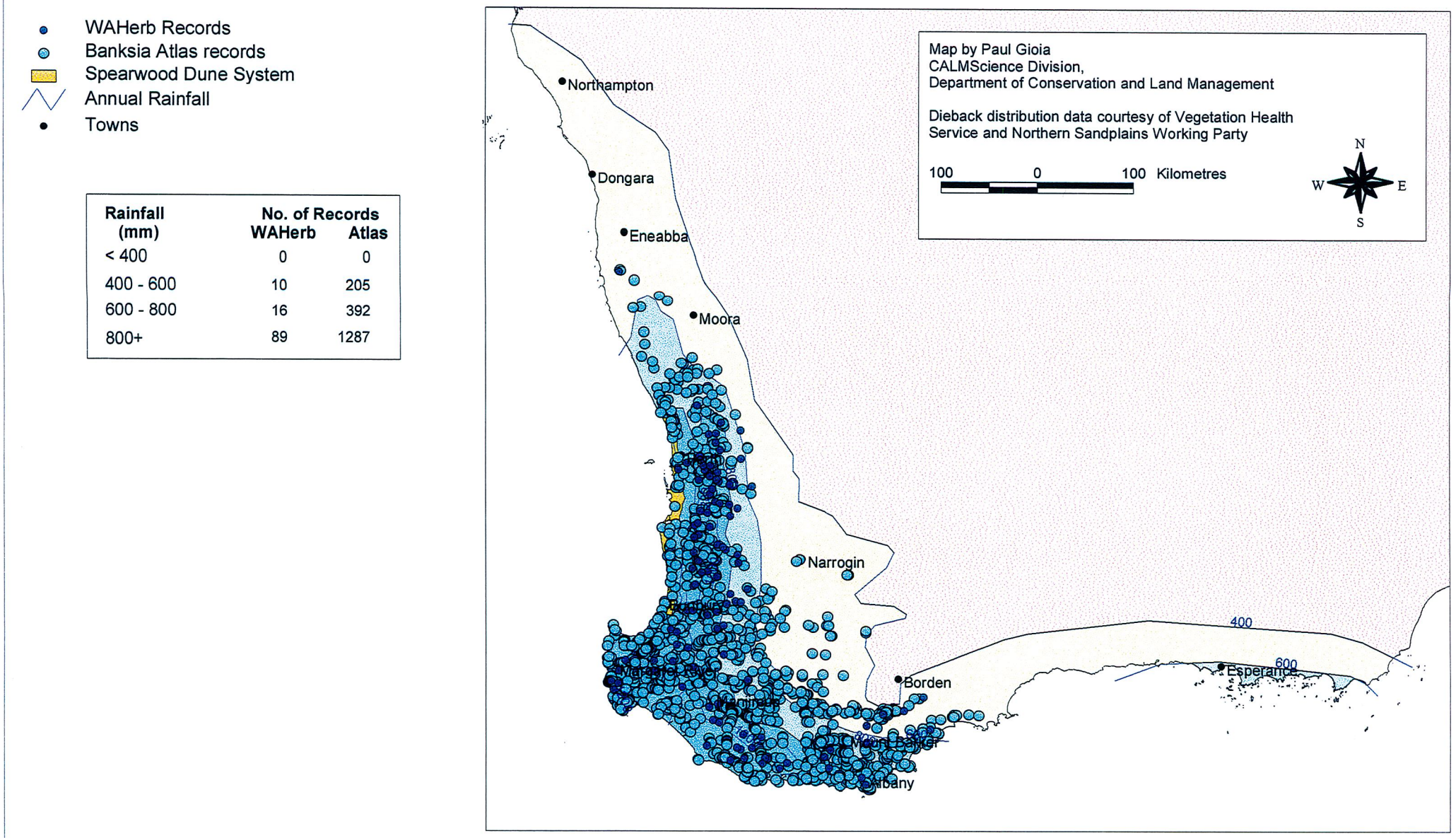



Figure 7. Distribution of records of *Banksia grandis* in relation to rainfall zones and the Spearwood dune system

### 4.6.2 *Banksia burdettii*

*B. burdettii* is restricted to an area between Eneabba and Mogumber. About a third of the populations listed in the Banksia Atlas were recorded in conservation reserves, whereas most other populations were found on road verges bordering agricultural land. Atlas records are confined to white or yellow sandplain as a component of shrubland or, less commonly, woodland (Taylor & Hopper, 1988).

Figure 5 shows the available records for *B. burdettii*, most of which relate to areas receiving between 400mm and 600mm annual rainfall. Application of the protocol (Section 4.5) leads to a positive response for Question 6 which states that populations in the 400-600mm rainfall zone are vulnerable unless they are located in topographically elevated positions on the landscape. Because *B. burdettii* occurs mainly in low lying areas, the threat of extinction posed to this species by *Phytophthora* appears to be moderately high, at least in a worst case scenario.

Although there are just seven herbarium records for *B. burdettii*, as opposed to eighty-one in the Banksia Atlas (Taylor & Hopper, 1988), only moderate range extension is associated with the latter. Two herbarium records from 1939 relate to populations in a relatively dry zone between Marchagee and Coorow. In the unlikely event that these two populations are extant, the threat of extinction would be significantly diminished.

Management options for protection of *B. burdettii* would require that all available records be examined to determine the topographic position of individual populations. Any records of up-slope populations would need to be confirmed in the field before possible control measures were investigated.

### 4.6.3 *Banksia coccinea*

Figure 6 shows the recorded occurrences of *B. coccinea*. This species has a near-coastal distribution on the southern sandplain of the SWLD with inland populations mostly located in the Stirling Ranges or the north-western corner of Fitzgerald River National Park. *B. coccinea*, which is highly susceptible to *P. cinnamomi*, prefers flat or gently undulating landforms, with one exception on Ellen Peak in the Stirling Ranges (Taylor & Hopper, 1988).

With reference to the protocol, all records of *B. coccinea* are for areas where annual rainfall exceeds 400mm, with almost half of those receiving more than 800mm. Most records for the 400-600mm zone relate to low-lying areas. *B. coccinea* should therefore be viewed as under an extremely high threat of extinction.

### 4.6.4 *Banksia grandis*

*B. grandis* has a widespread distribution throughout the coastal plain and Darling plateau, extending from Mt. Lesueur south to Augusta and east to Bremer Bay (Figure 7). Records from the Banksia Atlas indicate that the range of *B. grandis* extends as far inland as Badgebup and Dongolocking Nature Reserve, and that the species tends to occur on flat or gently sloping landforms (Taylor and Hopper, 1988).

All records of *B. grandis* are derived from zones receiving more than 400mm annual rainfall, with most relating to areas where the yearly average exceeds 800mm. Because the vast majority of recorded occurrences in the lower rainfall zone are also from low-lying sites, most populations of *B. grandis* are highly vulnerable to *Phytophthora*. One possible protective zone is the Spearwood Dune system for which a number of records occur, primarily in the Banksia Atlas (Taylor & Hopper, 1988).

Contrary to popular belief, the threat of extinction to *B. grandis* is much greater than might be expected, despite the widespread distribution of the species. The focus on geographically restricted taxa may sometimes tend to divert attention from the plight of currently widespread species that may ultimately become endangered.

# 5 OUTCOMES

- Methodology was successfully developed for the formulation of a protocol to evaluate threats of extinction posed by *Phytophthora* to taxa of native plants. The protocol was designed to assist management decisions in priority setting for application of limited resources essential to the protection of native biota under existing or immediate threat of extinction due to the activity of the pathogen.

- The protocol was based on the combination of expert knowledge obtained from a recognised authority on the pathology and management of *Phytophthora*, together with input from a specialist in the use of computer systems, particularly GIS. The advantage of an expert systems approach is that it obviates the requirement for modelling the complex behaviour of *Phytophthora* in natural environments, a task that is likely to be extremely difficult.

- Another advantage of this approach, is that application of the simple protocol described here does not require rigorous training or extensive prior knowledge. Thus, it can readily provide key assistance to land managers in the course of decision making for prioritisation of resources in plant protection.

- The study of *B. grandis* has raised the possibility that some widespread species of susceptible native plants might be under a significant threat of extinction in a worst case scenario in which *Phytophthora* eventually occupies all niches available to it, and where the distributions of the host and pathogen then coincide.

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# 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

***Barbara M. Komorek and Bryan L. Shearer, assisted by Melanie Blumberg, Colin Crane and Richard Fairman***

## 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 $\ell^{-1}$ and 400g $\ell^{-1}$, 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 60$\ell$ ha$^{-1}$ is insufficient to ensure disease control for more than one year.
- Application of 40% phosphonate at 30-60$\ell$ 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 15$\ell$ 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 30$\ell$ $ha^{-1}$ which resulted in an effective application rate of 60$\ell$ $ha^{-1}$.

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 60$\ell$ $ha^{-1}$. 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**

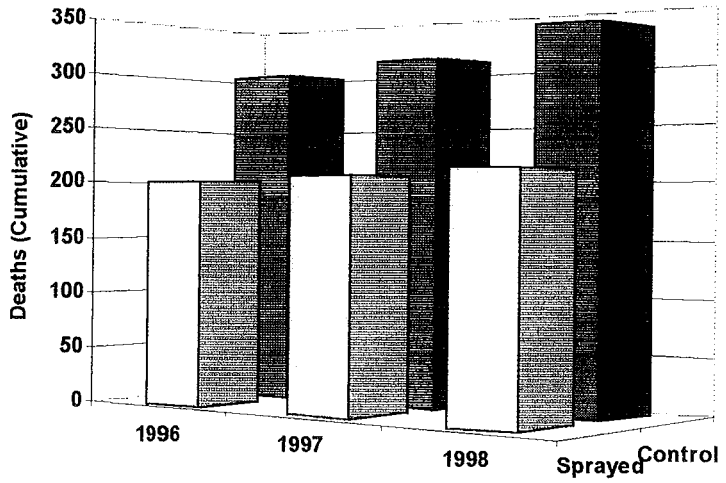


**Figure 1b. Mortalities in a stand of 15-year-old *Banksia coccinea* plants, treated or not (control) with 40% phosphonate**

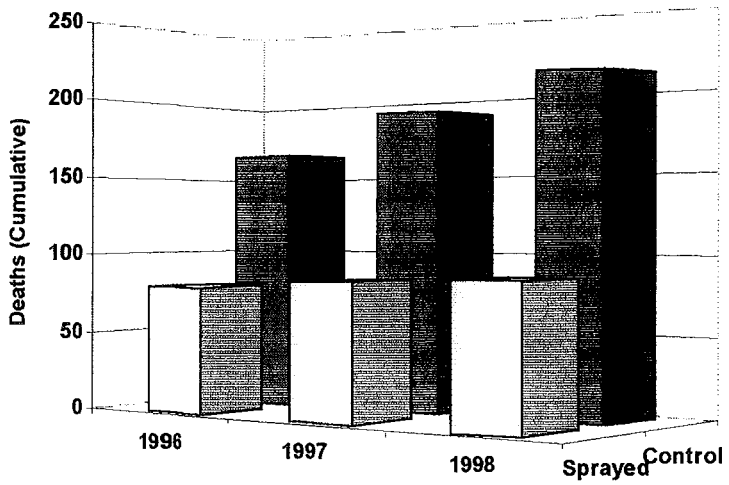
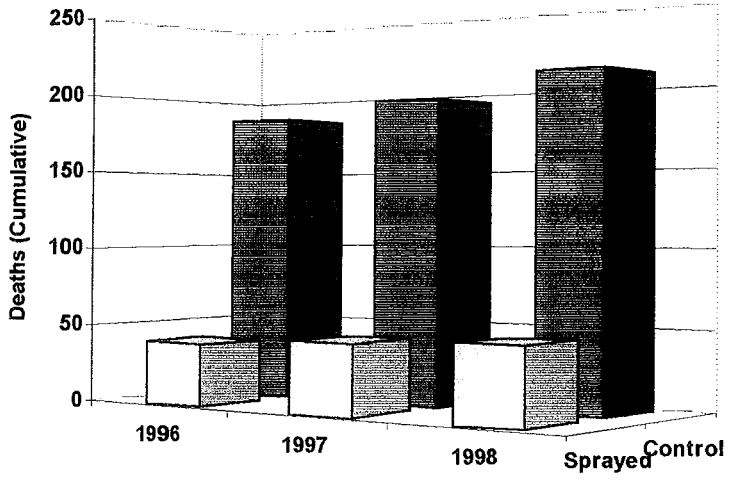



**Figure 1c. Mortalities in a stand of 8-year-old *Banksia coccinea* plants, treated or not (control) with 40% phosphonate**




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***

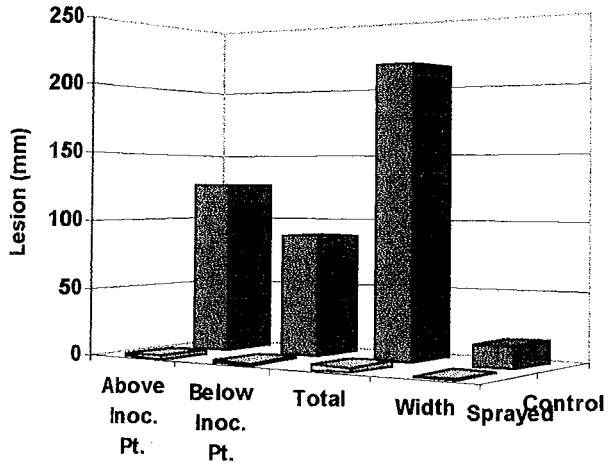



**Figure 2b. Lesion length and width on 15-year-old *Banksia coccinea* stems inoculated with *Phytophthora cinnamomi***

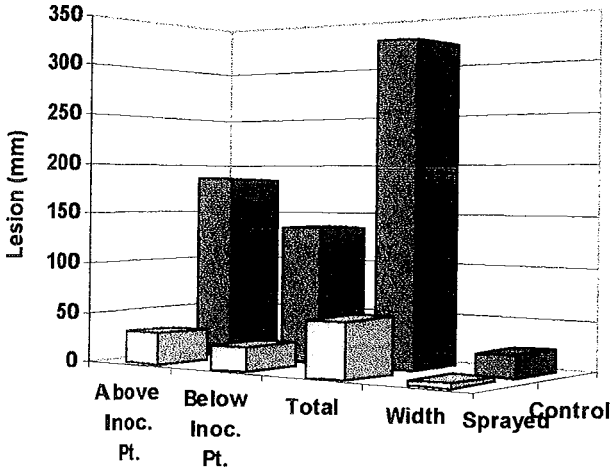
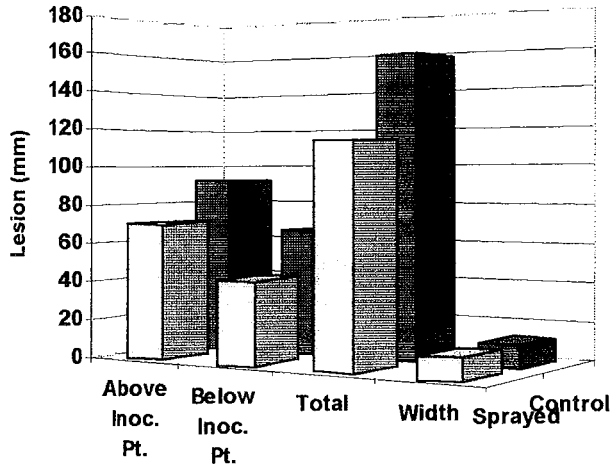


**Figure 2c. Lesion length and width on 8-year-old *Banksia coccinea* stems inoculated with *Phytophthora cinnamomi***




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**

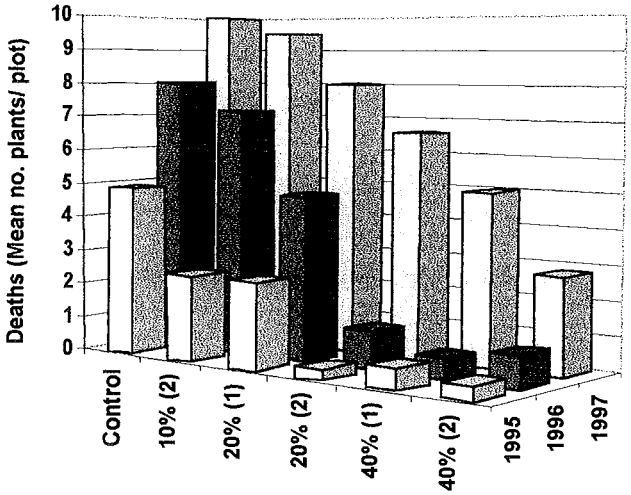
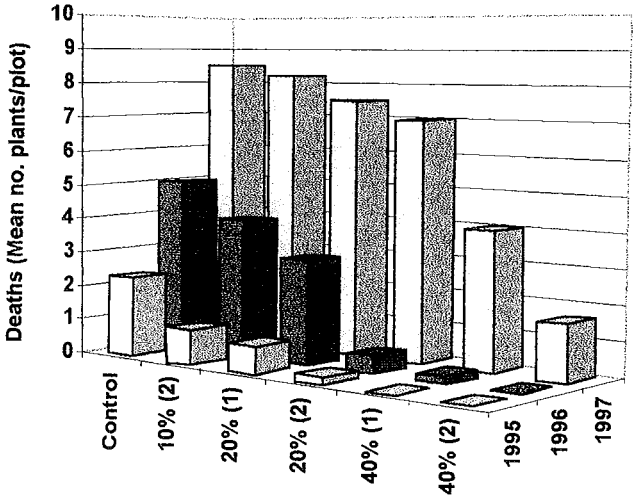



**Figure 4. Mortality (cumulative) of *Banksia menziessi* treated once (1) or twice (2) with phosphonate at stated concentrations**




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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RGC Mineral Sands were helpful with their information on dieback occurrence in the Eneabba area, providing free accommodation and allowing us to carry out field experiments at RGC.

# 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

***Sarah Barrett and Malcom Grant***

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 60$\ell$ 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 30$\ell$ $ha^{-1}$ and 60$\ell$ $ha^{-1}$, respectively. The 36kg $ha^{-1}$ treatment was applied using 30% phosphonate at 60$\ell$ $ha^{-1}$. 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 30$\ell$ $ha^{-1}$. 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 ($\mu g\ g^{-1}$) 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. ($\mu g\ g^{-1}$) | | | |
|---|---|---|---|---|
| | 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 ($\mu g\ g^{-1}$) 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. ($\mu g\ g^{-1}$) | |
|---|---|---|
| | 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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# SCOPE ITEM 5

# *EX SITU* CONSERVATION OF *PHYTOPHTHORA- AND* CANKER-THREATENED SPECIES OF WESTERN AUSTRALIAN NATIVE PLANTS

## PART A

## IDENTIFICATION, SEED COLLECTION AND GERMPLASM STORAGE OF THREATENED TAXA

***A. Cochrane, K. Brown and D. Coates***

## 1 INTRODUCTION

There is no doubt that germplasm storage has an extremely important role to play in the *ex situ* conservation of rare and threatened plant taxa. Threatening processes, particularly loss of habitat, weed invasion, dieback (*Phytophthora* spp.) and canker diseases continue to be major factors in the local extinction of native species in Western Australia. Loss of populations or a substantial reduction in population size may not necessarily lead to immediate extinction, but more commonly to a loss of genetic diversity. Where population extinction in the wild cannot be avoided, genebanks or germplasm storage facilities can be utilised as an interim solution to prevent loss of genetic diversity within the range of a species or, as a last resort, to prevent extinction of the species. One of the most cost effective methods for genebanking is the long term storage (minimum of 50 years) of seed at low (-20° C) or ultra-low (-196°C) temperatures.

Genebanking can be readily incorporated into an integrated strategy for conservation. The highly compact nature of seeds make them ideal for long term storage at low temperature and this strategy is more economical than the maintenance of living plant collections in botanic gardens. The success of germplasm conservation depends on the longevity of seed subjected to a particular storage regime and the capacity to regenerate adequate quantities of high quality seed without genetic change when

viability declines (Morse *et al.*, 1993). The Threatened Flora Seed Centre (TFSC) has been operating as a long term germplasm storage facility for threatened Western Australian plant taxa since its establishment by the Department of Conservation and Land Management (CALM) in 1992 (Cochrane, 1997).

The work reported here is concerned with Scope Item No. 5 for the *Phytophthora* and *Diplodina* Canker project (1997/98). This Scope Item requires:

- the continued identification of species that are susceptible to *Phytophthora* and *Diplodina* canker; and
- collection and storage of their seed as part of an *ex situ* strategy of germplasm conservation.

# 2 OBJECTIVES

The current objective of the TFSC, which addresses Scope Item No.5, is to ensure the maintenance of genetically representative seed collections of threatened, Western Australian flora under long term storage conditions as an interim solution to prevent the genetic degradation or local extinction of critically affected populations. An important objective of seed collection is to systematically capture 90-95% of the common alleles from each threatened taxon on a representative range-wide basis. This broad sample of genetic variation is essential if the stored material is to be effectively used for long term re-establishment of species in the wild following removal of any threats.

# 3 METHODS

## 3.1 SEED COLLECTION

For the past year, collection activity has focussed on Western Australia's critically endangered taxa and on the acquisition of seed from dieback- (*Phytophthora*) and canker-susceptible species in the south-west of the State. Ongoing consultation with Kings Park and Botanic Gardens has highlighted gaps in the *ex situ* conservation of critically endangered species. Accordingly, efforts are being made to secure germplasm of currently unrepresented taxa in the face of declining populations and possible extinctions. CALM's Declared Rare and Priority Flora (DRPF) List for Western Australia (Ken Atkins, 03/12/97) has been used for the most recent collections. The ranking of DRPF into three categories (Critically Endangered, Endangered and Vulnerable) by the Western Australian Threatened Species and Communities Unit of CALM has greatly assisted the formulation of management decisions regarding selection of taxa for collection. As of May 1998, 95 critically endangered plant taxa were recognised in Western Australia.

The TFSC seed collection protocols are derived primarily from work by Brown & Briggs (1991) and Brown *et al.* (1989a, 1989b). They are based on guidelines used by CSIRO's Australian Tree Seed Centre (ATSC) and Wakehurst Place, Royal Botanic

Gardens, Kew, U.K. The collection protocols have been extensively documented by Cochrane & Coates (1997).

### 3.2 SEED STORAGE, VIABILITY TESTING AND INVENTORY SYSTEM

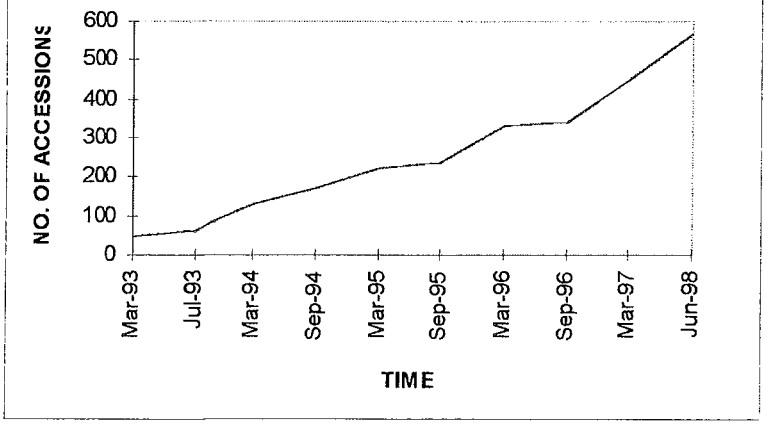
The TFSC laboratory protocols involve registration, cleaning, fumigation, testing, storage and monitoring of all collected seed. The design of genebanks and protocols recommended for use in genebanks have been formulated by the International Board for Plant Genetic Resources (Cromarty *et al.*, 1985; Ellis *et al.*, 1985a, 1985b). Information on the documentation, cleaning, fumigation, quantification, viability testing, moisture content determination, and reduction and drying of seeds at the TFSC is outlined in Appendix 1. A more detailed description of the various procedures is provided by Cochrane & Coates (1997). Data resulting from all TFSC activities are entered into the WASEED database. Considerable interest in WASEED has been shown by botanic gardens in the USA and the application is currently being trialed at the Berry Botanic Gardens in Portland, Oregon.

## 4 RESULTS AND DISCUSSION

### 4.1 SEED COLLECTION

Currently (June 1998), 567 accessions of rare and threatened flora are in storage at the TFSC (Figure 1 and Appendix 2). This represents 204 taxa in 47 genera in 18 families. These accessions represent a total of 435 seed collection sites.

**FIGURE 1:** No. of accessions held in storage, March 1993 to June 1998



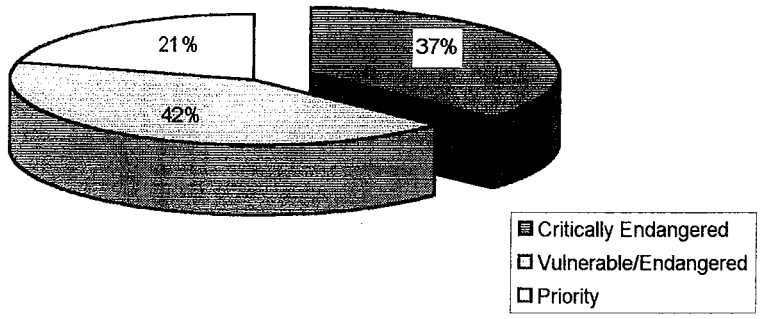

Good, genetically representative seed collections of 47 of the 95 critically endangered Western Australian plant taxa have been made by staff at the TFSC. Seed from at least some populations of an additional eight taxa are also held in long term storage. Of the other 40 critically endangered taxa, two are not known to produce seed and two appear to be extinct at known locations. Eleven taxa are in the family Orchidaceae and

research on germplasm storage of orchid seed is currently being conducted by the laboratory at Kings Park and Botanic Gardens. Seven of the critically endangered taxa have been listed as such only since May 1998. While populations of most other critical taxa have been surveyed, seed was not collected due to timing and/or low or sporadic fruit set, or because the majority of plants were still in a juvenile phase as a result of recent fire damage.

Seed of 70 endangered and vulnerable taxa (239 accessions) are presently held in long term storage and this is also true for 70 taxa (119 accessions) on Western Australia's priority flora list which comprises taxa that are poorly known and in need of further survey (Figure 2).

Since 1992, collection of seed from a number of rare and threatened taxa has proved very difficult due to small population size, low or sporadic seed production, high seed predation or disturbances such as disease, fire or drought which cause genetic fluctuations in the genepool. Differential fruiting of *Adenanthos* spp. has warranted the use of seed traps as a viable option for collection and this involves the assistance of the local community when long term continuous seed collecting is required.

**FIGURE 2: Conservation status of accessions held in TFSC storage.**




During the past year, research into reasons for low fruit production in one of the critically endangered *Acacia* spp.(*A. insolita* subsp. *recurva*) has been initiated by staff of the TFSC. The establishment and influence of herbivore exclusion cages, the regenerative response of *A. insolita* after fire, and an assessment of the soil seed-bank are some of the areas being investigated to increase our knowledge on the biology of this threatened taxon.

## 4.2 SEED STORAGE, VIABILITY TESTING AND INVENTORY SYSTEM

Monitoring of accessions is an integral part of seed storage. At the present time, more than 230 accessions representing 112 taxa have been tested for viability after one year in storage and 79% of these have maintained their initial viability. A relatively small number of accessions have shown a reduction in germinability after storage (21%). Further replication and testing of these taxa is needed to confirm whether some

species suffered a loss of viability, or whether dormancy was induced by drying and freezing. Of the accessions that showed a post-storage reduction in viability, 5% of cases were attributable to technical or procedural errors resulting in seed mortality. Protocols have since been established to ensure that the sequence of treatments prior to germination is standard. Fungal contamination during germination trials and small sample sizes also render comparison of pre- and post-storage germination results difficult. In addition, the narrow environmental tolerances of some wild seeds can present a potential problem in germination testing. Routine germination tests deplete seed accessions, and duplication of tests under different conditions is seldom possible due to the small size of samples.

In some cases it appears that the storage regime may have imposed dormancy on seeds. Although dormancy is considered to be biologically advantageous, it creates problems for research into the responses of seed to various storage regimes and it can limit the value of germination tests.

# 5 OUTCOMES

A long term germplasm storage facility capable of operating to international standards has been established at CALM's Western Australian Herbarium.

## 5.1 SEED COLLECTION

- An ongoing, well coordinated germplasm collection program has been established at the TFSC and a total of 567 accessions of rare and threatened Western Australian flora (representing 204 taxa) are currently held in the genebank. More than 75% of the State's threatened taxa, which are presumed susceptible to dieback and canker, are represented in low moisture, low temperature storage.

- New techniques for the collection of seed from differentially fruiting taxa have been developed. Seasonal re-sampling of populations associated with low levels of viable seed production has enabled sufficient quantities of seed of many taxa to be collected and stored.

- Effective communication links and collaborative activities have been fostered and maintained between the TFSC, CALM District staff and researchers, Kings Park and Botanic Gardens, and local community groups.

- A small proportion of the time spent on field work has been devoted to the collection of germplasm from various species for research into their population biology and to assess the taxonomic status of particular segments of the flora.

- During the last six years, our understanding of many of the variables that affect phenology, pollination, seed set, and seed ripeness has been enhanced in the course of field collections and research on seed biology. At the same time, essential

knowledge has been accumulated in regard to identification of seed from a diverse range of plant species.

## 5.2 SEED STORAGE, VIABILITY TESTING AND INVENTORY SYSTEM

- Research on various techniques designed to promote germination has resulted in a better understanding of the seed biology of many rare and threatened taxa. The use of growth hormones, smoke, heat treatment and scarification have provided useful information for assessment of seed viability and determination of the optimum methods for germination (Cochrane & Kelly, 1997). Research into seed storage, seed germination and dormancy-breaking mechanisms for the south-western flora has enhanced the operational effectiveness of the TFSC.

- Storage data are now available for a wide range of species. The effects of moisture content reduction and storage of germplasm in carbon dioxide at sub-zero temperatures are known for many taxa held in the genebank. Maintenance of high viability of stored seed has been demonstrated for the majority of accessions. In cases where high viability has not persisted, further research is required to determine a more appropriate storage regime. As fungal and bacterial infection may have contributed to the apparent loss of seed viability in many accessions, sterile conditions will be strictly implemented to enable accurate comparisons to be made between pre- and post-storage germination.

- For many taxa, knowledge of the optimum conditions for seed germination is still lacking and inability to break dormancy in some species limits efforts to store germplasm and maintain viability in storage.

- A comprehensive database is presently used to collate all aspects of the collection, processing, testing, storage, and monitoring of accessions. Detailed information on the phenology of a range of rare and threatened taxa will assist in the planning of fieldwork.

- Accessions will continue to be monitored on a yearly, then five-yearly basis until adequate knowledge of the flora's response to sub-zero storage is attained. A monitoring regime of ten years will then be implemented subject to viability figures.

## 5.3 OTHER DEVELOPMENTS

- Between June and September, 1997, the manager of the TFSC visited the International Plant Genetic Resource Institute in Rome (Italy), genebanks in the USA, the Seed Conservation Section of Wakehurst Place, Kew (UK) and seed biologists in South Africa. The impression gained from these visits was that the methods used in the TFSC for long term storage of conservation taxa are consistent with international standards. The overseas trip was supported by a Churchill Fellowship.

- Seedlings produced in the course of routine viability tests are being sent to researchers in CALM for assessment of susceptibility to *Phytophthora* spp.

- Germplasm stored in the TFSC is being used for a series of translocation proposals commencing in the current year. Seed from seven critically endangered taxa have already been germinated and subsequently cultivated at Kings Park and Botanic Gardens for planting in autumn 1998. This development equates with attainment of one of the major outcomes of the project, namely the provision of material for *ex situ* propagation as required in recovery programs.

- In March, 1998, staff of the TFSC conducted a preliminary workshop on the identification and collection of seed for CALM District personnel.

- In July, 1998, staff of the TFSC will conduct a national course on "Establishing a Seed-Based Genebank for Conservation Purposes". Participants attending the course represent botanic gardens and conservation agencies from around Australia.

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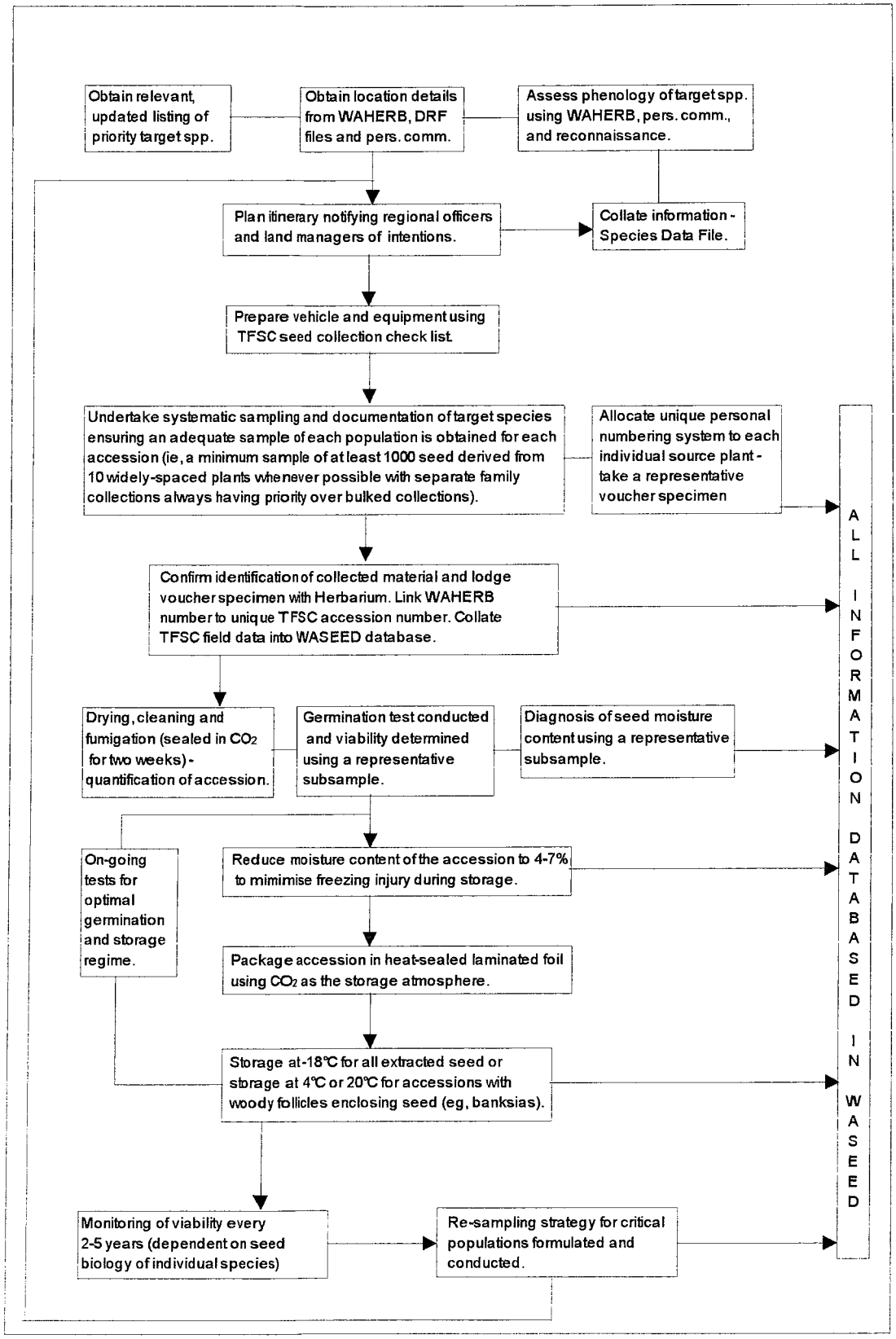
## Appendix 1. Seed collection and storage protocols for the Threatened Flora Seed Centre



# Appendix 2. Threatened Flora Seed Centre accessions to June 1998

| Accession | Date(s) of Collection | Species | Collection Type |
|---|---|---|---|
| 00196 V | 4/12/94 | *Acacia awestoniana* | B/7 |
| 00001 V | 9/08/87 | *Banksia brownii* | I/4 |
| 00002 V | 2/11/88 | *Banksia brownii* | I/14 |
| 00003 E | 9/02/88 | *Banksia cuneata* | I/6 |
| 00004 E | 9/02/88 | *Banksia cuneata* | I/10 |
| 00005 E | 9/02/88 | *Banksia cuneata* | I/13 |
| 00006 V | 21/04/89 | *Banksia brownii* | I/38 |
| 00007 V | | *Banksia brownii* | I/29 |
| 00008 V | 24/08/89 | *Banksia brownii* | I/49 |
| 00009 P2 | 26/04/88 | *Banksia epica* | I/45 |
| 00010 E | 9/02/88 | *Banksia cuneata* | I/30 |
| 00011 E | 9/02/88 | *Banksia cuneata* | I/7 |
| 00012 E | 8/02/88 | *Banksia cuneata* | B/23 |
| 00013 E | 23/05/88 | *Banksia cuneata* | I/23 |
| 00014 E | 24/09/90 | *Banksia cuneata* | I/29 |
| 00015 V | 1/06/86 | *Banksia verticillata* | I/6 |
| 00016 V | 10/05/86 | *Banksia verticillata* | I/11 |
| 00017 P3 | 15/03/94 | *Verticordia attenuata* | B/20 |
| 00018 V | 8/03/88 | *Banksia oligantha* | I/10 |
| 00019 V | 8/03/88 | *Banksia oligantha* | I/20 |
| 00020 V | 8/03/88 | *Banksia oligantha* | I/10 |
| 00021 V | 26/11/86 | *Banksia verticillata* | I/20 |
| 00022 V | 1987 | *Banksia verticillata* | I/9 |
| 00023 V | 10/05/86 | *Banksia verticillata* | I/13 |
| 00024 C | 28/11/90 | *Dryandra ionthocarpa* | I/19 |
| 00025 V | 9/08/90 | *Banksia oligantha* | I/14 |
| 00026 V | 9/08/90 | *Banksia oligantha* | I/11 |
| 00027 V | 9/08/90 | *Banksia oligantha* | I/10 |
| 00028 V | 9/08/90 | *Banksia oligantha* | I/30 |
| 00029 V | 7/06/85 | *Lambertia orbifolia* | I/9 |
| 00030 V | 13/12/92 | *Lambertia orbifolia* | B/15 |
| 00031 E | 12/12/92 | *Isopogon uncinatus* | I/16 |
| 00032 V | 12/12/92 | *Banksia verticillata* | I/10 |
| 00033 V | 13/12/92 | *Andersonia* sp. Two Peoples Bay | B/50 |
| 00034 P3 | 14/12/92 | *Andersonia echinocephala* | B/30 |
| 00035 P3 | 15/12/92 | *Andersonia grandiflora* | I/12 |
| 00036 E | 18/12/92 | *Adenanthos pungens* ssp. *Effusa* | B/10 |
| 00037 P3 | 22/01/93 | *Dryandra seneciifolia* | I/13 |
| 00038 P3 | 22/01/93 | *Andersonia grandiflora* | I/10 |
| 00039 C | 23/01/93 | *Dryandra ionthocarpa* | I/10 |
| 00040 V | 25/01/93 | *Banksia brownii* | I/22 |
| 00041 C | 29/01/93 | *Lambertia echinata* ssp. *Echinata* | I/3 |
| 00042 P2 | 30/01/93 | *Isopogon alcicornis* | I/10 |
| 00043 P4 | 31/01/93 | *Dryandra serra* | B/20 |
| 00044 V | 18/09/92 | *Banksia verticillata* | B/6 |
| 00045 P3 | 24/01/93 | *Thomasia solanacea* | I/5 |
| 00046 P3 | 24/01/93 | *Eucalyptus acies* | B/5 |
| 00047 P2 | 30/01/93 | *Melaleuca coccinea* ssp. *Eximia* | B/10 |
| 00048 V | 30/01/93 | *Eucalyptus merrickiae* | I/5 |
| 00049 P3 | 16/03/94 | *Verticordia attenuata* | B/30 |
| 00050 P3 | 16/03/94 | *Verticordia attenuata* | B/50 |
| 00051 V | 19/05/93 | *Banksia goodii* | I/5 |

| | | | |
|---|---|---|---|
| 00052 V | 9/05/86 | *Banksia goodii* | I/12 |
| 00053 V | 16/03/94 | *Verticordia plumosa* var. *ananeotes* | I/10 |
| 00054 G | 1/06/87 | *Banksia semi-nuda* ssp. *remanens* | I/7 |
| 00055 V | 4/04/93 | *Banksia verticillata* | I/25 |
| 00056 V | 6/04/93 | *Banksia verticillata* | I/15 |
| 00057 V | 7/04/93 | *Banksia verticillata* | I/25 |
| 00058 V | 15/05/93 | *Lambertia orbifolia* | B/15 |
| 00059 V | 15/05/93 | *Lambertia orbifolia* | B/21 |
| 00060 V | 18/05/93 | *Banksia brownii* | I/16 |
| 00061 V | 19/05/93 | *Banksia brownii* | I/41 |
| 00062 P1 | 20/07/93 | *Dryandra squarrosa* ssp. *argillacea* | I/10 |
| 00063 P1 | 21/07/93 | *Dryandra squarrosa* ssp. *argillacea* | I/17 |
| 00064 G | 22/07/93 | *Banksia semi-nuda* ssp. *remanens* | I/17 |
| 00065 G | 22/07/93 | *Banksia semi-nuda* ssp. *remanens* | I/14 |
| 00066 G | 23/07/93 | *Banksia semi-nuda* ssp. *remanens* | I/3 |
| 00067 G | 23/07/93 | *Banksia semi-nuda* ssp. *remanens* | I/18 |
| 00068 V | 24/07/93 | *Banksia brownii* | I/25 |
| 00069 P1 | 6/08/93 | *Isopogon scaber* | B/16 |
| 00070 C | 18/08/93 | *Dryandra anatona* | B/15 |
| 00071 P3 | 19/08/93 | *Dryandra seneciifolia* | I/16 |
| 00072 P2 | 19/08/93 | *Dryandra ferruginea* ssp. *pumila* | I/17 |
| 00073 P2 | 20/08/93 | *Andersonia* sp. Mt.Lindesay | B/10 |
| 00074 P4 | 21/08/93 | *Dryandra serra* | B/32 |
| 00075 P4 | 21/08/93 | *Dryandra serra* | I/11 |
| 00076 V | 21/08/93 | *Banksia brownii* | I/4 |
| 00077 V | 22/08/93 | *Banksia brownii* | I/15 |
| 00078 V | 23/08/93 | *Banksia verticillata* | I/8 |
| 00079 V | 23/08/93 | *Banksia verticillata* | I/16 |
| 00080 P3 | 24/08/93 | *Dryandra seneciifolia* | I/10 |
| 00081 P2 | 14/09/93 | *Dryandra acanthopoda* | I/12 |
| 00082 P1 | 14/09/93 | *Dryandra lepidorhiza* | B/20 |
| 00083 P3 | 15/09/93 | *Dryandra meganotia* | B/10 |
| 00084 P2 | 15/09/93 | *Dryandra octotriginata* | I/20 |
| 00085 P2 | 16/09/93 | *Dryandra erythrocephala* var. *inopinata* | I/21 |
| 00086 P2 | 16/09/93 | *Dryandra epimicta* | I/21 |
| 00087 P4 | 17/09/93 | *Dryandra conferta* var. *conferta* | I/17 |
| 00088 V | 21/09/93 | *Lambertia fairallii* | B/100 |
| 00089 E | 9/11/93 | *Dryandra mimica* | B/1? |
| 00090 V | 9/11/93 | *Lambertia orbifolia* | B/10 |
| 00091 P4 | 10/11/93 | *Astroloma* sp. Nannup | I/20 |
| 00092 C | 11/11/93 | *Dryandra ionthocarpa* | I/15 |
| 00093 C | 11/11/93 | *Dryandra ionthocarpa* | I/16 |
| 00094 V | 11/11/93 | *Lambertia fairallii* | B/50 |
| 00095 C | 3/11/93 | *Daviesia microcarpa* | B/12 |
| 00096 E | 25/11/93 | *Daviesia pseudaphylla* | I/11 |
| 00097 E | 25/11/93 | *Daviesia pseudaphylla* | B/25 |
| 00098 E | 25/11/93 | *Daviesia pseudaphylla* | B/20 |
| 00099 P3 | 26/11/93 | *Andersonia echinocephala* | I/22 |
| 00100 P4 | 28/11/93 | *Banksia laevigata* ssp. *laevigata* | I/20 |
| 00101 V | 14/01/98 | *Darwinia squarrosa* | B/80 |
| 00102 V | 30/11/93 | *Adenanthos ellipticus* | B/8 |
| 00103 V | 1/12/93 | *Daviesia megacalyx* | B/30 |
| 00104 P1 | 1/12/93 | *Dryandra corvijuga* | I/13 |
| 00105 P2 | 1/12/93 | *Dryandra folisissima* | I/10 |
| 00106 P2 | 2/12/93 | *Dryandra folisissima* | I/10 |

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| 00107 P1 | 2/12/93 | *Dryandra corvijuga* | I/10 |
| 00108 V | 2/12/93 | *Daviesia megacalyx* | I/14 |
| 00109 V | 2/12/93 | *Daviesia megacalyx* | I/5 |
| 00110 P3 | 3/12/93 | *Dryandra viscida* | I/23 |
| 00111 V | 3/12/93 | *Banksia sphaerocarpa* var. *dolichostyla* | I/20 |
| 00112 P4 | 4/12/93 | *Daviesia oxylobium* | I/8 |
| 00113 V | 1/11/90 | *Eucalyptus crucis* ssp. *crucis* | B/? |
| 00114 P4 | 8/09/93 | *Dryandra serra* | I/10 |
| 00115 V | 1/10/92 | *Lepidium catapycnon* | I/1 |
| 00116 E | 6/01/94 | *Adenanthos pungens* ssp. *effusa* | B/3 |
| 00117 V | 14/01/98 | *Darwinia squarrosa* | B/150 |
| 00118 E | 6/01/94 | *Adenanthos pungens* ssp. *pungens* | B/10 |
| 00119 V | 7/01/94 | *Adenanthos velutinos* | I/4 |
| 00120 V | 9/01/94 | *Banksia verticillata* | I/13 |
| 00121 V | 10/01/94 | *Adenanthos ellipticus* | B/40 |
| 00122 P4 | 11/01/94 | *Adenanthos labillardieri* | I/20 |
| 00123 E | 11/12/96 | *Acacia pygmaea* | B/10 |
| 00124 C | 14/01/94 | *Lambertia echinata* ssp. *echinata* | I/3 |
| 00125 E | 14/01/94 | *Myoporum turbinatum* | I/10 |
| 00126 P2 | 15/01/94 | *Astroloma* sp. Grass Patch | B/40 |
| 00127 P4 | 16/01/94 | *Daviesia campophylla* | I/21 |
| 00128 E | 7/07/93 | *Banksia cuneata* | B/? |
| 00129 P3 | 9/02/94 | *Andersonia echinocephala* | I/20 |
| 00130 P4 | 11/02/94 | *Dryandra* sp. Fitzgerald | I/10 |
| 00131 P4 | 13/02/94 | *Dryandra* sp. Fitzgerald | I/12 |
| 00132 C | 15/02/94 | *Andersonia* pinaster | I/12 |
| 00133 P2 | 15/02/94 | *Andersonia* sp. Mt. Lindesay | I/10 |
| 00134 P2 | 16/02/94 | *Banksia occidentalis* ssp. *formosa* | I/18 |
| 00135 V | 12/93-1/94 | *Daviesia spiralis* | B/25 |
| 00136 P4 | 1/09/93 | *Eucalyptus carnabyi* | B/5 |
| 00137 E | 15/03/94 | *Petrophile latericola* | B/40 |
| 00138 V | 20/04/94 | *Banksia verticillata* | I/13 |
| 00139 C | 20/04/94 | *Dryandra montana* | I/5 |
| 00140 E | 21/04/94 | *Verticordia harveyi* | I/16 |
| 00141 G | 22/04/94 | *Lambertia echinata* ssp. *citrina* | I/10 |
| 00142 G | 22/04/94 | *Lambertia echinata* ssp. *citrina* | I/6 |
| 00143 P2 | 26/04/94 | *Dryandra aurantia* | B/15 |
| 00144 V | 12/05/94 | *Lambertia fairallii* | B/8 |
| 00145 V | 1/05/85 | *Eucalyptus rhodantha* | I/6 |
| 00146 V | 1/04/85 | *Eucalyptus rhodantha* | I/21 |
| 00147 V | 1/04/85 | *Eucalyptus rhodantha* | I/32 |
| 00148 V | 1/08/85 | *Eucalyptus rhodantha* | I/14 |
| 00149 V | 15/04/85 | *Eucalyptus rhodantha* | I/8 |
| 00150 V | 12/07/93 | *Eucalyptus rhodantha* | I/11 |
| 00151 V | 13/07/93 | *Eucalyptus rhodantha* | I/6 |
| 00152 V | 2/09/93 | *Eucalyptus rhodantha* | I/35 |
| 00153 V | 23/06/93 | *Eucalyptus rhodantha* | I/34 |
| 00154 V | 13/07/93 | *Eucalyptus rhodantha* | I/4 |
| 00155 V | 17/12/92 | *Stylidium coroniforme* | B/6 |
| 00156 V | 16/12/93 | *Stylidium coroniforme* | B/9 |
| 00157 V | 16/12/93 | *Stylidium coroniforme* | I/13;B/15 |
| 00158 V | 15/12/93 | *Stylidium coroniforme* | I/11 |
| 00159 V | 15/12/93 | *Stylidium coroniforme* | I/25 |
| 00160 V | 16/12/93 | *Stylidium coroniforme* | I/14;B/27 |
| 00161 V | 1/06/87 | *Banksia verticillata* | I/19 |

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|---|---|---|---|
| 00162 P1 | 26/07/94 | *Dryandra fraseri* var. *oxycedrus* | I/14 |
| 00163 P1 | 26/07/94 | *Dryandra borealis* ssp. *elatior* | B/20 |
| 00164 V | 27/07/94 | *Leucopogon obtectus* | I/6 |
| 00165 P1 | 27/07/94 | *Dryandra stricta* | I/11 |
| 00166 V | 25/07/94 | *Dryandra serratuloides* | B/12 |
| 00167 V | 28/07/94 | *Dryandra serratuloides* | I/15 |
| 00168 P3 | 28/07/94 | *Dryandra pteridifolia* ssp. *vernalis* | I/10 |
| 00169 V | 28/08/94 | *Banksia verticillata* | I/13 |
| 00170 C | 1/09/94 | *Dryandra anatona* | B/6 |
| 00171 V | 1/06/94 | *Eucalyptus rhodantha* | I/5 |
| 00172 G | 28/09/94 | *Lambertia echinata* ssp. *citrina* | I/10 |
| 00173 V | Sept-Dec 93 | *Anigozanthus humilis* ssp. *chrysanthus* | I/44 |
| 00174 P2 | 18/11/94 | *Melaleuca ordinifolia* | I/10 |
| 00175 E | 19/11/94 | *Daviesia pseudaphylla* | B/30 |
| 00176 C | 16/11/94 | *Rulingia* sp. Trigwell Bridge | B/4 |
| 00177 V | 20/11/94 | *Banksia brownii* | B/10 |
| 00178 P3 | 20/11/94 | *Andersonia echinocephala* | I/13 |
| 00179 P4 | 21/11/94 | *Darwinia lejostyla* | B/100 |
| 00180 C | 21/11/94 | *Darwinia oxylepis* | B/100 |
| 00181 E | 21/11/94 | *Darwinia wittwerorum* | B/100 |
| 00182 V | 22/11/94 | *Verticordia helichrysantha* | B/200 |
| 00183 P2 | 22/11/94 | *Melaleuca sculponeata* | I/11 |
| 00184 V | 23/11/94 | *Daviesia megacalyx* | I/9 |
| 00185 V | 23/1194 | *Daviesia megacalyx* | B/10 |
| 00186 V | 23/11/94 | *Daviesia megacalyx* | B/50 |
| 00187 C | 14/12/94+28/12/94+13/02/95 | *Grevillea Mccutcheonii* | B/5 |
| 00188 P1 | 14/12/94 | *Hakea* aff. *varia* | I/8 |
| 00189 P1 | 14/12/94 | *Dryandra squarrosa* ssp. *argillacea* | I/10 |
| 00190 E | 15/12/94 | *Dryandra nivea* ssp. *uliginosa* | I/9 |
| 00191 E | 15/12/94 | *Petrophile latericola* | B/25 |
| 00192 P1 | 15/12/94 | *Hakea* sp. Williamson | B/30 |
| 00193 P1 | 16/12/94 | *Andersonia* aff. *latiflora* | B/100 |
| 00194 E | 16/12/94+28/12/94 | *Grevillea elongata* | I/22 |
| 00195 E | 15/12/94+28/12/94 | *Brachysema papilio* | B/5 |
| 00196 V | 4/12/94+12/12/94 | *Acacia awestoniana* | B/7 |
| 00197 E | 28/12/94 | *Grevillea elongata* | B/8 |
| 00198 C | 14/12/94 | *Andersonia pinaster* | B/10 |
| 00199 C | 14/12/94 | *Andersonia pinaster* | B/10 |
| 00200 C | 14/12/94+11/01/95 | *Andersonia pinaster* | B/10 |
| 00201 V | 14/11/94-29/01/95 | *Adenanthos pungens* ssp. *pungens* | I/10 (traps) |
| 00202 V | 9/01/95 | *Lambertia fairallii* | B/250 |
| 00203 P3 | 9/01/95 | *Andersonia grandiflora* | B/100 |
| 00204 E | 12/01/95 | *Isopogon uncinatus* | B/20 |
| 00205 E | 12/01/95 | *Isopogon uncinatus* | B/50 |
| 00206 P2 | 13/01/95 | *Andersonia* sp. Mt Lindesay | B/20 |
| 00207 V | 13/01/95 | *Verticordia fimbrilepis* ssp. *australis* | B/200 |
| 00208 R | 11/01/95 | *Hibbertia* sp. Porongorups | B/6 |
| 00209 G | 11/01/95 | *Lambertia echinata* ssp. *propinqua* | I/11 |
| 00210 V | 14/12/94 | *Eucalyptus rhodantha* | I/8 |
| 00211 V | 14/12/94 | *Eucalyptus rhodantha* | I/9 |
| 00212 V | 14/12/94 | *Eucalyptus rhodantha* | I/11 |
| 00213 V | 14/12/94 | *Eucalyptus rhodantha* | I/23 |
| 00214 C | 3/01/95 | *Verticordia albida* | B/8 |
| 00215 P2 | 28/01/95 | *Verticordia bifimbriata* | B/12 |
| 00216 P2 | 28/01/95 | *Andersonia bifida* | B/35 |

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|---|---|---|---|
| 00217 P1 | 13/02/95 | *Verticordia plumosa* var. *pleiobotrya* | I/27 |
| 00218 P3 | 13/02/95 | *Verticordia attenuata* | B/30 |
| 00219 E | 13/02/95 | *Dryandra nivea* ssp. *uliginosa* | I/10 |
| 00220 V | 14/02/95 | *Verticordia plumosa* var. *ananeotes* | B/30 |
| 00221 P1 | 15/02/95 | *Verticordia endlicheriana* var. *angustifolia* | B/50 |
| 00222V | 17/02/95 | *Kunzea pauciflora* | B/50 |
| 00223 E | 21/04/94 | *Banksia cuneata* | B/57 |
| 00224 E | 21/04/94 | *Banksia cuneata* | B/6 |
| 00225 E | 13/03/94 | *Banksia cuneata* | B/10 |
| 00226 E | 18/05/94 | *Banksia cuneata* | B/10 |
| 00227 E | 20/04/94 | *Banksia cuneata* | B/78 |
| 00228 E | 30/07/94 | *Banksia cuneata* | B/50 |
| 00229 E | 8/08/94 | *Banksia cuneata* | B/90 |
| 00230 V | 25/04/95+15/06/95 | *Lambertia orbifolia* | B/20 |
| 00231 V | 26/04/95 | *Banksia verticillata* | I/6 |
| 00232 E | 27/04/95 | *Verticordia harveyi* | B/200 |
| 00233 V | 28/04/95 | *Banksia verticillata* | I/12 |
| 00234 E | 11/05/95 | *Banksia cuneata* | B/3 |
| 00235 V | 15/06/95 | *Lambertia orbifolia* | B/10 |
| 00236 P1 | 13/06/95 | *Dryandra lepidorhiza* | B/60 |
| 00237 P2 | 13/06/95 | *Dryandra acanthopoda* | I/10 |
| 00238 P4 | 26/09/90 | *Banksia meisneri* var. *ascendens* | B/? |
| 00239 V | 24/08/95 | *Dryandra serratuloides* ssp. *serratuloides* | I/10 |
| 00240 E | 10/10/95 | *Verticordia staminosa* ssp. *staminosa* | I/15 |
| 00241 V | 10/10/95 | *Melaleuca sciotostyla* | I/10 |
| 00242 P2 | 15/10/95 | *Andersonia* sp. Mt. Lindesay | B/20 |
| 00243 E | 18/10/95 | *Eremophila denticulata* ssp. *denticulata* | B/20 |
| 00244 E | 19/10/95 | *Eremophila denticulata* ssp. *denticulata* | B/200 |
| 00245 P1 | 19/10/95 | *Eucalyptus preissiana* ssp. *lobata* | B/200 |
| 00246 C | 18/10/95 | *Daviesia microcarpa* | I/15 |
| 00247 V | 20/10/95 | *Eucalyptus insularis* | B/10+ |
| 00248 V | 20/10/95 | *Eucalyptus insularis* | B/5+ |
| 00249 C | 20/10/95 | *Lambertia echinata* ssp. *echinata* | I/3 |
| 00250 C | 21/10/95 | *Prostanthera carrickiana* | B/40 |
| 00251 V | 22/10/95 | *Eucalyptus platydisca* | I/17 |
| 00252 P1 | 24/10/95 | *Chamelaucium* sp. Hamersley | B/60 |
| 00253 V | 26/10/95 | *Myoporum cordifolium* | B/20 |
| 00254 V | 26/10/95 | *Myoporum cordifolium* | B/100 |
| 00255 V | 26/10/95 | *Verticordia helichrysantha* | B/1000 |
| 00256 V | 27/10/95 | *Grevillea maxwellii* | I/12 + B/5 |
| 00257 C | 6/11/95 | *Chamelaucium griffinii* | B/20 |
| 00258 E | 6/11/95 | *Grevillea calliantha* | B/14 |
| 00259 E | 6/11/95 | *Grevillea calliantha* | B/5 |
| 00260 E | 6/11/95 | *Grevillea calliantha* | B/10 |
| 00261 P1 | 8/11/95 | *Grevillea kenneallyi* | B/40 |
| 00262 E | 8/11/95 | *Gastrolobium hamulosum* | B/9 |
| 00263 V | 9/11/95 | *Microcorys eremophiloides* | B/10 |
| 00264 C | 9/11/95 | *Acacia pygmaea* | B/6 |
| 00265 E | 6/11/95 | *Grevillea calliantha* | B/4 |
| 00266 P4 | 1/04/97 | *Dryandra serra* | B/18 |
| 00267 E | 22/11/95 | *Darwinia acerosa* | I/10 |
| 00268 V | 30/11/95 | *Allocasuarina fibrosa* | I/24 |
| 00269 C | 1/12/95 | *Eremophila caerulea* ssp. *merrallii* | B/10 |
| 00270 P1 | 1/12/95 | *Jacksonia quairading* | B/100 |
| 00271 C | 4/12/95 | *Darwinia carnea* | B/10 |

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|---|---|---|---|
| 00272 E | 5/12/95 | *Acacia leptalea* | I/10 |
| 00273 E | 5/12/95 | *Acacia leptalea* | I/13 |
| 00274 V | 5/12/95 | *Verticordia staminosa* ssp. *cylindraceae* var. *cylindraceae* | I/7 |
| 00275 V | 6/12/95 | *Verticordia staminosa* ssp. *cylindraceae* var. *erecta* | I/10 |
| 00276 V | 6/12/95 | *Allocasuarina tortiramula* | I/10 |
| 00277 V | 6/12/95 | *Allocasuarina tortiramula* | I/11 |
| 00278 V | 7/12/95 | *Billardiera mollis* | B/20 |
| 00279 C | 9/12/95 | *Chamelaucium* sp. Gin Gin | I/10 |
| 00280 E | 9/12/95 | *Acacia* sp. Dandaragan | I/10 |
| 00281 P1 | 11/12/95 | *Calothamnus aff. quadrifidus* | I/11 |
| 00282 E | 12/12/95 | *Brachysema papilio* | B/10 |
| 00283 P1 | 12/12/95 | *Hakea aff. varia* | I/10* |
| 00284 E | 12/12/1995, 17/1/96 | *Dryandra nivea ssp. uliginosa* | I/12 |
| 00285 E | 12/12/95 | *Petrophile latericola* | B/10 |
| 00286 C | 12/12/95 | *Lambertia echinata ssp. septentrionale* | B/6 |
| 00287 E | 12/12/95 | *Petrophile latericola* | B/15 |
| 00288 V | 13/12/95 | *Lambertia orbifolia* | B/15 |
| 00289 V | 13/12/95 | *Lambertia orbifolia* | B/20 |
| 00290 V | 14/12/95+18/01/96 | *Lambertia orbifolia* | B/50 |
| 00291 V | 14/12/95 | *Kennedia macrophylla* | B/? |
| 00292 V | 14/12/95 | *Kennedia macrophylla* | B/20 |
| 00293 C | 9/01/96 | *Verticordia spicata ssp squamosa* | B/2 |
| 00294 C | 9/01/96 | *Eremophila nivea* | I/10 |
| 00295 C | 9/01/96 | *Eremophila nivea* | B/50+ |
| 00296 C | 9/01/96 | *Verticordia spicata ssp squamosa* | B/15 |
| 00297 C | 9/01/96 | *Verticordia spicata ssp squamosa* | I/1 |
| 00298 C | 9/01/96 | *Verticordia spicata ssp squamosa* | B/5 |
| 00299 C | 10/01/96 | *Verticordia albida* | B/500+ |
| 00300 C | 10/01/96 | *Verticordia albida* | B/10 |
| 00301 C | 10/01/96+22/01/96 | *Verticordia albida* | B/10 |
| 00302 C | 10/01/96+22/01/96 | *Verticordia albida* | B/16 |
| 00303 V | 18/01/96 | *Lambertia orbifolia* | B/100+ |
| 00304 V | 19/01/96 | *Lambertia orbifolia* | B/50+ |
| 00305 C | 22/01/96 | *Eremophila nivea* | B/13 |
| 00306 C | 23/01/96 | *Acacia sciophanes* | B/150+ |
| 00307 C | 23/01/96 | *Eremophila caerulea ssp merrallii* | I/10 |
| 00308 C | 24/01/96 | *Verticordia fimbrilepis ssp fimbrilepis* | B/10 |
| 00309 C | 24/01/96 | *Verticordia fimbrilepis ssp. fimbrilepis* | B/30 |
| 00310 C | 24/01/96 | *Verticordia fimbrilepis ssp. fimbrilepis* | B/150+ |
| 00311 C | 9/12/95 | *Eremophila caerulea ssp. merrallii* | B/15 |
| 00312 C | 9/12/95 | *Eremophila caerulea ssp. merrallii* | B/20 |
| 00313 C | 9/12/95 | *Eremophila caerulea ssp. merrallii* | B/42 |
| 00314 C | 18/01/94 | *Eremophila viscida* | I/10 |
| 00315 C | 17/01/94 | *Eremophila viscida* | I/10 |
| 00316 C | 20/02/96 | *Verticordia fimbrilepis ssp. fimbrilepis* | I/8 |
| 00317 C | 20/02/96 | *Eremophila veneta* | B/10 |
| 00318 V | 21/02/96 | *Darwinia collina* | B/10 |
| 00319 C | 21/02/96 | *Dryandra montana* | I/8 |
| 00320 E | 22/02/96 | *Isopogon uncinatus* | B/20 |
| 00321 E | 22/02/96 | *Isopogon uncinatus* | B/20 |
| 00322 V | 22/02/96 | *Verticordia fimbrilepis ssp. australis* | B/200 |
| 00323 V | 23/02/96 | *Lambertia orbifolia* | B/10 |
| 00324 V | 23/02/96 | *Lambertia orbifolia* | B/40 |
| 00325 x | 14/11/95 | *Hemigenia exilis* | I/10 |
| 00326 x | 14/11/95 | *Hemigenia exilis* | B/? |

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| 00327 x | 14/11/95 | *Hemigenia exilis* | B/15 |
| 00328 x | 14/11/95 | *Hemigenia exilis* | I/10 |
| 00329 x | 14/11/95 | *Hemigenia exilis* | B/15 |
| 00330 x | 14/11/95 | *Hemigenia exilis* | B/? |
| 00331 x | 13/11/95 | *Hemigenia exilis* | B/15 |
| 00332 x | 15/11/95 | *Hemigenia exilis* | I/5 |
| 00333 C | 21/04/96 | *Dryandra anatona* | B/12 |
| 00334 P1 | 25/07/94 | *Dryandra fuscobractea* | B/10 |
| 00335 E | 16/04/96 | *Verticordia harveyi* | B/30 |
| 00336 V | 18/04/96 | *Verticordia carinata* | B/100+ |
| 00337 E | 18/04/96 | *Verticordia harveyi* | B/500+ |
| 00338 P1 | 29/05/96 | *Melaleuca pritzellii* | I/13 |
| 00339 P1 | 6/08/96 | *Melaleuca pritzellii* | B/15 |
| 00340 P1 | 28/08/96 | *Daviesia* sp. Cunderdin | I/5 |
| 00341 P1 | 5/09/96 | *Dryandra fuscobractea* | B/11 |
| 00342 E | 3/10/96, 25/10/96,19/11/96 | *Verticordia staminosa* ssp. *staminosa* | B/50+ |
| 00343 E | 26/10/96 | *Grevillea dryandroides* ssp. *dryandroides* | B/10 |
| 00344 P2 | 27/10/96 | *Acacia recurvata* | B/50 |
| 00345 C | 27/10/96 | *Daviesia bursarioides* | B/5 |
| 00346 C | 27/10/96 | *Daviesia bursarioides* | B/8 |
| 00347 C | 27/10/96 | *Daviesia bursarioides* | B/9 |
| 00348 C | 27/10/96 | *Daviesia bursarioides* | I/10 |
| 00349 P2 | 28/10/96 | *Acacia recurvata* | B/100 |
| 00350 P2 | 28/10/96 | *Acacia wilsonii* | I/18 |
| 00351 C | 28/10/96 | *Grevillea humifusa* | B/20, I/18 |
| 00352 C | 29/10/96 | *Chamelaucium* sp. Gin Gin | B/50 |
| 00353 C | 29/10/96 | *Chamelaucium* sp. Gin Gin | B/50 |
| 00354 C | 14/10/96 | *Andersonia axilliflora* | B/6 |
| 00355 C | 15/10/96 | *Andersonia axilliflora* | B/42 |
| 00356 V | 11/11/96 | *Banksia brownii* | I/20, B/25 |
| 00357 V | 13/11/96 | *Banksia brownii* | I/21 |
| 00358 P1 | 14/11/96 | *Acacia brachypoda* | I/10 |
| 00359 V | 14/11/96 | *Adenanthos pungens* ssp. *pungens* | I/10 traps |
| 00360 C | 18/11/96, 11/12/96 | *Acacia pygmaea* | B/50 |
| 00361 V | 18/11/96 | *Microcroys eremophiloides* | B/50 |
| 00362 C | 19/11/96 | *Acacia vassallii* | I/13 |
| 00363 E | 18/11/96 | *Gastrolobium hamulosum* | B/11 |
| 00364 C | 19/11/96 | *Chorizema humile* | I/10 |
| 00365 C | 19/11/96 | *Acacia vassallii* | I/2 |
| 00366 C | 20/11/96, 12/12/96 | *Eremophila scaberula* | I/5, I/12 |
| 00367 V | 20/11/96 | *Gastrolobium hamulosum* | B/10 |
| 00368 P1 | 20/11/96 | *Acacia cochlocarpa* ssp. *cochlocarpa* | I/11 |
| 00369 C | 20/11/96 | *Hemiandra gardneri* | B/9 |
| 00370 C | 20/11/96 | *Hemiandra gardneri* | B/30 |
| 00371 P3 | 20/11/96 | *Acacia aprica* | B/30 |
| 00372 C | 21/11/96 | *Chorizema humile* | B/10 |
| 00373 P1 | 21/11/96 | *Grevillea murex* | B/50 |
| 00374 P1 | 21/11/96 | *Grevillea murex* | B/15 |
| 00375 C | 27/11/96 | *Calytrix breviseta* ssp. *breviseta* | B/40 |
| 00376 C | 2/12/96, 19/12/96 | *Orthrosanthus muellerii* | B/40 |
| 00377 E | 3/12/96, 19/12/96 | *Hibbertia* sp. Porongorups | B/50 |
| 00378 P2 | 10/01/97 | *Andersonia gracilis* | B/150 |
| 00379 V | 3/12/96, 19/12/96 | *Acacia awestoniana* | B/9 |
| 00380 V | 4/12/96 | *Lambertia fairallii* | B/50 |
| 00381 E | 4/12/96 | *Darwinia oxylepis* | B/100 |
| 00382 E | 4/12/96 | *Darwinia wittwerorum* | B/20 |

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|---|---|---|---|
| 00383 V | 5/12/96, 19/12/96 | *Acacia awestoniana* | B/50 |
| 00384 C | 5/12/96 | *Grevillea maxwellii* | I/8 |
| 00385 C | 5/12/96 | *Grevillea maxwellii* | I/10 |
| 00386 C | 6/12/96 | *Acacia insolita* ssp. *recurva* | B/5 |
| 00387 V | 6/12/96 | *Thomasia montana* | I/10 |
| 00388 C | 11/12/96 | *Jacksonia quairading* | B/60 |
| 00389 C | 12/12/96 | *Eremophila scaberula* | I/10 |
| 00390 C | 12/12/96 | *Eremophila scaberula* | I/12 |
| 00391 P2 | 13/12/96 | *Darwinia chapmanianna* | B/100 |
| 00392 P1 | 23/02/97 | *Verticordia plumosa* var. *vassensis* | B/40 |
| 00393 P1 | 13/12/96 | *Grevillea curviloba* ssp. *incurva* | B/??2 |
| 00394 P1 | 19/12/96 | *Acacia heteroclita* ssp. *valida* | B/20 |
| 00395 P2 | 20/12/96 | *Grevillea rara* | B/50 |
| 00396 V | 15/10/96 | *Banksia brownii* | B/10 |
| 00397 P1 | 17/12/96 | *Grevillea curviloba* ssp. *incurva* | B/10 |
| 00398 P1 | 17/12/96 | *Grevillea curviloba* ssp. *incurva* | B/30 |
| 00399 P1 | 17/12/96 | *Grevillea curviloba* ssp. *incurva* | B/7 |
| 00400 P1 | 17/12/96 | *Grevillea curviloba* ssp. *incurva* | B/20 |
| 00401 P1 | 17/12/96 | *Grevillea curviloba* ssp. *incurva* | B/30 |
| 00402 P1 | 17/12/96 | *Grevillea curviloba* ssp. *incurva* | B/10 |
| 00403 P1 | 13/01/97 | *Nemcia* aff. *rubra* | B/30 |
| 00404 V | 13/01/97 | *Darwinia macrostegia* | B/50 |
| 00405 V | 14/01/97 | *Darwinia squarrosa* | B/50 |
| 00406 V | 14/01/97 | *Darwinia collina* | B/50 |
| 00407 V | 14/01/97 | *Darwinia collina* | B/40 |
| 00408 C | 15/10/96, 14/01/1997 | *Dryandra montana* | B/10 |
| 00409 V | 14/01/97 | *Darwinia collina* | B/30 |
| 00410 P4 | 15/01/97 | *Darwinia lejostyla* | B/50 |
| 00411 C | 15/01/97 | *Dryandra montana* | I/1 |
| 00412 V | 16/01/97 | *Darwinia collina* | B/100 |
| 00413 C | 16/01/97 | *Andersonia axilliflora* | B/10 |
| 00414 V | 17/01/97 | *Darwinia squarrosa* | B/100 |
| 00415 P1 | 21/01/97 | *Jacksonia pungens* | B/20 |
| 00416 P1 | 21/01/97 | *Jacksonia pungens* | I/11 |
| 00417 C | 22/01/97 | *Verticordia spicata* ssp. *squamosa* | B/13 |
| 00418 P1 | 22/01/97 | *Verticordia comosa* | B/30 |
| 00419 C | 22/01/97 | *Verticordia spicata* ssp. *squamosa* | I/1 |
| 00420 C | 22/01/97 | *Verticordia spicata* ssp. *squamosa* | I/1 |
| 00421 C | 22/01/97 | *Verticordia spicata* ssp. *squamosa* | I/1 |
| 00422 C | 22/01/97 | *Verticordia spicata* ssp. *squamosa* | B/10 |
| 00423 C | 22/01/97 | *Verticordia spicata* ssp. *squamosa* | I/1 |
| 00424 C | 22/01/97 | *Verticordia albida* | B/12 |
| 00425 C | 22/01/97 | *Verticordia albida* | B/25 |
| 00426 C | 23/01/97 | *Verticordia albida* | B/7 |
| 00427 C | 23/01/97 | *Verticordia albida* | B/20 |
| 00428 V | 23/01/97 | *Eremophila microtheca* | I/10 |
| 00429 C | 30/01/97 | *Eremophila lactea* | B/50 |
| 00430 P1 | 30/01/97 | *Eremophila chamephila* | B/25 |
| 00431 C | 31/01/97 | *Lambertia echinata* ssp. *echinata* | I/3 |
| 00432 P1 | 31/01/97 | *Dryandra longifolia* ssp. *calcicola* | I/15 |
| 00433 P1 | 31/01/97 | *Dryandra longifolia* ssp. *calcicola* | B/30 |
| 00434 P4 | 2/02/97 | *Adenanthos labillardierii* | I/10 |
| 00435 V | 3/02/97 | *Verticordia fimbrilepis* ssp. *australis* | B/100 |
| 00436 C | 3/12/96, 4/02/1997 | *Dryandra anatona* | B/500 |
| 00437 C | 4/02/97 | *Dryandra anatona* | B/25 |

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|---|---|---|---|
| 00438 C | 5/02/97 | *Eremophila veneta* | B/2? |
| 00439 C | 5/02/97 | *Eremophila veneta* | B/20 |
| 00440 C | 5/02/97 | *Eremophila veneta* | B/50 |
| 00441 C | 4/02/97 | *Andersonia axilliflora* | I/17 |
| 00442 V | 18/02/97 | *Lambertia orbifolia* | I/10, B/80 |
| 00443 E | 19/02/97 | *Dryandra nivea* ssp. *uliginosa* | I/17, B/25 |
| 00444 E | 19/02/97 | *Dryandra nivea* ssp. *uliginosa* | I/12, B/40 |
| 00445 P1 | 20/02/97 | *Calothamnus* aff. *quadrifidus* | B/50 |
| 00446 P1 | 20/02/97 | *Calothamnus* aff. *quadrifidus* | B/50 |
| 00447 P1 | 20/02/97 | *Hakea* sp. Williamson | I/10 |
| 00448 P1 | 20/02/97 | *Calothanmus* aff. *quadrifidus* | B/20 |
| 00449 E | 20/02/1997, 7/4/97 | *Dryandra nivea* ssp. *uliginosa* | I/5+ I/6 |
| 00450 P1 | 21/02/1997, 7/4/97 | *Daviesia elongata* ssp. *elongata* | B/20 |
| 00451 C | 21/02/97 | *Lambertia echinata* ssp. occidentalis | B/7 |
| 00452 V | 21/02/97 | *Petrophile latericola* | B/4 |
| 00453 P1 | 4/03/97 | *Dryandra mucronulata* ssp. *retrorsa* | I/10 |
| 00454 C | 2/03/97 | *Verticordia fimbrilepis* ssp. *fimbrilepis* | B/50 |
| 00455 C | 2/03/97 | *Verticordia fimbrilepis* ssp. *fimbrilepis* | B/40 |
| 00456 C | 2/03/97 | *Verticordia fimbrilepis* ssp. *fimbrilepis* | B/25 |
| 00457 V | 9/04/97 | *Verticordia carinata* | B/400 |
| 00458 C | 29/01/97 | *Eremophila nivea* | I/1 |
| 00459 E | 9/04/97 | *Verticordia harveyi* | B/50 |
| 00460 E | 9/04/97 | *Verticordia harveyi* | B/50 |
| 00461 P1 | 10/04/97 | *Dryandra aurantia* | B/20 |
| 00462 P1 | 14/04/97 | *Dryandra aurantia* | B/40 |
| 00463 P1 | 8/04/97 | *Calothamnus* aff. *quadrifidus* | B/20 |
| 00464E | 15/04/97 | *Dryandra mimica* | B/20 |
| 00465 C | 3/03/97 | *Sphenotoma drummondii* | B/30 |
| 00466 C | 3/02/97 | *Dryandra montana* | I/2 |
| 00467 C | 17/04/97 | *Sphenotoma drummondii* | B/6 |
| 00468 C | 31/03/97 | *Dryandra ionthocarpa* | B/36 |
| 00469 C | 31/03/97 | *Dryandra ionthocarpa* | B/35 |
| 00470 E | 8/09/97 | *Dryandra mimica* | B/40 |
| 00471 P3 | 21/01/96 | *Eleocharis* sp. Kenwick | B/20 |
| 00472 P2 | 25/06/97, 1/07/97 | *Eremophila pinnatifida* | I/1, I/4 |
| 00473 E | 1/07/97 | *Dryandra mimica* | B/10 |
| 00474 C | 7/08/97, 28/08/97 | *Davesia cunderdin* | I/4 |
| 00475 V | 3/09/96 | *Banksia cuneata* | I/25 |
| 00476 C | 23/08/96 | *Banksia cuneata* | I/59 |
| 00477 C | 4/11/96 | *Banksia cuneata* | I/1 |
| 00478 C | 4/11/96, 26/11/96 | *Banksia cuneata* | I/76 |
| 00479 C | 16/10/96 | *Banksia cuneata* | I/28 |
| 00480 C | 25/02/98 | *Banksia cuneata* | I/38 |
| 00481 V | mid 1980's | *Kennedia glabrata* | I/10 |
| 00482 C | 16/10/97 | *Grevillea humifusa* | B/20 |
| 00483 C | 20/10/97 | *Grevillea maxwellii* | B/15 |
| 00484 C | 20/10/97 | *Grevillea maxwellii* | B/10 |
| 00485 C | 21/10/97 | *Grevillea maxwellii* | I/13, B/15 |
| 00486 C | 28/10/97, 14/11/97 | *Cyphanthera odgersii* spp. *occidentalis* | B/30 |
| 00487 V | 29/10/97 | *Hakea aculeata* | I/22 |
| 00488 V | 29/10/97 | *Hakea aculeata* | I/10 |
| 00489 V | 7/08/97 | *Hakea aculeata* | I/1 |
| 00490 V | 7/08/97 | *Hakea aculeata* | B/4 |
| 00491 V | various | *Adenanthos velutinos* | I/20 (traps) |
| 00492 V | various | *Adenanthos pungens* ssp. *pungens* | I/20 (traps) |
| 00493 E | 3/02/92 | *Villarsia calthifolia* | I/14 |
| 00494 E | 3/02/92 | *Villarsia calthifolia* | I/18 |

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|---|---|---|---|
| 00495 P5 | 27/10/97 | *Guichenotia seorsiflora* | B/4 |
| 00496 C | 12/12/95 | *Darwinia* sp. Williamson | B/5 |
| 00497 V | 13/12/95 | *Darwinia ferricola* | B/10 |
| 00498 P4 | 3/02/92 | *Villarsia marchantii* | I/11 |
| 00499 C | 11/11/97 | *Chamelaucium* sp. Gin Gin | B/50 |
| 00500 C | 11/11/97 | *Chamelaucium* sp. Gin Gin | B/50 |
| 00501 P2 | 11/11/97 | *Acacia aristulata* | B/30 |
| 00502 P2 | 12/11/97 | *Acacia aristulata* | B/60 |
| 00503 C | 12/11/97 | *Hemiandra gardneri* | B/15 |
| 00504 C | 12/11/97 | *Acacia cochlocarpa* ssp. *cochlocarpa* | B/30 |
| 00505 C | 12/11/97 | *Daviesia bursarioides* | B/30 |
| 00506 C | 12/11/97 | *Daviesia bursarioides* | B/7 |
| 00507 C | 12/11/97 | *Daviesia bursarioides* | B/8 |
| 00508 C | 12/11/97 | *Daviesia bursarioides* | B/5 |
| 00509 C | 13/11/97 | *Acacia aprica* | B/60 |
| 00510 C | 13/11/97 | *Acacia vassallii* | B/13 |
| 00511 C | 13/11/97 | *Chorizema humile* | B/11 |
| 00512 C | 13/11/97 | *Grevillea dryandroides* ssp. *dryandroides* | B/10 |
| 00513 C | 13/11/97 | *Acacia vassallii* | B/12 |
| 00514 C | 20/11/97, 5/12/97 | *Acacia insolita* ssp. *recurva* | B/12, I/44 |
| 00515 P1 | 5/11/97 | *Verticordia dasystylis* ssp. *oestopoia* | B/8 |
| 00516 C | 26/11/97 | *Grevillea althoferorum* | B/20 |
| 00517 C | 26/11/97 | *Darwinia carnea* | B/20 |
| 00518 C | 26/11/97 | *Darwinia carnea* | B/4 |
| 00519 C | 27/11/97 | *Chamelaucium* sp. Gin Gin | B/80 |
| 00520 C | 25/11/97 | *Daviesia euphorbioides* | I/4 |
| 00521 C | 2/12/97 | *Darwinia* sp. Williamson | B/30 |
| 00522 C | 2/12/97 | *Brachysema papilio* | B/20 |
| 00523 C | 2/12/97 | *Lambertia echinata* ssp. *occidentalis* | B/5 |
| 00524 C | 4/12/97 | *Orthrosanthus muellerii* | B/80 |
| 00525 C | 4/12/97 | *Adenanthos pungens* ssp. *effusa* | I/14, B/10 |
| 00526 V | 5/12/97 | *Adenanthos pungens* ssp. *pungens* | I/10, B/30 |
| 00527 C | 11/12/97 | *Hemiandra* sp. Watheroo | B/100 |
| 00528 C | 11/12/97 | *Hemiandra* sp. Watheroo | B/60, I/11 |
| 00529 C | 25/11/97 | *Acacia pharangites* | B/? |
| 00530 C | 25/11/97 | *Acacia auratiflora* | B/5 |
| 00531 C | 13/11/97 | *Gastrolobium hamulosum* | B/? |
| 00532 E | ? | *Eucalyptus balanites* | I/1 |
| 00533 E | 3/02/92 | *Villarsia calthifolia* | I/10 |
| 00534 C | 1/01/1998, 2/2/98 | *Grevillea McCutcheonii* | B/2 |
| 00535 C | 1/01/98 | *Grevillea elongata* | B/20 |
| 00536 E | 30/11/97 | *Grevillea christinae* | B/? |
| 00537 C | 1/12/97 | *Acacia insolita* ssp. *recurva* | B/7? |
| 00538 C | 20/01/98 | *Verticordia fimbrilepis* ssp. *fimbrilepis* | B/30 |
| 00539 C | 22/01/98 | *Petrophile latericola* | B/50 |
| 00540 C | 22/01/98 | *Verticordia plumosa* var. *vassensis* | B/30 |
| 00541 P1 | 22/01/98 | *Daviesia elongata* ssp. *elongata* | B/15 |
| 00542 P1 | 23/01/98 | *Daviesia elongata* ssp. *elongata* | B/4 |
| 00543 P1 | 23/01/98 | *Daviesia elongata* ssp. *elongata* | B/80 |
| 00544 V | 23/01/98 | *Dryandra squarrosa* ssp. *argillaceae* | I/30 |
| 00545 P2 | 2/02/98 | *Grevillea brachystylis* ssp. *australis* | B/5 |
| 00546 E | 2/02/98 | *Verticordia plumosa* var. *vassensis* | B/20 |
| 00547 C | 2/02/98 | *Petrophile latericola* | B/50 |
| 00548 C | 3/02/98 | *Verticordia plumosa* var. *ananeotes* | B/150 |
| 00549 E | 3/02/98 | *Dryandra nivea* ssp. *uliginosa* | I/12 |

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|---|---|---|---|
| 00550 C | 9/02/94 | *Darwinia oxylepis* | B/50 |
| 00551 C | 21/1/98, 3/2/98, 2/03/98 | *Verticordia fimbrilepis* ssp. *fimbrilepis* | B/10 |
| 00552 C | 4/02/98 | *Verticordia fimbrilepis* ssp. *fimbrilepis* | B/250 |
| 00553 C | 4/02/98 | *Verticordia fimbrilepis* ssp. *fimbrilepis* | B/15 |
| 00554 C | 10/02/98 | *Eremophila nivea* | B/8 |
| 00555 C | 11/02/98 | *Eremophila nivea* | I/21 |
| 00556 C | 11/02/98 | *Jacksonia pungens* | B/50 |
| 00557 C | 10/02/98 | *Verticordia spicata* ssp. *squamosa* | B/11 |
| 00558 P1 | 18/02/98 | *Jacksonia* sp. Collie | I/11 |
| 00559 P1 | 18/02/98 | *Jacksonia* sp. Collie | I/20 |
| 00560 C | 4/03/98 | *Sphenotoma drummondii* | I/18 |
| 00561 C | 5/03/98 | *Sphenotoma drummondii* | I/2 |
| 00562 C | 10/03/98 | *Dryandra mimica* | B/? |
| 00563 E | 2/10/96 | *Darwinia* sp. Carnamah | B/30 |
| 00564 E | 2/10/96 | *Darwinia* sp. Carnamah | B/10 |
| 00565 C | 11/02/98 | *Eremophila nivea* | I/1 |
| 00566 C | 13/10/96 | *Sphenotoma drummondii* | B/2 |
| 00567 V | 7/04/98 | *Verticordia carinata* | B/100 |

## SCOPE ITEM 5

# *EX SITU* CONSERVATION OF *PHYTOPHTHORA*- AND CANKER-THREATENED SPECIES OF WESTERN AUSTRALIAN NATIVE PLANTS

## PART B

# SUSCEPTIBILITY OF RARE AND THREATENED FLORA TO *PHYTOPHTHORA CINNAMOMI*

***B. L. Shearer, C. E. Crane and A. Cochrane***

## 1 INTRODUCTION

*Phytophthora cinnamomi* infection is a major threatening process affecting the viability and genetic diversity of populations of rare and endangered flora of south-western Australia. In 1992, the Threatened Flora Seed Centre (TFSC) was established by the Department of Conservation and Land Management as part of an integrated conservation strategy for the genebanking of threatened and rare flora.

While considerable progress has been made in the collection and storage of seed, little is known about the susceptibility of the stored taxa to *Phytophthora*. Current estimates of the susceptibility of rare taxa are usually of an empirical nature or based on observations of family susceptibility. However, species within susceptible families such as the Proteaceae, Papilionaceae and Myrtaceae, vary greatly in their sensitivity to *P. cinnamomi* infection (Shearer & Dillon, 1995; 1996). There is a need to systematically test taxa in the TFSC collection to determine their susceptibility to *Phytophthora* and thereby facilitate prioritisation of endangered species according to requirements for protection from the pathogen. Seeds in the TFSC collection are routinely tested for germination and are available for this work.

## 2 OBJECTIVES

This study is concerned with Scope Item No. 5 for the *Phytophthora* and *Diplodina* canker project (1997/98) and more specifically, with determination of the susceptibility of endangered flora (held in the TFSC) to *P. cinnamomi* in order to:

- rank the taxa in the TFSC according to susceptibility to *P. cinnamomi*;
- identify intra-specific variation in susceptibility to *P. cinnamomi* and the potential for selection of resistant individuals;
- determine intra-familial variation in susceptibility to *P. cinnamomi* between endangered species; and
- determine inter-familial variation in susceptibility to *P. cinnamomi.*

## 3 METHODS

Seedlings were established in pots as germinated seeds became available from the TFSC.

Specimens of *P. cinnamomi* were recovered from the vicinity of endangered flora. Appropriate isolates for inoculation of particular hosts were selected on the basis of origin and grown on pine plugs (8-15mm diam.; 20-30mm long) derived from young, debarked branches of *Pinus radiata.* After autoclaving, the plugs were inoculated with *P. cinnamomi* and incubated for one month at 25° C. Thick aerial mycelium covered the plugs by the end of three weeks. Pots were inoculated centrally with three plugs, located lengthwise on top of one another. Controls were non-inoculated pots for each species-isolate combination, and inoculated pots of susceptible *Banksia* spp. including *B. grandis* and the rare and endangered *B. brownii.*

Seedlings from 85 species in 15 families were established in pots as germinated seeds became available from the TFSC. In March, 1998, 17 species in 6 families were inoculated with *P. cinnamomi* (Table 1).

The periods of time between (a) inoculation and first appearance of a root collar lesion and (b) inoculation and plant mortality were recorded. Diseased tissue from dead plants was surface sterilised and incubated on selective agar to re-isolate and confirm the presence of the pathogen.

## 4 RESULTS

Results are not yet available as monitoring of plant death and infection are still continuing. A second inoculation trial will commence in spring, 1998.

**Table 1. Native flora included in pathogenicity tests with *Phytophthora***

| Family | No. of Species Planted | No. of Species Inoculated |
|---|---|---|
| Casuarinaceae | 2 | |
| Dilleniaceae | 1 | 1 |
| Epacridaceae | 1 | |
| Iridaceae | 1 | |
| Lamiaceae | 3 | 1 |
| Menyanthaceae | 2 | |
| Mimosaceae | 10 | 4 |
| Myoporaceae | 1 | |
| Myrtaceae | 24 | 2 |
| Papilionaceae | 12 | 2 |
| Pittosporaceae | 1 | |
| Proteaceae | 22 | 7 |
| Solanaceae | 1 | |
| Sterculiaceae | 3 | |
| Stylidiaceae | 1 | |
| Total | 85 | 17 |

# 5 OUTCOMES

The information gained from this study will address the current deficiency in knowledge of the susceptibility of endangered flora to *P. cinnamomi* infection and assist the prioritisation of taxa that require protection.

# 6 REFERENCES

**Shearer, B.L. & Dillon M.** (1995). Susceptibility of plant species in *Eucalyptus marginata* forest to infection by *Phytophthora cinnamomi*. *Australian Journal of Botany* **43**, 113-134.

**Shearer, B.L. & Dillon M.** (1996). Susceptibility of plant species in *Banksia* woodlands on the Swan Coastal Plain, Western Australia, to infection by *Phytophthora cinnamomi*. *Australian Journal of Botany* **44**, 433-445.

# SCOPE ITEM 6

# REHABILITATION OF *PHYTOPHTHORA*-DEGRADED PLANT COMMUNITIES IN SOUTH-WEST WESTERN AUSTRALIA

***D.I.L. Murray***

## 1 OBJECTIVE

The purpose of this document is to address Scope Item No. 6 for the *Phytophthora* and *Diplodina* Canker project (1997/98). This entails a review of factors likely to be important for the *in situ* rehabilitation of *Phytophthora*-damaged native plant communities and the associated restoration of habitat structure for dependent flora and fauna. A limited literature search was conducted using CD ROM TREE CD (1973-present; CAB International) to locate relevant publications. It is emphasised that a comprehensive review of the subject area could not be attempted within the period of time allotted for completion of this study.

## 2 INTRODUCTION

Several species of *Phytophthora* have been causally associated with dieback disease of native plant communities in the South West Land Division (SWLD) of Western Australia. These soil- or water-borne, root-infecting fungi include *P. cinnamomi, P. citricola, P. cryptogea,* (syn. = *P. drechsleri*), *P. megasperma* var. *megasperma, P. megasperma* var. *sojae* and *P. nicotianae* (Shearer *et al.*, 1991). While interactions between the various pathogens and native plant communities have differing degrees of significance, there is no doubt that *P. cinnamomi*, the cause of epidemic dieback of jarrah and other components of the indigenous flora (Podger, 1972), is by far the most important *Phytophthora* sp. in terms of the magnitude of damage that it inflicts on a broad spectrum of hosts in the SWLD. Accordingly, this document focuses on consideration of *P. cinnamomi* and its impact on native vegetation in Western Australia.

*P. cinnamomi* has been the subject of extensive research in Western Australia for more than a quarter of a century. During this period, considerable knowledge has accumulated regarding the distribution (Shearer, 1994), pathology and life cycle of the fungus, particularly in relation to the northern jarrah forest environment (Shearer & Tippett (1989). The pathogen has been reported to infect more than 900 species of higher plants many of which are important components of Western Australian plant communities (Zentmyer, 1980; Shearer & Hill, 1989; Shivas, 1989).

*P. cinnamomi* has a widespread though disjunct distribution, not only in the jarrah forest, but also in *Banksia* woodlands of the Swan Coastal Plain and kwongan communities on the northern and southern sandplains. The fungus occurs in an area extending south from Eneabba, inland beyond Dryandra, and around the south-west corner of the State continuing adjacent to and along the coast past Esperance in the east (Shearer, 1994). Podger (1972) reported that *P. cinnamomi* was killing most of the overstorey and shrub layers in *Banksia* woodlands. More recently, Hill (1990) and Shearer & Dillon (1996a; 1996b) studied the impact of *P. cinnamomi* in *Banksia* woodlands on the Swan Coastal Plain and listed many species as susceptible to the fungus. Wills (1993) assessed the susceptibility of native flora in the Stirling Range National Park and found that the pathogen was causing mortalities in 36% of 330 species examined. Many Declared Rare or Priority Flora are killed by the fungus in affected woodland or heath communities.

# 3 DEGRADATION OF PLANT COMMUNITIES

Significant alteration of floristic composition and reduction of structural complexity are consistent features of forest, woodland and sandplain communities following establishment of *P. cinnamomi* and natural progression of disease in susceptible vegetation. For example, in healthy jarrah forest, species of Proteaceae, Myrtaceae, Epacridaceae, Papilionaceae, Dilleniaceae and Xanthorrhoeaceae constitute a major segment of the understorey and shrub layers. *B. grandis* and many other of these species are killed by *P. cinnamomi* and their loss, together with mortality of jarrah, culminates in a marked decrease in floristic diversity (Shearer & Tippett, 1989). In terms of structural change, forest dieback sites infected 20-50 years ago have often become open woodlands bearing regrowth marri and a relatively simple ground cover dominated by sedges where a complex assemblage of mainly dicotyledonous species once prevailed.

Similarly, the structure and floristic diversity of *Banksia* woodlands are degraded by infections of *P. cinnamomi* on the coastal sandplain. Dominant tree species such as *B. attenuata, B. ilicifolia,* and *B menziesii* are killed in some areas leaving scattered eucalypts and a shrub layer in which species richness may be significantly diminished together with reductions in plant biomass of up to 90% (Shearer & Hill, 1989). *P. cinnamomi* is primarily a pathogen of woody perennials, whereas annuals, geophytes and herbaceous perennials are usually unaffected by the fungus (Zentmyer, 1980; Wills, 1993). Increased abundance of the latter plant forms can thus be expected as the floristic composition of an affected community changes after disease establishment.

Wills (1993) studied the ecological impact of *P. cinnamomi* in the Stirling Range National Park and pointed out that changes in habitats induced by modification of community structure or composition may adversely affect plants that are not directly attacked by the pathogen. This was illustrated by reference to *Stylidium scandens* which appears to be resistant to *P. cinnamomi*. Specimens of the triggerplant were common in healthy areas of the park but absent in adjacent, diseased sites. *S. scandens* is usually found in dense understorey beneath healthy stands of jarrah and it was suggested that its absence in infested areas was due to loss of canopy shading associated with understorey mortalities. Various components of the flora, resistant to

*P. cinnamomi* but sensitive to specific habitat alterations, may also be indirectly disfavoured by the results of pathogen activity while others, for example, introduced annuals, may benefit from such disturbance.

There is no doubt that degradation of native plant communities, caused by *P. cinnamomi*, has the potential to severely affect the abundance and composition of associated faunal populations (Wilson *et al.*, 1994). A decrease in canopy cover would be expected to directly impact on habitat availability for avifauna or arboreal marsupials. Changes to floristic composition in the overstorey, understorey and/or shrub layers might result in the loss of specific food (e.g., seeds, pollen or nectar) or habitat requirements (e.g., shelter from predators). Reduction in canopy together with thinning or removal of ground cover, leaf litter and exposure of soil surfaces may equate with severe alteration to, or local loss of habitat for some small ground-dwelling animals including litter invertebrates. Conversely, fauna adapted to more open habitats may be favoured (Wilson *et al.*, 1994). In exposed situations, the soil biota are likely to be affected by increased fluctuations in sub-surface temperatures as are soil microorganisms. The latter, including *P. cinnamomi*, may also be influenced by changes in floristic composition at least in the vicinity of plant roots (Murray *et al.*, 1985; Murray, 1987).

Indirect effects of *P. cinnamomi* on pollinators may further exacerbate the decline of relatively rare *Banksia* spp. and other animal-pollinated plants in some affected communities. For example, the local survival of two species of nectarivorous possums, *Cercartetus concinnus* and *Tarsipes rostratus*, are threatened by loss of nectar sources as a result of dieback disease in the Stirling Range National Park. If populations of the possums diminish in infested areas, this in turn could adversely affect the reproductive activity of remaining plants (Shearer *et al.*, 1991; Wills, 1993; Wills & Keighery, 1994). Further examples of interactions between pathogen, flora and fauna are cited by Shearer (1990) and Shearer *et al.*, (1991). Associations of this nature would be an important consideration in regard to rehabilitation of degraded communities.

# 4 REHABILITATION OF PLANT COMMUNITIES

With the exception of minesite studies, the literature search conducted at the onset of this work failed to locate any reports of *in situ* rehabilitation of *Phytophthora*-degraded native plant communities in Western Australia or elsewhere. Although mining activities obviously result in major physical disruption of the environment some aspects of bauxite mine rehabilitation are of interest in relation to the restoration of native plant communities devastated by *P. cinnamomi.*

## 4.1 MINESITE REHABILITATION

Alcoa of Australia Ltd. has been involved in open cut bauxite mining and subsequent rehabilitation of minesites in the jarrah forest since 1963 and 1966, respectively (Koch *et al.*, 1994; Ward *et al.*, 1997; Grant & Koch, 1997). At the Jarrahdale and Willowdale sites, mining occurs in predominantly dieback-affected forest (Colquhoun,

1992). Mining entails the removal of topsoil (5-15cm), overburden (0-1m), cemented caprock and bauxite ore to a depth of 2-5m. Pit walls are levelled during the initial stage of physical rehabilitation, and the overburden and topsoil are then replaced in that order before mechanical ripping to a depth of 1.5m (Nichols *et al.*, 1989; Ward *et al.*, 1996).

Early attempts at rehabilitation included the planting of exotic pines or eastern states eucalypts (resistant to *P. cinnamomi*) without understorey seeding or ripping of pit floors (Nichols & Bamford, 1985; Nichols *et al.*, 1989). The outcome of this approach was a single stratum of plantation-like vegetation with poor development of understorey or ground cover (Nichols & Bamford, 1985).

As rehabilitation techniques gradually evolved in tandem with increased understanding of pathogen-host-environment interactions, it became apparent that jarrah and other species considered susceptible to *P. cinnamomi* could be used for minesite rehabilitation. The re-establishment of these species was probably favoured, and the pathogen disfavoured, by alteration of environmental conditions including removal of caprock, reduced soil compaction and improved drainage associated with deep ripping (Colquhoun, 1992). It is interesting to speculate whether cost-effective methods for modification of the soil environment could be developed to accommodate the reintroduction of dieback-susceptible species in woodland, sandplain or unmined forest areas infested with the pathogen.

An objective of Alcoa's mine rehabilitation program is to establish a self-sustaining ecosystem (Ward *et al.*, 1997) with floral, faunal and soil characteristics of the indigenous jarrah forest (Nichols *et al.*, 1991). Since 1989, Western Australian native eucalypts including jarrah have been used exclusively for overstorey rehabilitation. Moreover, the current policy is to use only local species for the understorey seed mix that supplements the seed-bank in returned topsoil. To assist achievement of this objective, a seed collection provenance zone has been established for each mine (Koch *et al.*, 1994).

Mortality rates of reintroduced jarrah on minesites are low despite the presence of *P. cinnamomi*. At Eneabba, on the northern sandplain, highly susceptible species of *Banksia* have been used successfully in the rehabilitation of infested land mined by RGC Mineral Sands Ltd. (Colquhoun & Peterson, 1994). While the survival of sensitive host plants in these areas may be favoured by environmental disturbance associated with mining, it is also possible that insufficient time has elapsed (since revegetation) to allow the biomass of susceptible root tissue in the soil to attain a level conducive to rapid build up of pathogen inoculum.

Collins *et. al.* (1985) studied re-colonisation of restored bauxite minesites by birds and reported that older sites, lacking understorey or planted with pines, supported smaller populations and fewer species of avifauna than sites that had been revegetated using more advanced methods. Furthermore, population densities and numbers of species at the latter sites were similar to those recorded for healthy forest communities. Old sites rehabilitated only with overstorey trees were also unsuitable for most reptiles (Nichols & Bamford, 1985). There is evidence that as floral communities on rehabilitated minesites mature and plant diversity increases, animal species absent in the early stages

of colonisation may return as specific habitat requirements become available. Examples include birds that nest in hollows or in the canopy of tall trees. According to Nichols *et al.* (1991), it is unnecessary and impractical to physically reintroduce fauna back into restored minesites as their successful establishment will be dependent on the existence of suitable habitats, a key element of which is vegetation.

## 4.2 INVENTORY

Determination and documentation of the pre-infection floristic composition and structure of *Phytophthora*-degraded plant communities are essential prerequisites to rehabilitation if conservation values are to be restored in affected areas. Disease-induced alterations to the jarrah forest understorey are not well documented (Shearer & Tippett, 1989) although the original work of Podger (1968), the site-vegetation relationships elucidated by Havel (1975a; 1975b) and more recent studies by Shearer & Dillon (1995) provide some indication of likely changes following disease establishment in forest areas. Alterations to floristic composition and structural complexity of *Banksia* woodland on the Swan Coastal Plain are reported by Shearer & Dillon (1996b). Further assessments of plant community structure and composition need to be undertaken for healthy areas of forest, woodland and sandplain, particularly those under imminent threat of infestation by *Phytophthora*. In some cases, extrapolation of results from healthy to adjacent diseased areas may be the only avenue available for determination of pre-infection community composition.

A comparison between pre- and post-infection assessments of animal populations can provide some idea of the success of a particular rehabilitation procedure as the disappearance of certain species in the latter assessment may indicate a lack of suitable habitats (Nichols *et al.*, 1991).

## 4.3 REHABILITATION STRATEGIES

Three options are available in relation to selection of appropriate plant species for rehabilitation of *Phytophthora*-damaged communities in forest, woodland or sandplain situations. These are:

1. Reintroduction of the native species eliminated previously by *P. cinnamomi*.

2. Introduction of non-local (surrogate) species.

3. A combination of 1 and 2.

The first option is essentially that adopted by Alcoa for minesite rehabilitation. If feasible, it is certainly the most attractive alternative since it should lead ultimately to the development of a similar native plant community to that existing before infection. This assumes that niches occupied by the original flora are still open, or could be made so by removal of annual weeds or other invaders prior to revegetation. Faunal recolonisation would proceed as suitable habitats again became available in the

developing plant community. Various aspects of the reintroduction of native species are considered below, in greater detail.

If a decision is made in favour of using non-local or exotic flora (Option 2), it is important that certain properties are demonstrated for candidate plant species prior to their introduction to native communities. Selected species should be field resistant to *P. cinnamomi* and to any other significant pathogens or pests likely to be encountered. They should be site compatible in terms of climatic, edaphic and biotic factors but not likely to become invasive or weedy at any stage of development. Their response to fire and their ability to withstand grazing should not differ significantly to that of the original flora. Finally, each species should be phenotypically similar to that which it replaces in the community. Implicit in the last requirement is the need for introduced species to act as surrogates, to re-establish the structure and quality of habitats for dependent flora and fauna, thus ensuring the availability of adequate nutrition, shelter, reproductive or other necessary conditions at levels comparable to those prevailing before community degradation.

At some locations it might be expedient to use surrogate species to replace only those elements of a community that are particularly susceptible to *P. cinnamomi*, for example, various *Banksia* spp. This approach (Option 3), which would be expected to significantly reduce the quantity of pathogen inoculum available for infection of other hosts, might assist the reintroduction of relatively insensitive components of the native flora. Irrespective of which option is chosen, testing of candidate species under field conditions and monitoring in the long term would be needed to evaluate the efficacy of any rehabilitation strategy.

## 4.4 REINTRODUCTION OF NATIVE SPECIES

Reintroduction of susceptible native species into an area infested by *P. cinnamomi* is unlikely to be successful unless the environment can be manipulated to create conditions unfavourable for host infection or alternatively, the reintroduced plants can be protected by cost-effective application of a persistent, systemic fungicide such as phosphonate. A third possibility is the selection of genetic resistance to *P. cinnamomi* in the native species to be used for the rehabilitation process.

Manipulation of floristic composition by prescription burning was suggested more than two decades ago as a means of ameliorating the impact of *P. cinnamomi* in the jarrah forest (Shea & Malajczuk, 1977). Moreover, there is evidence that plant establishment in rehabilitated minesites can be enhanced by fire (Grant *et al.*, 1997) and it is possible that controlled burning may in some circumstances have a role to play in the restoration of degraded plant communities. However, physical alteration of the soil environment to a degree resembling that noted for mining operations would probably be prohibitively expensive.

Phosphonate is a non-toxic, systemic fungicide considered to be capable of controlling most species of *Phytophthora* including *P. cinnamomi*. The chemical is thought to enhance host resistance to the pathogen and it is reported to protect *Banksia* spp. from infection for at least four years after application (Shearer *et al.*, 1991). Therefore,

phosphonate treatment would appear to be a potent tool for assisting the reintroduction of susceptible native plants into areas where previously they were eliminated by the pathogen.

High levels of genetic resistance to *P. cinnamomi* have been demonstrated to exist in *Eucalyptus marginata* (jarrah) and technology is now available for the micropropagation of selected lines that may be useful in the rehabilitation of degraded sites in the jarrah forest (McComb *et al.*, 1994; Stukely & Crane, 1994). Some evidence of resistance to *P. cinnamomi* has also been found for *Banksia coccinea* and *B. hookerana*, two species that are regarded as highly susceptible to the pathogen (McCredie *et al.*, 1985). Detection of genetic resistance and propagation of resistant lines of native species currently being destroyed by *P. cinnamomi* is possible. However, the time required to develop screening and propagation techniques will probably restrict the use of this approach to priority species under threat of extinction from the fungus (McComb *et al.*, 1994) at least in the short term.

There is evidence in the literature to suggest that, whenever possible, local provenance material should be used for revegetation programs (Taylor *et al.*, 1994; van Leeuwen, 1994; Coates & van Leeuwen, 1996). This is based on the premise that considerable genetic variation exists in a species (over its geographic range) which reflects not only the evolutionary history of the species, but also the ecological conditions to which it has been exposed. It follows that the population of a species best adapted to a particular locality will be that which evolved there. Accordingly, provenance seed from locally occurring species is likely to be most suitable for the rehabilitation of a degraded plant community (Coates & van Leeuwen, 1996). While accepting the validity of this argument, it is clear that local species did not evolve in the presence of *P.cinnamomi* and that susceptible taxa are not well adapted to coexist with the pathogen. If local provenance material is to be used for the reintroduction of susceptible native flora, then plants will require protection in the form of phosphonate application unless resistant lines can be identified. This probably represents the best strategy currently available for rehabilitation of native plant communities damaged by *P. cinnamomi.* As indicated earlier, populations of fauna should return as the plant community develops, assuming that there are no barriers to migration from surrounding areas.

# 5 OUTCOME

- This review represents a first step towards synthesis of the knowledge base required for *in situ* rehabilitation of *Phytophthora*-damaged native plant communities in Western Australia, and for the associated restoration of habitat structure that is essential for conservation of dependant flora and fauna.

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# SCOPE ITEM 7

# IMPACTS OF DIEBACK-INDUCED VEGETATION CHANGES ON NATIVE FAUNAL COMMUNITIES IN SOUTH-WEST WESTERN AUSTRALIA

***O.G. Nichols***

## 1 INTRODUCTION

In February 1998, the Department of Conservation and Land Management (CALM) commissioned Environmental Management and Research Consultants (represented here by the writer) to review impacts of dieback-induced vegetation changes on fauna. The specific objective of the work was to review existing knowledge on the impact of *Phytophthora cinnamomi*, and of management practices for its control, on the welfare of native animal species and communities with a view to identifying opportunities and constraints for the abatement of those impacts.

In the course of the review it was found that little detailed research had been carried out in the subject area. Apart from work on mammals in Victoria, most studies had been conducted in Western Australia. Accordingly, the review concentrates mainly on the situation in the latter State and, to a lesser degree, in Victoria. Existing knowledge is summarised, results are synthesised and interpreted, and from the conclusions, recommendations are developed for the minimisation of dieback impacts and the conservation of potentially threatened species.

## 2 DIEBACK-INDUCED VEGETATION CHANGES

*P. cinnamomi* is a phytopathogenic fungus that causes serious root-rot and dieback of many Australian native plant species. The disease has significantly damaged various plant communities including tropical rainforests in Queensland, eucalypt forests, woodlands and heathlands in Victoria and the jarrah forest and heathlands of south Western Australia. The pathogen spreads autonomously by movement of water-borne zoospores in the soil and mycelial growth in infected plant roots, or through vectored spread of infested soil by man and other animals.

The distribution and severity of infection in plant communities is influenced by temperature, soil type, nutrient status and water availability (Weste & Marks, 1987; Marks & Smith, 1991; Wilson *et al.*, 1994), thus the magnitude of disease impact varies between sites. Greatest impact generally occurs where soils are infertile and drainage is poor. Communities growing on fertile soils, high in organic matter, may suffer relatively little damage (Shearer & Tippett, 1989).

*Eucalyptus marginata* (jarrah) is the dominant tree species in forests of south Western Australia and it is the only eucalypt in the jarrah forest that is susceptible to *P. cinnamomi*. Healthy jarrah forest has a dense understorey and shrub layer which is floristically rich and includes a diverse flora with nectar-producing flowers that are pollinated by small mammals, birds or insects. Many of these plant species, including *Xanthorrhoea preissii* and members of the Proteaceae, Dilleniaceae, Papilionaceae (Podger, 1968) and Epacridaceae (Keighery, 1988) are particularly susceptible to dieback. *Banksia grandis* is also highly susceptible and mortality of this species is one of the first indicators of infestation by *P. cinnamomi* (Shearer & Tippett 1989).

In the Proteaceae-dominated heathlands along the south coast of Western Australia, the impact of dieback is particularly severe, resulting in marked structural changes and decline in floristic diversity.

The effect of dieback infection on plant communities is similar in Victoria, although different species are present there. The cover provided by tree crowns decreases and the composition of the understorey is altered. Susceptible species such as *Xanthorrhoea australis* disappear or are greatly reduced in abundance while resistant species, particularly grasses and sedges, take over. In some situations, severe erosion occurs as a result of vegetation losses (Kennedy & Weste, 1986).

Many parts of the jarrah forest were severely affected by dieback between the 1950's and the 1980's. Frequently, all or most of the jarrah and much of the understorey was killed and replaced by other plant species. Resistant marri (*Eucalyptus calophylla*) recolonised affected sites which eventually became open woodland with a ground cover dominated by sedges (Shearer & Tippet, 1989). By comparison with the original forest, floristically impoverished communities developed in which the lower stratum consisted predominantly of plants that lacked nectar-producing flowers.

# 3 SUMMARY OF EXISTING KNOWLEDGE

## 3.1 MAMMALS

No specific studies on the impacts of dieback-induced vegetation changes on mammals have been carried out in Western Australia. Several of the Alcoa studies discussed in sections 3.2 and 3.3 have included mammal trapping, but for most species the numbers trapped were too low to detect any differences that might have existed.

Friend (1992) hypothesised that the Honey Possum (*Tarsipes rostratus*) could become a species under threat as a result of dieback impacts on floral communities, particularly in south coast heathlands. Since many of the plant species on which the Honey Possum feeds are susceptible to *P. cinnamomi*, dieback infestation could significantly deplete the animal's food resource.

Wilson *et al.* (1994) speculated that habitat changes caused by *P. cinnamomi* and *Diplodina* (a cause of canker disease) could potentially threaten the Dibbler (*Parantechinus apicalis*) in south-west Western Australia by decreasing the number of prey invertebrates inhabiting flowers and the deep litter layer. They also concluded that *Pseudomys shortridgei*, a restricted mammal species which depends on floristically rich

vegetation, could be detrimentally affected by the spread of dieback in the Victorian Grampians.

Specific research on the impacts of dieback-induced vegetation changes on mammalian fauna appears to have been conducted only in Victoria. Laidlaw & Wilson (1994) studied small mammals in open forest, woodland and heathland in Angahook-Lorne State Park, near Anglesea where some areas were affected by dieback. The study did not find any relationship between dieback impact and the diversity or abundance of small mammals. However, the authors noted the probability that insufficient sites were examined to allow detection of significant differences.

Wilson *et al.* (1994) cite an unpublished study by Laidlaw & Wilson as finding lower abundances of several small mammal species such as the Swamp Rat (*Rattus lutreolus*), Bush Rat (*R.. fuscipes*) and the Brown Antechinus (*Antechinus stuartii*) in dieback-affected heathland near Anglesea in Victoria. Mean species richness of small mammal communities was also found to be lower at infected sites.

Detailed studies were also carried out by Newell & Wilson (1993) who investigated the relationship between dieback, vegetation changes and the abundance of *A. stuartii* in Victoria's Brisbane Ranges. Trapping indicated that the species was significantly less abundant in sites infested with *P. cinnamomi* than in uninfested sites. The volume of vegetation at the 0-20 and 20-24cm heights was also significantly reduced. When the abundance of *A. stuartii* was regressed against vegetation, a positive significant correlation was found at both of the above heights. These results and other observations clearly indicated a link between dieback impacts, structural decline of vegetation and numbers of *A. stuartii*. Radio tracking studies by Newell (1994) indicated that *A. stuartii* showed a high degree of overlap with areas that were unaffected by dieback while the species largely avoided affected areas.

Newell & Wilson (1993) investigated the relationship further and concluded that the decline of the grass tree (*X. australis*) was a major contributing factor in the reduction of *A. stuartii* abundance, possibly because of the decrease in shelter associated with loss of the tree. Other studies conducted by Newell (1997) did not indicate significant declines in the abundance of ground-dwelling invertebrates, so reduced food availability would appear to be an unlikely reason for the decreased abundance of *A. stuartii*.

Wilson *et al.* (1994) provided a table of predicted effects on fauna associated with the presence of *P. cinnamomi* in plant communities. This is shown in Table 1 where it can be seen that widespread changes in the vegetation are possible with consequent effects on fauna. The predicted effects on fauna are discussed in more detail in Section 4.

## 3.2 BIRDS

Very little research has been published on the effects of dieback-induced vegetation changes on avifauna. Several research projects, conducted in the jarrah forest by Alcoa of Australia Ltd., have focused on comparisons between avifaunal recolonisation of rehabilitated bauxite minesites and bird communities in unmined forest. Unmined

control plots included areas of healthy and dieback-affected forest, and were intended to represent a cross-section of pre-mining vegetation types. Useful comparative data on the avifauna of healthy and diseased forest were obtained in the course of monitoring. The following results were extracted from published work by Nichols & Watkins (1984) and from unpublished Alcoa data and reports.

**Table 1. Predicted effects on flora and fauna due to the presence of *P. cinnamomi* in plant communities in Victoria (from Wilson *et al.*, 1994)**

| Effects on Vegetation | Effects on Fauna |
|---|---|
| 1. Loss of susceptible plant species in the understorey or midstorey. | • Direct loss of food sources, *e.g.*, seeds, pollen.<br>• Indirect loss of food sources, *e.g.*, invertebrates. |
| 2. Decline in species richness and diversity. | • Loss of food for species that prefer floristically rich vegetation.<br>• Loss of seasonal food availability. |
| 3. Decrease in plant cover; increase in bare ground and erosion. | • Loss of habitat for species dependent on thick ground cover.<br>• Increased predation risk.<br>• Changes to microclimate. |
| 4. Decrease in canopy cover. | • Loss of food for arboreal species.<br>• Loss of habitat for arboreal species. |
| 5. Decrease in litter fall. | • Decline in litter invertebrates (dry conditions).<br>• Decline in invertebrate food sources for insectivores. |
| 6. Post-infection increase in frequency of field resistant plant species, *e.g.*, sedges. | • Increase in food for specialist herbivores. |

In 1981 a long term monitoring program commenced at Alcoa's Jarrahdale mine. Birds were surveyed within 20m of transects located in two healthy and two dieback-affected areas of forest. Methods are described by Nichols & Watkins (1984). The surveys were repeated in 1987 and 1993. In all years, monitoring was undertaken in summer. Impacts in both dieback-affected sites were severe, although some rehabilitation (understorey establishment and planting of resistant trees) was carried out in Dieback Site 1 in 1994. Results are summarised in Table 2 and complete data for each year is given in Appendix 1. Although insufficient sites were monitored to allow statistical

analyses of results, the data suggest that several differences exist between the bird communities in healthy and dieback-affected forest.

**Table 2. Numbers of bird species, densities (number/ha) and Shannon-Wiener Diversity (relative abundance) recorded in surveys of healthy and dieback-affected, unmined forest at Jarrahdale in 1981, 1987 and 1993 (results from Armstrong & Nichols, in prep.)**

| | Healthy 1 | Healthy 2 | Dieback 1 | Dieback 2 |
|---|---|---|---|---|
| No. of species | | | | |
| 1981 | 16 | 16 | 9 | 14 |
| 1987 | 18 | 20 | 13 | 10 |
| 1993 | 27 | 28 | 21 | 16 |
| Total density | | | | |
| 1981 | 13.2 | 12.6 | 6 | 13.5 |
| 1987 | 9.5 | 13.2 | 6.2 | 6.2 |
| 1993 | 12.2 | 8 | 8.2 | 8 |
| Diversity | | | | |
| 1981 | 1.09 | 1.08 | 0.88 | 0.99 |
| 1987 | 1.13 | 1.18 | 1.06 | 0.89 |
| 1993 | 1.03 | 1.15 | 1.04 | 1.04 |

The results summarised in Table 2 indicate that total numbers of bird species were consistently less in sites affected by dieback than in healthy forest. Bird density in dieback sites was also lower for some but not all surveys. In most cases, diversity (which compares the relative abundance of species) was reduced in the dieback-affected sites. These results indicate that dieback-induced vegetation changes have some affect on avifaunal communities.

The data in Appendix 1 give some indication of what those changes might be. The White-naped Honeyeater was recorded in healthy forest on most occasions but never in the dieback-affected sites. The Western Spinebill was usually recorded in healthy forest sites and in dieback Site 2, but not in Site 1. No honeyeaters were recorded at dieback Site 1 during any surveys. Since forest-dwelling honeyeaters commonly feed on susceptible plant species such as *B. grandis, Adenanthos barbigerus* and *Grevillea spp.*, these results suggest a link between dieback impacts and a local decline in honeyeaters.

Indirect effects on bird species are also possible. Many birds are insectivorous, thus any decline in insect numbers due to vegetation changes would be expected to affect the numbers of species that feed on insects. In Appendix 1 it can be seen that the

Rufous Treecreeper, a bird that eats insects on tree trunks, was usually recorded in healthy forest but never in diseased sites. The Western Yellow Robin, which forages for insects in the middle and lower strata, and the Grey Shrike-thrush, a mid-stratum forager, also tended to be more common in healthy than in dieback-affected areas. However, numbers of other insectivores such as the Striated Pardalote, a leaf gleaning species, and the Grey Fantail were similar in both healthy and dieback-affected forest. These results indicate that numbers of some insectivorous bird species may decline in severely diseased areas, but others are not so obviously affected. Further studies are needed to clarify the situation.

Structural changes in the vegetation may also have a negative effect on some bird species. However, the relationship is not clear from currently available data.

The impacts on avifauna are not all negative. Numbers of some species appear to increase. For example, birds that usually utilise more open areas, such as the White-winged Triller and the Rainbow Bee-eater, tend to be most common in dieback-affected forest. The Willy Wagtail and Yellow-rumped Thornbill, which are usually recorded only in open farmland and rarely in forest, were recorded in dieback Site 1.

The results of this work suggest that forest, severely affected by dieback, supports fewer species of avifauna and lower bird densities and diversity than healthy forest. Species that appear to decline include some honeyeaters and insectivores, while some other bird species characteristic of open areas tend to proliferate.

**Table 3. Numbers of bird species and Shannon-Wiener Diversity (relative abundance) recorded in healthy (F) and dieback-affected (D), unmined forest sites near Alcoa's Jarrahdale (J) and Huntly (H) mines (Alcoa: unpubl. results)**

| | Year | FJ1 | FJ2 | [A]DJ1 | [B]DJ2 | FH1 | FH2 | [C]DH1 | [C]DH2 |
|---|---|---|---|---|---|---|---|---|---|
| Summer | | | | | | | | | |
| No. spp. | 1992 | 19 | 13 | - | 8 | - | - | - | - |
| | 1995 | 19 | 16 | 27 | 7 | 18 | 18 | 11 | 14 |
| | 1998 | 18 | 11 | 25 | 12 | 7 | 11 | 16 | 14 |
| Diversity | 1995 | 1.99 | 2.42 | 2.19 | 0.69 | 2.42 | 2.41 | 1.74 | 1.88 |
| | 1998 | 2.26 | 1.96 | 2.44 | 1.63 | 1.59 | 1.73 | 2.15 | 2.24 |
| Winter | | | | | | | | | |
| No. spp. | 1992 | 18 | 8 | - | 7 | 18 | 12 | 21 | 15 |
| | 1995 | 20 | 9 | 22 | 14 | 17 | 11 | 13 | 13 |
| Diversity | 1995 | 1.85 | 0.50 | 1.71 | 1.94 | 2.22 | 1.91 | 1.97 | 1.72 |

Impact of disease in affected sites was [A]low, [B]high or [C]moderate

A second Alcoa monitoring program commenced in 1992. This again concentrated on faunal recolonisation of rehabilitated areas and it included both healthy and dieback-affected, unmined forest control sites. Monitoring was conducted every three years in both summer and winter. At each site, birds were surveyed three times along two 250m transects. All birds within 20m of the transects were counted. Results are shown in Tables 3 and 4.

**Table 4. Densities (number/ha) of stated bird species recorded in 1995 in healthy (F) and dieback-affected (D) sites in unmined forest near Jarrahdale (J) or Huntly (H) (Alcoa: unpubl. data)**

| Species | FJ1 | FJ2 | [A]DJ1 | [B]DJ2 | FH1 | FH2 | [C]DH1 | [C]DH2 |
|---|---|---|---|---|---|---|---|---|
| Summer | | | | | | | | |
| White-naped Honeyeater | 0.17 | 0.33 | | * | * | 0.33 | 0.33 | 0.33 |
| New Holland Honeyeater | | | | * | * | | * | * |
| Western Spinebill | | 0.33 | 0.17 | * | 0.17 | * | | * |
| Winter | | | | | | | | |
| White-naped Honeyeater | 0.83 | * | 0.67 | 0.17 | * | 0.17 | 0.5 | * |
| New Holland Honeyeater | * | * | | * | * | * | * | * |
| Western Spinebill | * | * | 0.33 | | 0.33 | * | * | * |

*indicates that the species was present but not recorded in density estimates.
Impact of disease in affected sites was [A] low, [B] high or [C] moderate. Sites are the same as those listed in Table 3.

Considerable variation was noted between the avifaunas of healthy forest sites and this tended to mask any effects due to dieback (Table 3). Nevertheless, some trends were apparent. The low impact site at Jarrahdale (DJ1) appeared to support greater numbers of bird species than the corresponding healthy forest control sites. The reasons for this are unclear but may be related to partial opening of the canopy leading to a situation where species that inhabit open areas, for example, magpies, colonise before those requiring healthy forest are excluded.

In summer, species richness and diversity of birds at the high impact site were lower than the corresponding values for either healthy forest or the low impact site. However, the difference was not apparent in winter. No consistent differences were evident in the avifauna of forest that was moderately affected by dieback.

No honeyeaters were recorded for density counts at the high impact site in summer (Table 4). Neither the moderate nor the low impact sites showed any consistent differences in numbers of honeyeater species when compared to healthy forest sites in either summer or winter.

Other unpublished data from the 1995 study show variation in the degrees to which insectivorous species utilise dieback-affected sites. The Rufous Treecreeper was not recorded in the high impact dieback site but it was noted in a moderately affected site. The Western Yellow Robin was not found in any dieback-affected site in summer, but was present in all categories of dieback sites in winter. The Striated Pardalote showed no consistent differences between sites in any season, while numbers of the Grey Fantail tended to be least in moderately or severely affected sites in summer.

In summary, although the results have not been analysed statistically, trends indicate that in summer, severely diseased forest supported fewer species of avifauna with less diversity than healthy forest. Species thought to be affected include some honeyeaters and insectivores. The numbers of several bird species characteristic of open areas tended to increase. No declines were apparent in the low impact dieback site. In moderately affected sites, reductions in species numbers and diversity were apparent in some surveys but not in others. There were no consistent differences between the avifaunas of healthy and dieback-affected sites in winter. The large variation observed in avifaunal communities between sites with similar dieback impact indicates that more work is required to provide an adequate understanding of the interactions between bird species, vegetation changes, season, and other relevant factors.

In a separate study conducted by Wykes (1983), densities of birds in healthy and dieback-affected jarrah forest were compared. Wykes used a strip transect method similar to the two Alcoa surveys mentioned above. The dieback site was described as severely affected. Surveys were conducted every two months commencing in June 1981. Mean densities for each species are shown in Table 5 and total bird densities per count are shown in Figure 1.

**Figure 1. Seasonal changes in density of bird species in healthy and dieback-affected jarrah forest (adapted from Wykes, 1983)**

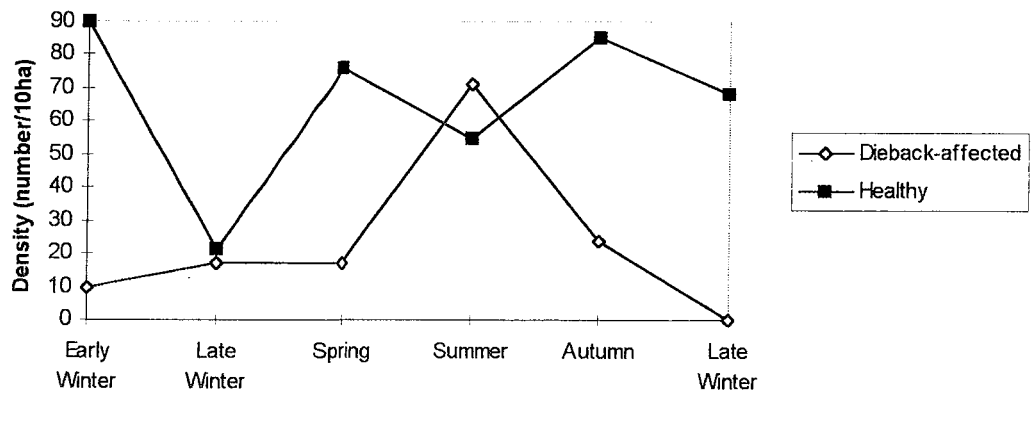

**Table 5. Bird densities (number/10ha) calculated by Wykes (1983) using strip transect methods in healthy and dieback-affected sites in the jarrah forest**

| Guild | Species | Healthy | Dieback |
|---|---|---|---|
| Insectivore: | | | |
| a) Ground foraging | Scarlet Robin | 1.9 | 0.9 |
| | Western Yellow Robin | 1.6 | |
| | Splendid Wren | 4.7 | 2.9 |
| | White-browed Scrub-wren | 0.3 | |
| b) Shrub foraging | Western Thornbill | 9.7 | 1.4 |
| | Red-winged Fairy Wren | 1.9 | |
| | Grey-breasted White-eye | 3.8 | 2.8 |
| c) Tree foraging | Broad-tailed Thornbill | 6.5 | 3.1 |
| | Golden Whistler | 3.3 | |
| | Western Shrike-thrush | 0.4 | |
| | Striated Pardalote | 5.8 | 0.8 |
| | Spotted Pardalote | 1.0 | |
| | White-naped Honeyeater | 3.6 | 0.6 |
| d) Bark foraging | Varied Sittella | 1.0 | |
| e) Air foraging | Tree Martin | 1.0 | 0.3 |
| | Grey Fantail | 6.8 | 1.2 |
| | Western Flyeater | 2.5 | 1.8 |
| Carnivore | Little Eagle | 0.5 | 0.3 |
| | Laughing Kookaburra | 0.6 | |
| Nectarivore | Western Spinebill | 2.1 | |
| Graminivore | Western Rosella | 0.4 | 0.9 |
| | Red-capped Parrot | 3.8 | |
| | Port Lincoln Parrot | 1.6 | 0.8 |
| Others | seven species | 0.7 | 0.3 |
| Total ( ± s.d.) | | 65.5 ± 23.9 | 18.1 ± 18.8 |

Each value is the mean of six surveys conducted between June 1981 and August 1982. Total bird density was significantly greater in the healthy forest site, as was the density of the Western Thornbill, Golden Whistler and White-naped Honeyeater.

Wykes (1983) found statistically significant differences between the total bird densities at the two sites and also in the numbers of Western Thornbills, Golden Whistlers and White-naped Honeyeaters (Table 5). In all cases, densities were greatest in healthy forest. The results also suggest that the densities of many other species may be higher in healthy, than in diseased forest. Figure 1 shows that in most seasons the total number of birds were greater in healthy forest than in the site affected by dieback. The greatest differences were noted in winter.

These results accord with Alcoa data which indicate that total bird densities are generally least in areas severely affected by dieback. In both studies, densities of honeyeaters and some insectivores tended to be lower in diseased than in healthy forest and the differences were statistically significant. However, seasonal trends in bird densities do not necessarily follow those in species richness. In the work conducted by Alcoa, the difference in species richness between healthy and dieback sites was greatest in summer. In the Wykes study, the difference in density was greatest in winter.

## 3.3 REPTILES

The only documented information available on utilisation of dieback-affected areas by reptiles is that collected during two long term monitoring projects conducted by Alcoa. These were carried out in the same areas as the avian studies described above.

Appendix 2 shows the results obtained from sites monitored in 1981, 1987 and 1993. Methods are described in detail by Nichols & Reynolds (in prep). At healthy forest Site 1 and dieback Site 2, 10 PVC pipes (100mm deep x 150mm diam.) and 40 plastic containers (110mm depth and diam.) were installed. In addition, 10 medium and 4 large Elliott traps were used. At healthy forest Site 2 and dieback Site 1, half this number of traps were installed. To correct for the difference in sample areas, densities for each species were calculated and expressed as the number of individuals per hectare. Trapping was conducted in summer (December-January). Hand collecting and visual searches were also undertaken to obtain records of presence or absence of species. Equal periods of time were spent at each site. Although the results for sites cannot be compared statistically and must be interpreted with caution, given the variation in trap numbers, they do give some indication of whether large differences are likely to exist between healthy and severely affected dieback sites.

The total numbers of species, both recorded and trapped, differed considerably between sites and over time, but in any one year were generally highest in healthy forest. The same applied to the number of individual reptiles trapped.

Species such as the skink, *Ctenotus labillardieri*, were regularly recorded in healthy forest but never in dieback-affected sites. *C. labillardieri* is a ground forager which shelters in log or stump crevices. In any given year, several other species including *Bassiana triliniata, Hemiergis initialis* and *Morethia obscura* were usually more common in healthy, than in diseased forest. All forage amongst litter with *B. trilineata* common in shaded areas and *H. initialis* in moist sites and under logs or rocks. Other species including the skink (*Tiliqua rugosa*) and the goanna (*Varanus gouldii*) were more often recorded in healthy forest than in dieback-affected vegetation. However,

**Table 6. Presence (*) and abundance (number collected) of reptile species in 1992 and 1995 in healthy (F) or dieback-affected (D) sites at Jarrahdale (J) and Huntly (H) (Alcoa: unpubl. data)**

| Species | FJ1 | FJ2 | [A]DJ1 | [B]DJ2 | FH1 | FH2 | [C]DH1 | [C]DH2 |
|---|---|---|---|---|---|---|---|---|
| Geckos | | | | | | | | |
| *Diplodactylus polyophthalmus* | | | (1) | (*) | | | | (2) |
| Skinks | | | | | | | | |
| *Bassiana trilineatum* | 1 | (1)2 | 2 | | | | (1) | 1 |
| *Cryptoblepharus plagiocephalus* | (*) | | (*)1 | (1)* | * | | | |
| *Ctenotus delli* | | | | (1) | | | * | |
| *C. labillardieri* | (*)* | | (*) | | (2)* | (*)3 | | (2) |
| *E. napoleonis* | | 3 | | | 1 | | * | (1)* |
| *Hemiergis initialis* | (1) | * | | | 1 | (3)1 | 1 | |
| *Lerista distinguenda* | 1 | | 1 | 2 | | | | |
| *Menetia greyi* | 1 | | (1) | | 1 | | 2 | (1)* |
| *Morethia obscura* | (1)3 | (1)2 | 1 | (1)1 | (*)1 | (1)3 | 1 | 2 |
| *Tiliqua. Rugosa* | | | | (*) | | | | |
| Blind Snakes | | | | | | | | |
| *Ramphotyphlops australis* | (1) | | | | | | * | |
| Elapid Snakes | | | | | | | | |
| *Pseudonaja affinis* Dugite | | | | | | | | * |
| No. of species 1992 | 5 | 2 | 4 | 5 | 2 | 3 | 1 | 4 |
| No. of species 1995 | 5 | 4 | 4 | 3 | 6 | 3 | 6 | 5 |

Results for 1992 are given in parentheses. Presence includes winter observations while abundance refers only to summer trapping.

Disease impact in affected sites (identified in Table 3) was [A] low, [B] high or [C] moderate.

the densities of some species such as the skinks *Menetia greyi* and *Cryptoblepharus plagiocephalus*, were similar in both healthy and dieback-affected forest. *M. greyi* often forages in open areas while *C. plagiocephalus*, which is commonly known as the Fence Skink, lives on logs, posts and stumps. It is often seen foraging in sunlight.

The other long term monitoring data (Table 6) were obtained using different trapping methods. At each site, traps included 10 large pit traps (150 mm diam.) with seven metre flywire drift fences, two large Elliott traps and eight medium Elliott traps. The trapping work was supplemented by hand collecting and visual searches.

Total numbers of species again differed both between sites and sampling dates, but no consistent differences were found between healthy forest and any of the dieback sites. *C. labillardieri* and *H. initialis* were more often recorded in healthy forest than in areas affected by dieback. However, at Jarrahdale, *C. plagiocephalus* was most frequently noted in dieback sites. No clear differences were apparent for other species.

Although these data are by no means definitive they do suggest a number of trends that could be investigated in more detail. These include:

- Whether total numbers of reptile species are reduced in severely affected dieback sites;

- Whether species which require litter, moist sites and shady situations are relatively common in healthy forest; and

- Whether species which forage in open areas, or on logs and stumps in exposed sunlight, are more common in areas affected by dieback than in healthy parts of the forest.

Future studies should investigate links between the presence of species and microhabitat, food, predation and competition. Predicted effects of severe dieback impacts on reptile fauna are discussed in Section 4.

## 3.4 FROGS

No studies appear to have been conducted on the effects of dieback-induced vegetation changes on frogs.

## 3.5 AQUATIC FAUNA

Horwitz *et al.* (1997) indicate that research has not been carried out on the effects of dieback impacts on aquatic fauna and suggest that changes in a plant community due to dieback may affect hydrological regimes. They also state that logging may cause

hydrological changes and exacerbate the spread of dieback, thus further influencing hydrological regimes. Although recognising that the effects of such disturbances on aquatic macroinvertebrates and fish have not been investigated, Horwitz *et al.* (1997) conclude that any change in the hydrological regime would influence the composition of aquatic communities. In the absence of any detailed research it is not possible to speculate what links, if any, may exist between dieback and aquatic fauna.

## 3.6 TERRESTRIAL INVERTEBRATES

Several studies have investigated the degree to which terrestrial invertebrate communities differ in dieback-infected and healthy plant communities.

Postle *et al.* (1986) studied soil and litter invertebrates in healthy and dieback-infected jarrah forest. Over a 15 month period, soil and litter were sampled from 10 randomly selected points in both healthy and diseased forest. The study involved detailed measurements of litter biomass and rates of litterfall. Annual litterfall was 48% less in dieback-affected than in healthy forest. Litter biomass, total leaves, twigs/bark and fruit/flowers were all considerably reduced in the diseased area.

The abundance of most taxa was found to be relatively low in dieback-affected forest for at least part of the year and particular species were sometimes absent. The greatest differences in densities at some times of the year were noted for soil and litter Symphyla and Pauropoda, litter Araneae, Diplopoda, Coleoptera, Pseudoscorpionida and Heteroptera, and soil Chilopoda. Postle *et al.* (1986) were uncertain whether these differences were caused by changes in microclimate or by decreased availability of food. However, it was noted that proportionally more soil taxa were depleted in January and March than in any other month.

Certain invertebrate taxa were most abundant in dieback-affected sites during some seasons. These included cockroaches, ants, fly and beetle larvae and Psocopterans. It was postulated that these insects might be responding to an altered food base, but no results were provided to support this.

The conclusion to be drawn from this study is that most invertebrate taxa living in soil and litter are less abundant in dieback-affected areas than in healthy communities This is probably related to decreased litter biomass although the exact nature of the link has yet to be been determined.

Nichols & Burrows (1985) conducted a survey of predatory invertebrates in the jarrah forest as part of a study investigating recolonisation following bauxite mine rehabilitation. Predatory invertebrates were selected because it was assumed that they might reflect differences in those components of the invertebrate community which constitute their prey. The study comprised two unmined forest sites; one infected with dieback, the other free of the disease. Survey methods included pitfall trapping as well as tree beats, herb and shrub sweeps and hand collecting.

Similar numbers of spider, scorpion and ant species were recorded in both dieback and healthy sites. Numbers of centipede and earwig species were greatest at the latter site

while numbers of pitfall-trapped and total species recorded were marginally lower. However, Shannon-Weiner diversity was highest in the dieback site. Abundance of herb- and shrub-inhabiting species, and all species excluding ants were greater in the dieback-affected site while the opposite was true for the abundance of all individuals.

Hamilton-Brown (1994) examined the two sites again in 1994, 13 years after the first survey was conducted. She found no large differences between the numbers of ant species in diseased and healthy areas. The number of spider species and total spider numbers were least in dieback-affected forest as were counts of total species and total number of individuals.

These results, like those of Postle *et al.* (1986), indicate that the effects of dieback-induced vegetation changes vary between invertebrate groups and between surveys. The abundance of some groups increases, possibly in response to more open ground (lower litter cover) providing better opportunities for foraging. Others such as centipedes, appear to decline. Overall, species numbers and abundance seem to decrease. For certain groups, the findings of Postle *et al.* (1986) appear to differ from those of Nichols & Burrows (1985). In some months, Postle *et al.* (1986) found that spider abundance was greatest in dieback-free forest, while Nichols & Burrows (1985) reported similar results for both sites. A possible factor contributing to the difference in findings is that dissimilar methods were used in the two studies.

The Alcoa long term fauna monitoring program discussed in Sections 3.2 and 3.3 included ant pitfall trapping in healthy and dieback-affected jarrah forest. At each site, 20 pitfall 42mm diam. traps containing Galt's solution were opened for one week in late summer and early autumn. The results from 1992 and 1995 are shown in Table 7. There was no evidence that either numbers of ant species or diversity were reduced in any of the dieback-affected sites. Distribution of individual species has not been analysed in detail. However, in 1995 it was found that *Cardiocondyla nuda*, an early coloniser that was usually apparent in the first stages of bauxite mine rehabilitation, was present in dieback affected Site DJ1, but not in any of the healthy forest sites.

Majer (1977) also conducted a study in the jarrah forest and concluded that the ant fauna of dieback-infected forest included species that were characteristic of healthy areas as well as those more commonly found in disturbed or open sites. This supports some of the findings of the Alcoa study discussed above.

Newell (1997) studied the abundance of ground-dwelling invertebrates in a Victorian forest affected by dieback. The site was located in the Brisbane Ranges, north-west of Melbourne. The study was conducted as part of the research on *Antechinus stuartii* described in Section 3.1. For the invertebrate assessment, Newell selected two low, open forest sites. The first site included areas of uninfected, recently infected and long term infected forest, while the second supported uninfected and long term infected vegetation. Fifty pitfall traps were placed in each area and sampled for one week in each of four seasons for three years. Samples were identified (usually to order) and grouped as macroinvertebrates or microinvertebrates according to the food preference of *A. stuartii*.

**Table 7. Diversity and number of ant species recorded for the 1992 and 1995 Alcoa long term monitoring program in healthy (F) or dieback-affected (D) forest sites at Jarrahdale (J) and Huntly (H)**

| | FJ1 | FJ2 | [A]DJ1 | [B]DJ2 | FH1 | FH2 | [C]DH1 | [C]DH2 |
|---|---|---|---|---|---|---|---|---|
| 1992 | | | | | | | | |
| No. species | 14 | 14 | 20 | 19 | 13 | 19 | 19 | 22 |
| 1995 | | | | | | | | |
| No. species | 17 | 7 | 18 | 18 | 12 | 11 | 13 | 14 |
| Diversity | 2.3 | 1.7 | 1.7 | 1.5 | 2.2 | 2.1 | 2.3 | 2.0 |

Disease impact in affected sites was [A]low, [B]high or [C] moderate.

Few statistically significant differences between the various categories of dieback-affected forest were detected for any invertebrate group. Parametrical statistical tests showed that some differences between infection groups were observed for Collembola, with higher numbers present in dieback-affected sites. In spring, abundance was highest in recently infected forest. Occasional significant differences were also observed for Coleoptera and Dermaptera, but again numbers were highest in dieback-affected sites. Few significant differences were noted for any other taxa. Non-parametric tests showed differences between infection categories for Araneae, Orthoptera, Hemiptera, Diptera and unidentified larvae on some sample dates. For all of these groups, apart from larvae, abundance was greater in infected sites than in uninfected ones. No significant differences were detected in the abundance of morpho-species of ants. Higher abundances of microinvertebrates were detected in infected than in uninfected sites for most years in both study areas.

The sampling design allowed Newell (1997) to compare interactions between seasons and dieback infection. The only statistically significant link between the two variables was found for Collembola.

Invertebrate diversity (measured using the Shannon-Weiner Index) did not differ significantly between infection categories on one site, while the long term diseased sites had the higher index of this parameter.

Detailed assessment of vegetation followed by statistical analyses were conducted to determine which factors influenced the abundance of particular invertebrate groups. Some significant relationships, both negative and positive, were found to exist at one site between the abundance of smaller invertebrates and lower structural variables such as *Xanthorrhoea australis* and litter cover. Positive correlations were found between litter cover and both Diptera and Collembola, vertical diversity and Coleoptera, and larvae and *X. australis*. Negative correlations were found between *X. australis* and Coleoptera, Collembola and Diptera, and between larvae and vertical diversity. Stepwise multiple regression analysis revealed several significant links between low

vegetation parameters and invertebrate abundance, but the correlations were both positive and negative. This lead Newell (1997) to conclude that abundances within taxa were weakly associated with ground level habitat features, and therefore the impact of *P. cinnamomi* on vegetative structure and floristics was not reflected in different abundances of ground-dwelling invertebrates.

This does not necessarily mean that links between some invertebrate groups and vegetation changes do not occur. For example, a possible link existed between a temporary increase in litter cover following infection, and the abundance of Collembola. This increase in litter may be due to mortality of *X. australis*. However, the lack of any difference in litter cover between long term infected and uninfected sites may partly explain why differences in the invertebrate communities were generally insignificant.

The observation that abundance of some groups, for example, Blattodea, was correlated positively with the extent of bare ground may be an artifact of the survey method in that the mobility of individuals could be greatest on open ground, thereby increasing their chances of capture.

*Summary:*

Although the link between terrestrial invertebrates and dieback impact has been investigated in some detail, there are notable differences between results of the various studies. This probably reflects seasonal effects, identities of groups examined, survey methods used, identification and analyses (e.g., whether species numbers or abundance were compared) and possible differences between ecosystems (e.g., Western Australian v Victorian forests). Consistent findings are therefore few. Despite this, individual studies provide useful indications of the links that may exist.

In studies conducted in the jarrah forest, many groups appeared to decline in dieback-affected areas. These included centipedes, beetles and pseudoscorpions. This tends to result in a decrease in total invertebrate abundance. However, increases in some groups reduce the extent of this decline. Numbers of ants and ant species generally do not appear to decline, although some changes in species composition may occur with taxa more characteristic of open areas replacing those that require a healthy forest habitat.

In Victoria's Brisbane Range forest, widespread declines were not apparent. However, results of the work reviewed here suggested links between increased litter cover in recently affected sites and the abundance of Collembola. This group was also found to be more abundant in dieback-affected than in healthy jarrah forest in wetter months.

Almost all of the studies conducted to date have concentrated on ground-dwelling invertebrates. Virtually nothing is known about the extent to which changes induced by dieback might affect groups such as Lepidoptera, Orthoptera, wasps and native bees, most other pollinators and any species which feed in the canopy.

# 4 SYNTHESIS

## 4.1 IMPACTS OF VEGETATION CHANGES ON FAUNA

The studies described above are very limited both in number and in the areas and groups of fauna which they consider. None were carried out in Western Australia's southern heathlands or northern sandplains. Apart from several Victorian studies on mammals and ground-dwelling invertebrates, no work in these areas was reported for other states. In some cases, different methods were used and this limited the extent to which results for various sites could be compared.

As well as limitations imposed by the paucity of relevant studies, the nature of the disease and variation in factors relating to its spread and impact tend to complicate the measurement and estimation of dieback effects on fauna. Infested areas vary widely in the degree to which they provide habitats for particular species. This is well illustrated by considering the factors that are likely to influence impacts of dieback-induced vegetation changes on fauna. For the jarrah forest, where the majority of dieback research has been conducted, these include:

- The extent of infestation, i.e. patch size. The area infested may occupy a small part of the home range of a larger predator such as the Chuditch, *Dasyurus geoffroii*, but may cover the whole home range of a smaller mammal or bird species. Patch number and size in relation to a species' home range are likely to influence the extent of impact.

- The shape of an infested area. Narrow, linear areas such as those along stream zones are less likely to impact on some species of fauna than more rounded areas. However, the reverse may be true if the linear area coincides with the distribution of an important food species, such as *Banksia littoralis*.

- The location of infested areas. Some species may not be able to inhabit dieback-affected areas due to a lack of suitable food or shelter, or both. For these species, the extent and distribution of uninfected areas may be important because affected areas may act as barriers.

- Time since infection. The extent of vegetation and related changes vary with time. A typical sequence of impact in jarrah forest and some likely effects on fauna are illustrated in Table 8.

- Degree of impact. Some sites can be described as high impact. In these, all susceptible understorey and tree species have died, significantly changing both the structure and floristic composition of the site. In extreme cases, open ground may be present following the decomposition or burning of the litter layer. In low impact

sites, the most susceptible species die but the long term survival of resistant understorey species and jarrah trees confers the appearance of low impact. More details are given in Shearer & Tippett (1989). It was previously assumed that the extent of impact could be related to Havel vegetation site type (Havel, 1975). For example, P type was considered to be high hazard, i.e. infection inevitably resulted in severe impact, while T type was considered to be low hazard. Recent observations (F. Podger, pers. comm.) have shown that this relationship is not as strong as hitherto believed Thus, the concept of hazard is no longer used in dieback management.

- Plant species present. Some animal species feed on particular plant species for part or all of the year. The Western Spinebill on *Adenanthos barbigerus* is one example. In cases where an animal is strongly dependant on one or more dieback-susceptible plant species, introduction of the disease can have a significant, local impact on the animal.

- Spread by indigenous species of fauna. It is believed that *P. cinnamomi* can be spread by species such as the Western Grey Kangaroo, but quantitative data are not available. The extent to which other native species may spread the disease is unknown.

- Spread by feral species (e.g., pigs). It has been demonstrated that pigs can spread *P. cinnamomi*. The extent to which they do this has not been quantified. The effectiveness of pig control programs is likely to influence the rate of spread.

- Predation by feral species such as the fox and cat. Feral predators have a significant impact on fauna. Whether this impact is increased in more open dieback-affected areas is not known but would seem possible. Also, it is reasonable to postulate that for some species the combined effects of feral predators and widespread dieback infestation are greater than the impacts of either in isolation.

- Floristic diversity. Following the introduction of dieback, the fauna habitat value of sites possessing predominantly Proteaceous species will be lower than that of sites which contain a greater floristic diversity including dieback-tolerant species.

- Hydrological changes. At present the link between dieback, hydrology and stream fauna is largely unknown. It is possible that if changes in stream flow, seasonality, turbidity and conductivity occur they may have some effect on aquatic fauna.

- Sub-surface drainage. The link between this and the extent of dieback impact is described in Shearer & Tippett (1989). While the general principles are well understood, in the absence of site-specific studies, it is not generally possible to

accurately predict the magnitude of impact on fauna in the event of dieback introduction in a particular situation.

- Cumulative impacts. Few studies have considered the extent to which impacts of forest land uses and disturbances may be cumulative. Disturbances such as planned and unplanned fires, as well as land uses including bauxite mining and logging are all known or likely to have a temporary impact on some animal species. The total impact on a particular species will be greater if it is impacted by a number of these land uses or disturbances. Nichols & Bamford (1985) and Nichols & Watkins (1984) concluded that for birds and reptiles, the impacts of bauxite mining and dieback are generally not cumulative because the two habitats support different species.

- Regrowth of tolerant species. Marri (*E. calophylla*) eventually establishes in some areas that have been severely affected by dieback. This assists partial restoration of tree canopy cover although the process may take place over many decades. Dieback-resistant sedges also appear to be relatively abundant on many severely affected sites and they can provide ground cover for small species of fauna. The extent to which these plants spread into areas vacated by the death of susceptible species has not been measured.

- The evolutionary history of the fauna. Most of the fauna of south-western Australia are adapted to recurring disturbances such as fire and drought. This might make some species more tolerant to the types of changes that result from introduction of dieback.

In determining and managing the degree of dieback impacts, we need to first consider the nature of any given impact. These may be impacts on particular indicator species, rare species, whole communities, specific fauna groups, or a particular parameter such as species numbers, density or diversity. In the following summary of impacts, all of the above are considered.

### 4.1.1 Impacts on Mammals

In the jarrah forest, it is difficult to clearly define what the long term impacts of dieback changes on mammal species will be. One reason for this is that unpublished studies by Alcoa and CALM have recently shown that numbers of species such as the Chuditch and Southern Brown Bandicoot have increased significantly due to fox baiting as part of Operation Foxglove. Other species that may be increasing, or are expected to increase, include the Western Brush Wallaby, Brush-tailed Phascogale and Quokka. Reintroduction of the Woylie at a number of sites has been particularly successful, with second generation animals now being captured (P. deTores, pers. comm.). These changes in abundance make it extremely difficult to assess the extent of localised, patchy changes due to dieback.

**Table 8. Predicted sequence of vegetation changes in a typical S/P type jarrah forest (Havel, 1975) following the introduction of dieback, with likely impacts on fauna over time (It should be noted that many of the impacts are speculative.)**

| Vegetation Change | Impact on Mammals | Impact on Birds | Impact on Reptiles |
|---|---|---|---|
| 1. Deaths of *Banksia* and other highly susceptible species. | Decreased food resource for W. Pygmy-possum and Honey Possum. | Decreased food resource for honeyeaters and some insectivores. | Decreased food resource for arboreal geckos, temporary increase in exfoliating bark shelter. |
| 2. Deaths of other susceptible understorey species. | Decreased shelter availability for small mammals. | Decline in wrens and White-browed Scrub-wren due to open understorey. Decline in some insectivores. | Decreased cover and shading, unfavourable microclimate. Decline in some skink species, possible increase in others. |
| 3. Deaths of jarrah and *Allocasuarina fraseriana.* | Decreased food availability for arboreal insectivores, e.g., Brush-tailed Phascogale. | Decline in some insectivores, e.g., Rufous Treecreeper, Western Yellow Robin. | Changes in microclimate disfavouring species requiring shaded sites. |
| 4. Increase in litter. | Possible temporary increase in food availability for ground dwelling insectivores. | Not known. | Increased food resource for some species. |
| 5. Opening of the canopy following tree deaths. | Increased raptor predation. Change in microclimate. | Decline in some insectivores, increase in species favouring open habitat, e.g., White-winged Triller. Increased suitability for raptors. | Increased raptor predation. Unfavourable microclimate for some species. |

**Table 8 (Cont.).**

| Vegetation Change | Impact on Mammals | Impact on Birds | Impact on Reptiles |
|---|---|---|---|
| 6. Possible temporary increase in hollows and logs following tree deaths. | Provision of shelter for species which require hollows and logs. | Provision of hollow nesting sites, e.g., for owls, pardalotes. | Increased shelter and foraging sites for some species, e.g., *C. plagiocephalus*. |
| 7. Decrease in litter due to decomposition and burning. | Decreased food resource (e.g., reptiles and invertebrates). | Decreased food resource for ground foraging insectivores. | Decreased cover and food resource for ground foraging species. |
| 8. Decrease in logs due to fire. | Decreased shelter availability e.g. for Chuditch and Bandicoots. | Possible decreased food availability for raptors. | Decreased shelter for several species ie. *Egernia napoleonis*, *Ctenotus labillardieri*. |
| 9. Decrease in hollows due to trees falling. | Decreased shelter availability, e.g., for Brush-tailed Phascogale, Possum. | Decrease in hollow nest sites. | |
| 10. Increase in marri density. | Partial increase in food availability for arboreal insectivores. | Increase in food resource for some insectivores. Seasonal increase in food availability for nectarivores. | Increase in shading and less extreme microclimate would favour some species. |
| 11. Possible small increase in sedges, ground cover and litter. | Possible partial increase in food resource for small insectivores. More favourable microclimate. | Possible partial increase in food resource for ground foraging insectivores. | Possible increase in food resource and protection from raptors for ground dwelling species. |

Certain aspects of dieback-induced vegetation changes would be expected to impact on some mammalian species. For example, tree deaths would not initially decrease the number of available hollows for the arboreal Brush-tailed Phascogale, but eventually some impact would be expected. The extent and duration of the impact would partly depend on the rate at which dieback-tolerant marri replaced jarrah. The food resource for the Brush-tailed Phascogale would also be expected to be lower in severely affected dieback areas. However, as the Phascogale also forages for invertebrates on a number of dieback-tolerant species (e.g., marri and wandoo), only localised declines would be expected.

The abundance of logs on the ground would possibly increase in some dieback-affected sites following tree deaths. It might then decline after fire. Reduced log abundance may diminish a habitat's suitability for species such as the Chuditch, Southern Brown Bandicoot and Numbat. However, decreased fox predation may lessen the species' requirement for logs to shelter from predators so the impact of reduced log abundance might not be as great.

Dieback impacts on susceptible plants would be expected to have some localised effect on some species of mammals, by decreasing nectar and (possibly) insect availability. Species such as the Honey Possum, which inhabit heathland in the Mt. Saddleback State Forest, would probably be affected if widespread deaths of nectar-producing Proteaceous plants occurred. Insectivores such as the Mardo (*Antechinus flavipes*) may be locally affected in the event of reduced abundance of suitable invertebrates. However, insufficient research has been conducted to determine the likely magnitude of any declines in food availability. Species such as the Western Pygmy-possum (*Cercartetus concinnus*) feed on both insects and nectar. In trapping programs conducted by Alcoa, both Mardos and Western Pygmy-possums have been trapped in dieback-affected forest However it is possible that these animals were itinerant individuals passing through the area. Whatever the case, the results suggest that dieback-affected sites do not form barriers to movement between remnant areas of healthy forest.

Research has shown that as a general rule, the jarrah forest's mammalian species recolonise after bauxite mining rehabilitation (Nichols, in prep.) and burning (Christensen & Abbott, 1989). Research at Kingston Block has not demonstrated either large or long term declines in the abundance of any mammal species. It can therefore be assumed that provided these and other forest activities are managed responsibly, their cumulative impacts together with dieback should be manageable.

In summary, it would seem that no mammal species in the jarrah forest is seriously threatened by dieback-induced vegetation changes, particularly in the light of increased numbers due to fox baiting. Nevertheless, some species might decline and as the extent of any such decline is unclear, the wisest course of action would be to limit the spread of dieback and manage other disturbances and land uses to minimise the overall impact of *P. cinnamomi*.

In areas other than the jarrah forest, impacts on mammalian species may be more serious. Friend (1992) speculated that the introduction of dieback into areas of

southern heathland, including parts of the Fitzgerald River National Park (FRNP), is likely to cause a significant decline in the abundance of Honey Possums. This may well be correct and impacts on the Dibbler are also possible. Research investigating the likely extent of impacts would produce useful information as other values such as flora conservation and tourism are also threatened. The most urgent priority is to limit the spread of the disease in such areas.

In Victoria, sufficient impacts on small mammal species have been identified to conclude that introduction of dieback causes localised declines in faunal abundance. It is too early to say whether any species would be threatened as a result of these declines.

### 4.1.2 Impacts on Birds

Localised changes in the abundance of particular bird species in the jarrah forest are likely in areas impacted by dieback. These species would include honeyeaters and some insectivores. The changes would result in decline of total numbers of species in areas severely affected by dieback. Some impact on hollow nesting species might occur if the total number of hollows declines.

The Rufous Treecreeper is relatively uncommon in the jarrah forest. It forages for insects on trunks of jarrah and other trees or in log piles. The studies referred to in Section 3.2 did not record this species in severely affected dieback areas. Nor has it been recorded in rehabilitated bauxite minesites (Nichols, in prep.). Although the Rufous Treecreeper is not rare, further studies are warranted on its ecology and sensitivity to the impacts of disturbances including dieback.

The mobility of birds reduces the extent to which dieback-affected areas might act as barriers to movement between uninfected sites.

In conclusion, it is not apparent at this stage that the status of any jarrah forest inhabiting bird species is seriously threatened. However, a number of species have declined in severely affected areas, resulting in changes to the avifaunal community.

Given the absence of any relevant studies, it is difficult to make definitive statements on the likely impacts of dieback-induced changes on birds in areas outside the jarrah forest. Some rare species such as the Noisy Scrub Bird occur in areas known to be infected with dieback (e.g., Two Peoples Bay Nature Reserve; J. Blythe, pers. comm.). Others, such as the Rufous Bristlebird and Western Whipbird occur in heathland rich in Proteaceae, for example in the FRNP. The major structural and floristic changes that would follow dieback infestation in such areas could have a potentially significant impact on these rare species.

The potential impact of dieback on another rare bird species, the Western Ground Parrot, has been considered in the Species Interim Recovery Plan (Burbidge *et al.*, 1997). It was postulated that a decline in woody Proteaceous perennials could have an adverse impact on Western Ground Parrot habitat in some areas. However, the authors also recognised that such changes might actually improve the habitat by removing large

*Banksia spp.* and shrubs and by increasing the dominance of sedges with a possible increase in food availability for the parrot. The conclusion is that at present, the likely effects of vegetation changes on the species are unknown and further studies are required.

### 4.1.3 Impacts on Reptiles

The results of limited research conducted in the jarrah forest indicate that a number of reptile species are likely to decline in areas severely affected by dieback. These seem to be species that rely on an intact litter layer, moist conditions, shelter such as crevices and exfoliating bark, shade, or combinations of the above. The result of these declines is likely to be a decrease in total species numbers and reptile density in severely damaged forest areas. None of the reptile species likely to be affected is regarded as seriously threatened or at risk of extinction.

No research has been conducted on the link between dieback impacts and the reptile fauna of the southern heathlands. *Banksia* woodland north of Perth supports a species-rich reptile community. This is also likely to be true of the southern sandplains. Dieback-susceptible Proteaceae constitute a significant component of plant communities in these areas, both in terms of structure and floristic diversity. Changes to the vegetation following the introduction of dieback would be expected to significantly alter the litter, microclimate and invertebrate community with probable impacts on some reptile species. Insufficient information is available to speculate on the likely extent of these impacts on particular species.

### 4.1.4 Impacts on Frogs

The lack of studies carried out to date makes it impossible to provide a meaningful assessment of the impact that dieback-induced vegetation changes might have on frogs. In some situations such as the broad sandy valleys of the eastern jarrah forest, vegetation changes due to dieback may alter site hydrology and result in prolonged surface water retention. This may benefit some species which require shallow ponds, but disadvantage burrowing species of the genus *Helioporus* due to early flooding of burrows. More work is needed to determine the nature of any impacts.

The potential of dieback-induced hydrological changes to affect rare frog species, such as the Sunset Frog, is unknown but should be investigated.

### 4.1.5 Impacts on Aquatic Fauna

It is not possible to speculate on the impacts of dieback on aquatic fauna beyond the conclusions already drawn in Section 3.5.

### 4.1.6 Impacts on Terrestrial Invertebrates

Some impacts on terrestrial invertebrates occur following the introduction of *Phytophthora*. These are most notable in severely affected areas. Reductions in litter cover and depth cause declines in numbers of centipedes, beetles and pseudoscorpions. Other groups probably decline, but the type and magnitude of changes would be expected to vary between sites and over time. A reduction in small, litter dwelling invertebrates is likely to cause a decrease in larger predatory species which feed on them.

The extent to which pollinating insects are dependent on particular dieback-susceptible plant species is unknown.

As the status of most invertebrates is unknown, it is impossible to conclude whether any species may be threatened as a result of dieback-induced vegetation changes. However, the possibility exists that some may be, particularly in areas of heathland where widespread infestation has occurred or where entire remnant patches have been infected.

## 4.2 MANAGING DIEBACK IMPACTS ON FAUNA

### 4.2.1 Constraints

Constraints that limit the extent to which the impact on fauna associated with dieback-induced vegetation changes can be managed, fall into three broad categories:

- Limitations in the amount of information available on impacts on particular species and groups of fauna.

- Limitations in the extent to which the spread of dieback can be controlled.

- The irreversible nature of changes.

*Limitations in knowledge:*

Clearly, there is a need for more information on the effects of dieback-induced changes on fauna. Studies to date have focused on few groups in limited areas. The extent to which dieback may threaten the conservation status of many species, particularly invertebrates, is either poorly known or unknown. Changes in the composition of bird and reptile communities are understood to a limited extent in some jarrah forest sites, but have not been studied in other ecosystems. Additional limitations in knowledge are noted in Section 3.

*Limitations in controlling spread:*

The application of phosphonate has proved to be an effective means of controlling the impact of disease. However, costs and the need for repeat applications may limit its use to critical areas. Spread can occur in three ways:

- Autonomous spread, which includes spread along roots, growth of mycelia, movement of zoospores and spread via ground or surface water movement. Once the disease is established control of autonomous spread is virtually impossible.

- Spread via animal vectors including native and feral species. Apart from control of pigs, which is only partially effective, limiting spread due to animals is not practicable.

- Spread due to the activities of man, specifically those that involve movement of infected soil. This is the area, where with sufficient commitment, appropriate management practices and adequate resources, further spread of the disease can be controlled.

Thus, it is apparent that only limited control of spread is possible. Introduction to new areas and autonomous spread from existing infections will continue to result in vegetation changes that will impact on fauna as discussed in Sections 3 and 4.1.

*Irreversible changes:*

Available evidence strongly suggests that the changes in plant communities induced by *P. cinnamomi* are irreversible. The fungus does not die out in an area after dieback has swept through and susceptible plant species have been killed. Although some re-invasion by tolerant species such as marri and various grasses and sedges may occur (Shearer & Tippett, 1989; Kennedy & Weste, 1986), plant communities on affected sites do not regain their original structure or the floristic diversity that existed prior to infection.

Some instances of susceptible species re-invading infested areas have been reported, for example, *X. australis* in Victoria's Brisbane Ranges (Dawson *et al.*, 1985). However, it is possible that the observed plants were growing in patches which had escaped infection.

### 4.2.2 Recommended Remedial Actions and Opportunities

There are two implications stemming from the limited availability of information on the impacts of dieback-induced vegetation changes on fauna. Firstly, research needs to be focused on animal species and groups most likely to be affected. Both management and broader research efforts need to be directed to minimisation of further spread and reduction of impacts on fauna.

Priority should be given to understanding and managing impacts on the following fauna values:

- Rare, threatened or restricted species where there is thought to be a reasonable chance of decline due to dieback-induced vegetation changes. Examples include the Dibbler, Western Whipbird and Rufous Bristlebird in Western Australia and *Pseudomys shortridgei* in Victoria.

- Species for which there is some evidence of localised decline in impacted areas and which might serve as indicators of impacts. Examples include some honeyeater, insectivore, reptile and litter-inhabiting species in Western Australia and *A. stuartii* in Victoria.

- Species and groups of fauna for which no information is currently available, but knowledge of their biology and disease impacts indicate a high probability of localised and possibly serious decline. Examples include the Honey Possum and some invertebrate pollinators such as native bees.

- Structure of faunal communities, particularly in areas where conservation is a priority land use (e.g. nature reserves and national parks).

Broader dieback research which is likely to be of significant benefit to fauna includes but is not limited to:

- Continuing research into the cost-effective application of phosphonate.

- Research into the development of dieback-resistant jarrah and other key susceptible species, including research into understanding the mechanism of resistance.

- In situations where significant populations of a rare or uncommon animal species are located and found to be at risk, there may be merit in investigating operational techniques for establishing dieback-tolerant tree and understorey species.

- Any operational research which is directed towards reducing the introduction of the disease during operations involved with land uses such as logging, mining, flower picking, beekeeping, seed collecting and tourism.

Management efforts need to be directed towards minimisation of further spread and reduction of impacts on fauna. Priority should be given to:

- Developing and implementing cost-effective procedures which will minimise the spread of the disease due to the activities of man.

- Prioritising areas that require the greatest amount of protection with respect to conservation of fauna values.

- Developing a program for planting dieback-resistant jarrah in priority conservation areas when sufficient affordable stock becomes available and where there is likely to be significant benefit to fauna and flora.

- Controlling spread of inoculum by feral species, particularly pigs.

- Controlling feral predators, particularly the fox and, where necessary, the cat (when cost effective control measures become available). This has been shown to significantly increase numbers of many species and would reduce the impacts of dieback-induced vegetation changes.

- Translocation and re-establishment of populations of some rare species known to be at threat from dieback impacts.

- Integrated studies are needed to investigate the combined impacts on fauna over time, of forest management practices such as burning, land uses such as logging and mining, and dieback. Variations on the intensive monitoring and research program being conducted at Kingston Block in the jarrah forest would be the most appropriate way to address this need.

# 5 OUTCOME

This document reviews available information relevant to the understanding and management of impacts on Western Australian fauna associated with structural and floristic changes to native plant communities caused by *Phytophthora cinnamomi*. Constraints on the management of these impacts are considered and remedial actions and opportunities for impact abatement are discussed. Recommendations are made for future research directions.

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**Appendix 1. Mean densities of avifauna (birds $ha^{-1}$) in healthy and dieback-affected jarrah forest**

Figures in round ( ) and square [ ] parentheses are density values from 1981 and 1987, respectively. Values not enclosed in parentheses are for 1993. Each value is the mean density derived from three surveys. Data are from Armstrong & Nichols (in prep.).

* Species observed on site in 1993 but not during survey periods.

| Species | Healthy Forest | | Dieback-affected Forest | |
|---|---|---|---|---|
| | Site 1 | Site 2 | Site 1 | Site 2 |
| Emu *Dromaius novaehollandiae* | | * | | |
| Little Eagle *Aquila morphnoides* | | [0.33] | | |
| Collared Sparrowhawk *Accipiter cirrhocephalus* | 0.17 | 0.33 | 0.17 | |
| Brown Falcon *Falco berigora* | * | | | |
| Common Bronzewing *Phaps chalcoptera* | [0.33] | [0.67] | 1 | * |
| Red-tailed Black Cockatoo *Calyptorhynchus magnificus* | * | | [0.67] 0.83 | [0.5] 0.33 |
| Baudin's Cockatoo *Calyptorhynchus baudinii* | | 1.17 | | |
| Australian Ringneck *Platycercus zonarius* | (0.67) [0.33] * | 0.5 | 0.33 | 0.33 |
| Red-capped Parrot *Platycercus spurius* | (1.5) [0.83] 0.33 | (0.5) [0.5] 0.17 | (0.67) 0.83 | (1.17) 0.5 |
| Western Rosella *Platycercus icterotus* | (2.5) 0.67 | [0.17] * | * | (0.17) |
| Horsfield's Bronze Cuckoo *Chysococcyx basalis* | | 0.17 | | |
| Laughing Kookaburrra *Dacelo gigas* | * | (0.34) [0.67] 0.17 | 0.17 | 0.83 |
| Sacred Kingfisher *Halcyon sancta* | [0.17] | * | (0.34) | |
| Rainbow Bee-eater *Merops ornatus* | | * | (1.84) | (3.17) |
| Tree Martin *Cecropis nigricans* | | (1.84) * | [0.33] | (1.33) |
| Black-faced Cuckoo-shrike *Coracina novaehollandiae* | * | | | (0.17) |
| White-winged Triller *Lalage sueurii* | | | | (0.83) |
| Scarlet Robin *Petroica multicolor* | [0.33] * | 0.17 | (0.34) [0.33] * | [1] * |
| Western Yellow Robin *Eopsaltria griseogularis* | (0.84) 0.83 | (0.34) [0.17] 0.5 | * | |
| White-breasted Robin *Eopsaltria georgiana* | [0.83] | [0.5] | [0.5] | [0.33] |
| Golden Whistler *Pachycephala pectoralis* | (0.17) 0.33 | [1.5] * | | 0.33 |

## Appendix 1 (Cont.).

| Species | Healthy Forest | | Dieback-affected Forest | |
|---|---|---|---|---|
| | Site 1 | Site 2 | Site 1 | Site 2 |
| Rufous Whistler *Pachycephala rufiventris* | 0.17 | [0.17] 0.5 | * | 0.33 |
| Grey Shrike-thrush *Colluricincla harmonica* | (0.5) [0.17] 0.5 | (0.17) [0.17] 0.33 | | |
| Willie Wagtail *Rhipidura leucophrys* | | | * | |
| Grey Fantail *Rhipidura fuliginosa* | (0.84) [0.5] 0.83 | (1) [1.83] 0.33 | [1] 0.33 | [0.5] 0.83 |
| Weebill *Smicrornis brevirostris* | (0.17) | (0.34) | (0.34) [0.17] * | |
| Western Gerygone *Gerygone fusca* | (0.17) [1] 0.33 | (0.17) [0.17] 0.83 | (0.67) [0.33] 1.33 | (0.33) [0.83] 0.17 |
| Inland Thornbill *Acanthiza apicalis* | (0.67) [0.83] 0.17 | (0.34) 0.5 | [0.33] 1 | (0.17) [0.33] 0.83 |
| Western Thornbill *Acanthiza inornata* | (2.17) [1.5] 4 | (0.5) [0.5] 1.83 | (0.67) [0.67] 0.5 | (1) [1.83] 1.67 |
| Yellow-rumped Thornbill *Acanthiza chrysorrhoa* | | | * | |
| White-browed Scrubwren *Sericornis frontalis* | | | | [0.17] |
| Red-winged Fairy-wren *Malurus elegans* | [0.17] 1 | | [0.17] | 0.5 |
| Splendid Fairy-wren *Malurus splendens* | [0.5] | (1.17) * | [0.33] 0.33 | |
| Varied Sitella *Daphoenositta chrysoptera* | * | (1.5) | | (0.83) |
| Rufous Tree-creeper *Climacteris rufa* | (0.67) 0.33 | (0.17) [0.33] 0.33 | | |
| Striated Pardalote *Pardalotus striatus* | (0.84) [1] 0.33 | (2.17) [1.33] 0.67 | (0.5) [0.83] 0.83 | (2.5) [0.5] 0.5 |
| Spotted Pardalote *Pardalotus punctatus* | (0.5) | | | |
| Silvereye *Zosterops lateralis* | [0.17] | * | | (1.17) |
| Brown Honeyeater *Lichmera indistincta* | | [0.17] | | |
| White-naped Honeyeater *Melithreptus lunatus* | (0.5) [0.17] 0.67 | (1.17) [1.17] | | |
| New Holland Honeyeater *Phylidonyris novaehollandiae* | | [0.67] | | |
| Western Spinebill *Acanthorhynchus superciliosus* | (0.5) 1.33 | (0.84) [1.5] 0.67 | | (0.5) [0.17] 0.83 |
| Little Wattlebird *Anthochaera carunculata* | 0.33 | * | | (0.17) |
| Dusky Woodswallow *Artamus cyanopterus* | | | (0.67) | |
| Australian Magpie *Cracticus tibicen* | [0.67] * | | [0.5] 0.5 | * |
| Grey Currawong *Strepera versicolor* | * | * | | |
| Australian Raven *Corvus coronoides* | * | 0.33 | * | |

## Appendix 2. Numbers of reptiles per hectare in healthy and dieback-affected jarrah forest

* Survey 1=1981, 2=1987 and 3=1993.

\+ Species observed but not trapped. Results are from Nichols & Reynolds (in prep.).

| Species | Healthy Forest | | | | | | Dieback-affected Forest | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Site 1 | | | Site 2 | | | Site 1 | | | Site 2 | | |
| *Survey | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 | 1 | 2 | 3 |
| **Gekkonidae** | | | | | | | | | | | | |
| *Diplodactylus polyophthalmus* | 8 | | | 16 | | | | | | | | |
| *Phyllodactylus marmoratus* | + | | | | | | | | | | | |
| **Agamidae** | | | | | | | | | | | | |
| *Pogona minor (minor)* | + | | | | | | 64 | | | | | 8 |
| **Varanidae** | | | | | | | | | | | | |
| *Varanus gouldii* | + | + | | + | + | | + | | | + | | |
| **Scincidae** | | | | | | | | | | | | |
| *Bassiana trilineata* | + | 24 | 8 | | 16 | 48 | | 32 | | | 8 | 8 |
| *Cryptoblepharus plagiocephalus* | + | 16 | 16 | | 32 | 16 | 32 | 32 | | 40 | 16 | 16 |
| *Ctenotus delli* | + | | 8 | | | | | | | | | |
| *Ctenotus labillardieri* | 16 | 8 | 8 | 32 | 32 | 48 | | | | | | |
| *Egernia napoleonis* | 32 | | + | | 16 | 32 | + | 16 | | | 8 | |
| *Hemiergis initialis* | + | 8 | 24 | 32 | | 16 | | | 16 | | 8 | + |
| *Lerista distinguenda* | 112 | 48 | 24 | 480 | 32 | 48 | 32 | | 208 | 64 | | 104 |
| *Menetia greyii* | 24 | 24 | 56 | 256 | 16 | 16 | 48 | 16 | 16 | 32 | 32 | 8 |
| *Morethia obscura* | 352 | 176 | 48 | 224 | 32 | 80 | 64 | 64 | 112 | 16 | | 32 |
| *Tiliqua rugosa* | 32 | | + | + | 16 | | 16 | | | | | + |
| **Elapidae** | | | | | | | | | | | | |
| *Notechis scutatus* | + | | | | | | | | | | | |
| *Pseudonaja affinis* | + | | + | | | | | + | | | | |
| *Rhinoplocephalus nigriceps* | | | | + | | | | | | | | |
| No. of species recorded | 16 | 8 | 11 | 9 | 9 | 8 | 8 | 6 | 5 | 5 | 5 | 8 |
| No. of species trapped | 7 | 7 | 8 | 6 | 8 | 8 | 6 | 5 | 4 | 4 | 5 | 6 |
| No. of individuals trapped | 576 | 304 | 192 | 1040 | 192 | 304 | 256 | 160 | 352 | 152 | 72 | 176 |

# SCOPE ITEM 8

# IDENTIFICATION AND EVALUATION OF THREATS POSED BY *PHYTOPHTHORA* TO THE NATIVE BIOTA OF SOUTH-WEST WESTERN AUSTRALIA

***Stephen A. Carstairs and Larissa E. Carstairs***

## 1 INTRODUCTION

*Phytophthora* occupies an interesting position in the Oomycetes, as it forms a natural link between the saprobic watermoulds (Saprolegniales) at one extreme, and the obligate, parasitic downy mildews (Peronosporaceae) at the other. Anton de Bary (1876) combined the Greek *phyto* (plant) and *phthora* (destruction) to synthesise the generic name *Phytophthora* which literally means plant destroyer. Although all species of *Phytophthora* are saprophytic to some degree, 90% of the crown diseases of woody plants may be attributable to members of the genus (Tsao, 1990).

*Phytophthora cinnamomi* and its interactions with Western Australian native plant communities have been the subject of extensive research for around thirty years. A considerable body of knowledge now exists on the activity of this pathogen, particularly in causing epidemic dieback of *Eucalyptus marginata* and many other components of the native jarrah forest community. Some measure of the importance of *P. cinnamomi* is reflected by the fact that it is one of only five key threatening processes identified in the Endangered Species Protection Act of 1992 (Commonwealth). Much less is known about the threats that other species of *Phytophthora* pose to the native biota of the South West Land Division (SWLD) of Western Australia and it is that with which the current study is concerned.

### 1.1 IDENTIFICATION OF *PHYTOPHTHORA* TAXA THAT POSE THREATS TO NATIVE FLORA

During the early part of the nineteenth century, citrus trees and many other exotic plants were shipped around the world in the absence of quarantine restrictions. Not surprisingly, at least some of the *Phytophthora* spp. (including *P. cinnamomi*) known to infect citrus (Erwin & Ribeiro, 1996) were carried on the roots of imported trees to parts of the SWLD. Fraser (1956) first reported *P. cinnamomi* on Australian native vegetation in New South Wales. Podger *et al.* (1965) subsequently isolated and identified the pathogen from dieback-affected jarrah forest in Western Australia.

In order to evaluate the extent to which taxa of *Phytophthora* pose a threat to native plant communities, it is necessary to consider certain aspects of the host-pathogen interaction. Significant features of the ancient, dry sclerophyll flora of the SWLD are its richness in woody perennials and a high degree of endemicity. These characteristics are common to most species of the Myrtaceae, Proteaceae, Papilionaceae, Dilleniaceae and Epacridaceae, which represent major segments of heath, woodlands and forests of the SWLD. *P. cinnamomi* is a non-host-specific pathogen of woody perennials (Zentmyer, 1980) and some other *Phytophthora* spp. (see below) are similar in that regard. Secondly, the highly endemic nature of the native flora and the probable absence of most if not all species of *Phytophthora* until recent times, means that the putative hosts and pathogens would not have evolved together. Thus, insufficient time may have elapsed for many native plant species to adapt and develop resistance to *Phytophthora* spp.

Of 62 taxa that are presently recognised in *Phytophthora* (Erwin & Ribeiro, 1996), pathogens fall into two broadly defined categories:

- specifically (vertically) pathogenic: fungi that parasitise only one host species, or are limited to a narrow range of hosts usually included within a single genus; and
- generally (horizontally) pathogenic: fungi that attack a diverse range of hosts, usually including members of more than one family.

Our intention was to identify those taxa of *Phytophthora* that (like *P. cinnamomi*) are generally pathogenic to woody plants and as such, would be expected to pose a threat to the flora of the SWLD. We also consider the possibility that currently undescribed taxa in that category may be discovered overseas or in natural Western Australian ecosystems.

## 1.2 DETECTION OF *PHYTOPHTHORA* TAXA THAT ARE GENERALLY PATHOGENIC ON WOODY PLANTS

A number of factors may impinge upon the pathologist's ability to recover *Phytophthora* from natural ecosystems. These include:

- the sampling intensity employed to achieve some predetermined confidence level of detection;
- the effectiveness of substrate(s) selected for use as baits;
- seasonal and site effects on recovery; and
- incubation of host plant tissue on agar, as opposed to the use of substrate baits for detection of the fungus in samples containing soil and plant material.

In dieback-affected sites, inoculum levels of *P. cinnamomi* do not remain constant but vary from season to season (Shea *et al.*, 1980; Shearer & Shea, 1987). Differences between floristic composition in different areas may also influence inoculum levels (Shea *et al.*, 1978; Murray *et al.*, 1985).

Carstairs & Stukely (1996) examined the relationship between sampling intensity and the number of samples shown to be positive for *Phytophthora* at a particular site. They found a positive though weak relationship ($r^2 = 0.36$) for 28 infested sites. It was also found that if six samples/site were assayed and if all proved negative for *Phytophthora*, a site could then be deemed free of the fungus within confidence limits of 95%.

While detection and recovery of *Phytophthora* from recently infected plant tissue is simple, isolation from material in advanced stages of decay is difficult or impossible unless special techniques are used. In the past 25 years, development of baiting techniques and selective media for isolation of *Phytophthora* spp. from old, diseased tissues and soils, have made these pathogens among the most readily detectable (Tsao, 1990).

Since the mid 1980's, the Department of Conservation and Land Management (CALM) have managed a root-rot disease thought to be caused by an undescribed species of *Phytophthora* in the Fitzgerald River National Park (FRNP). This species, which appears to have some affinity with *P. megasperma*, is subsequently referred to as *P.* aff *megasperma* Black Point. Detection of the pathogen was difficult when techniques designed for isolation of *P. cinnamomi* were used and two factors that emerged as critical were sample collection time (season) and sample type (M. Grant, J. Webster, and R. Hart pers. comm.).

## 1.3 IDENTIFICATION OF PATHOGENIC *PHYTOPHTHORA* TAXA AND QUANTIFICATION OF THE DAMAGE THEY CAUSE

It has been known for about 30 years that species of *Phytophthora* other than *P. cinnamomi* are present in natural ecosystems in the SWLD. In the mid 1980's, Shearer *et al.* (1987; 1988) compared the pathogenicity of a range of *Phytophthora* spp. and found that some grew in jarrah as aggressively as *P. cinnamomi*. However, in *Banksia grandis*, *P. cinnamomi* was by far the most aggressive pathogen and it girdled plant stems before any host resistance could be expressed. In contrast, other species of *Phytophthora* were confined to lesions by active host resistance (Shearer *et al.*, 1988).

Since then, results obtained with analytic molecular techniques including RFLP and isoenzyme analysis, have demonstrated that certain *Phytophthora* spp., eg. *P. citricola*, *P. cryptogea*, *P. drechsleri*, and *P. megasperma*, are artificial species comprising a number of discrete but morphologically indistinguishable biological taxa (Oudemans & Coffey, 1991; Oudemans *et al.*, 1994). This suggests that some of the findings of early pathogenicity studies should be viewed with caution. Recently, pathogenicity tests have been conducted with allozyme taxa (taxa distinguished by isoenzyme analysis) on *B. grandis* in the southern karri forest (Podger & Carstairs, unpubl.). Preliminary results of that work are included in Section 4.3.

Plant diseases may be described in terms of epidemic progress curves and epidemics can be divided into those that fit simple interest (SI) type curves, or those that fit compound continuous interest (CCI) curves (van der Plank, 1963). The progress curve of avocado root-rot disease caused by *P. cinnamomi* has been described as a CCI type of epidemic (MacKenzie *et al.*, 1983).

# 2 OBJECTIVES

The primary objective of this work is to address Scope Item No. 8 for the *Phytophthora* and *Diplodina* canker project (1997/98). This entails evaluation of the significance of *P. megasperma* as a threat to the native biota, and investigation of the identity and importance of other *Phytophthora* spp. found in native vegetation.

Attainment of the stated objective involved work in the areas noted below:

1. Identification of *Phytophthora* taxa that are generally pathogenic to woody plants and thus represent a threat to native flora of the SWLD.

2. Evaluation of the possibilities; (a) that any new, generally pathogenic species of *Phytophthora* (on woody plants) will be discovered in natural ecosystems in the SWLD or elsewhere, and (b) that taxa of *Phytophthora* reported from Australia are representative of the genus, worldwide.

3. Assessment of methods for the detection and isolation of *Phytophthora* taxa noted in 1 (above).

4. Evaluation of the relative abilities of different taxa of *Phytophthora* to cause disease and/or mortality in native vegetation of the SWLD.

5. Preparation of a host list for *P.* aff. *megasperma* Black Point and description of an epidemic progress curve for this pathogen in natural ecosystems.

# 3 METHODS

## 3.1 IDENTIFICATION OF *PHYTOPHTHORA* TAXA THAT POSE THREATS TO NATIVE FLORA

Sixty-two taxa of *Phytophthora* (Erwin & Ribeiro, 1996) were assessed to determine their pathogenic characteristics and each was assigned to one of the following classes:

- host not determined;
- specifically pathogenic on herbaceous plants;
- generally pathogenic on herbaceous plants;
- specifically pathogenic on woody plants; or
- generally pathogenic on woody plants.

Taxa were deemed to be generally pathogenic to woody plants if they had been reported as pathogenic on three or more families of vascular plants and if the majority of their hosts were woody species.

## 3.2 DETECTION OF *PHYTOPHTHORA* TAXA THAT ARE GENERALLY PATHOGENIC ON WOODY PLANTS

Information on baiting techniques for the isolation of 15 *Phytophthora* taxa (general pathogens of woody plants) was selected from the literature (Erwin & Ribeiro, 1996) and updated with data reported for Western Australia

In the FRNP, taxa of *Phytophthora* were detected in March and August, 1997, by baiting roadside pond water with 2-6 nylon covered pears. In March, 11 ponded sites and the Gairdner River were baited for 2-4 days with 24 pears (2 pears/site). In August, 8 ponded sites were baited with 2-6 pears.

After retrieval, the nylon covers were removed and the pears were maintained in the laboratory in plastic dishes for 2-5 days to allow development of lesions. Portions of lesions were excised and incubated for a period on selective agar in Petri dishes. Mycelium of *Phytophthora* that grew from the lesions was subcultured to corn meal agar (CMA) and checked for purity after two days. The isoenzyme characteristics of pure cultures were determined using methods described by Carstairs & Stukely (1996). Up to three isolates of each allozyme class were examined microscopically to establish the morphology of their sporangia and oospores. A Contingency Chi Square test was used to compare the relative abundance of different *Phytophthora* taxa isolated from pond water in autumn and spring.

Ninety-three samples of soil and root tissue, from sites infested with *P. cinnamomi*, were assessed for the presence of the pathogen by direct plating and baiting methods. The direct method involved incubation of root material on selective agar, and subsequent isolation and identification of fungi growing from the roots. The second technique entailed incubation of *Eucalyptus sieberi* cotyledon baits in soil (850g) from each sample, followed by transfer of lesioned baits to selective agar and identification of fungi growing from the baits. These procedures were also used to assay 85 samples from sites infested with *P.* aff. *megasperma* Black Point. Contingency Chi Square tests were applied to compare *Phytophthora* recovery rates obtained with different methods.

## 3.3 IDENTIFICATION OF PATHOGENIC *PHYTOPHTHORA* TAXA AND QUANTIFICATION OF THE DAMAGE THEY CAUSE

Some heath vegetation at the side of Point Anne Road in the FRNP is affected by aerial cankers and by root-rot caused by *Phytophthora*. In order to minimise the requirement for artificial inoculation experiments, and to provide circumstantial evidence as to the identity of the causal organism(s), we decided to investigate whether any one species of *Phytophthora* was consistently present in the tissues of diseased plants. To further assist this objective, we determined whether isolates recovered from diseased plants at the roadside were conspecific with those isolated from soil or water in which the hosts were growing.

For 2-4 days, ponded water at the edge of Point Anne Road was baited for *Phytophthora* with eight nylon-covered pears. The pears were returned to the

laboratory, processed in the manner indicated already and isolations of *Phytophthora* were identified (Section 3.2).

Eleven Proteaceous plants with symptoms characteristic of root rot were excavated together with 2kg of soil adjacent to the roots. In the laboratory, plants and soil were separated for each sample. Under aseptic conditions, 12 pieces of root and shoot from each plant were placed on selective, antibiotic agar in Petri dishes and incubated for five days. Fungi growing from the plant material were subcultured to fresh CMA. The allozyme class identity of each culture of *Phytophthora* was determined using the isoenzyme technique described by Carstairs & Stukely (1996).

The soil collected with each plant was divided into two portions and placed in plastic dishes. Distilled water was added to cover the soil to a depth of 20-30mm. A pear fruit was then placed in each dish of soil and these were left on the laboratory bench for 5-7 days. Taxa of *Phytophthora* were isolated from the pears and identified as before. The Fisher-Irwin Exact Test was used to determine whether numbers of *Phytophthora* taxa recovered from ponded water, soil and plant tissues were the same.

# 4 RESULTS AND DISCUSSION

## 4.1 IDENTIFICATION OF *PHYTOPHTHORA* TAXA THAT POSE THREATS TO NATIVE FLORA

Of the 62 *Phytophthora* taxa reported worldwide, 31 are pathogens of herbaceous plants and 28 parasitise woody hosts. The latter include roughly equal numbers of specific and general (non-specific) pathogens (Table 1). It is notable that none of the specific pathogens of woody plants have been reported from Australia. In contrast, all of the general pathogens of woody species are present in this country (Tables 1and 2).

**Table 1. Numbers of *Phytophthora* taxa reported worldwide and in Australia, classified in terms of host specialisation**

| Class | Number of Taxa | |
|---|---|---|
| | Worldwide | Australia |
| Host not determined | 3 | 0 |
| Specifically pathogenic on herbaceous hosts | 22 | 5 |
| Generally pathogenic on herbaceous hosts | 9 | 4 |
| Specifically pathogenic on woody hosts | 13 | 0 |
| Generally pathogenic on woody hosts | 15 | 15 |
| Totals | 62 | 24 |

**Table 2. Occurrence of species of *Phytophthora* in Western Australia and elsewhere in Australia**

| Species | Western Australian Ecosystems | | Eastern States and Territories |
|---|---|---|---|
| | Natural | Other[1] | |
| **General pathogens on woody hosts** | | | |
| Non-caducous | | | |
| *P. cambivora* | NR[2] | + | + |
| *P. cinnamomi* | + | + | + |
| *P. citricola* | + | + | + |
| *P. citrophthora* | NR | + | + |
| *P. cryptogea* | + | + | + |
| *P. drechsleri* | + | + | + |
| *P. gonapodyides*[3] | + | NR | NR |
| *P. megasperma* | + | + | + |
| *P. nicotianae* | + | + | + |
| *P. syringae*[4] | NR | NR | + |
| Caducous | | | |
| *P. boehmeriae* | + | NR | + |
| *P. cactorum* | NR | + | + |
| *P. heveae* | NR | NR | + |
| *P. hibernalis* | NR | + | NR |
| *P. palmivora* | NR | NR | + |
| Caducity unknown | | | |
| *Phytophthora* spp. | + | + | + |
| **Specialist pathogens on herb. hosts** | | | |
| *P. clandestina* | NR | + | + |
| *P. macrochlamydospora* | NR | NR | + |
| *P. medicaginis* | NR | NR | + |
| *P. sojae* | NR | NR | + |
| *P. vignae* | NR | NR | + |
| **General pathogens on herb. hosts** | | | |
| *P. erythroseptica* var. *erythroseptica* | NR | + | + |
| *P. fragariae* var. *fragariae* | NR | NR | + |
| *P. infestans* | NR | + | + |
| *P. porri* | NR | NR | + |

[1] Other ecosystems: horticultural and pasture.

[2] NR = Not reported.

[3] *P. gonapodyides* has been recovered from karri forest soil from the temperate SWLD of WA.

[4] Although not reported for Western Australia, *P. syringae* has been recorded from apple in South Australia and it has been reported from New Zealand.

When Contingency Chi Square analysis was used to compare the number of taxa worldwide (for each host class) with that in Australia, the two samples were found to differ significantly from one another ($\chi^2_{obs.}=14.68>\chi^2_{0.05}=7.81$; d.f.=3). Although there has been considerable speculation about the geographic origin of particular species of *Phytophthora*, the literature contains little in regard to the possible origin of the genus. Our conclusion is that *Phytophthora* originated in the Indo-China region where it evolved with the Fabaceae before migrating with members of that family into the Americas. Other members of the genus migrated westward into the Mediterranean basin and some of these specialised to become the Peronosporaceae and Albuginaceae. It follows that Australia would have few endemic species of *Phytophthora*. This accords with the observation that the assemblage of *Phytophthora* taxa in Australia is not typical of the genus worldwide. The disproportionate occurrence and success in Australia of *Phytophthora* taxa that are general pathogens of woody hosts is not unexpected in view of the preponderance of woody species in the native flora.

The 15 recognised general pathogens of woody hosts all occur in Australia (Table 1) and eight of these have been recovered from natural ecosystems in Western Australia. The latter comprise seven of ten non-caducous species and one of five caducous species (Table 2). Application of the Fisher-Irwin Exact test to the data indicated that observed recovery rates in Western Australia were unlikely ($p = 0.01$). The three non-caducous species yet to be reported from natural ecosystems in this State are *P. cambivora*, *P. citrophthora* and *P. syringae*.

An explanation for the paucity of caducous, general pathogens of woody hosts in natural Western Australian ecosystems may reside in the mode of dispersal of the asexual phase of these fungi which requires environmental conditions unlike those prevalent in the SWLD of the State. We conclude that among the 62 recognised taxa of *Phytophthora,* only those ten that are both generally pathogenic on woody plants and non-caducous pose a threat to native biota in the SWLD.

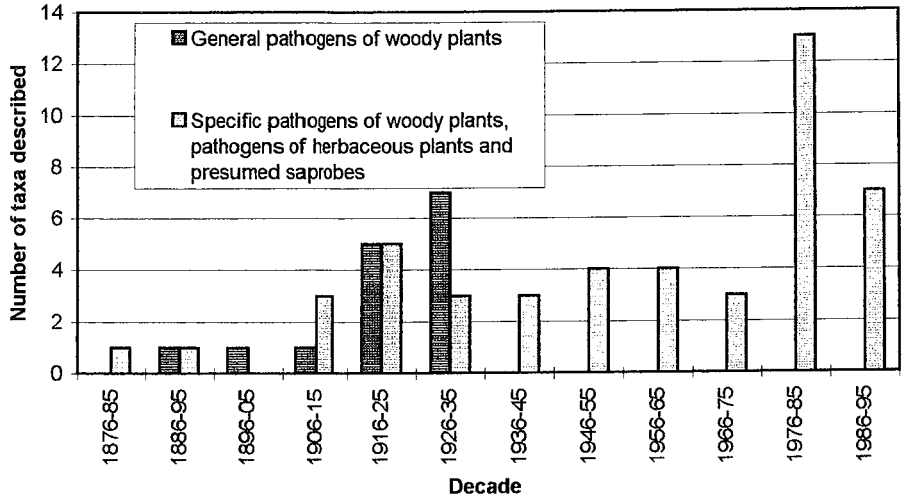
Until recently, Western Australian laboratories have relied on traditional alpha taxonomic methods for the identification of *Phytophthora* spp. based on microscopic examination of morphological characters. However, a molecular technique (CAGE of isoenzymes) has now been developed by CALM (in collaboration with the Minerals and Energy Research Institute of Western Australia) for identifying species of *Phytophthora* (Carstairs & Stukely, 1996). This technique has facilitated the assignment of some isolates to species previously unrecorded in Western Australia. These are *Phytophthora boehmeriae* (D'Souza *et al.*, 1997) and *Phytophthora gonapodyides* (Carstairs & Podger, in prep.).

The isozyme technique has also been used successfully to identify the presence of discrete taxa within the *P. megasperma* species complex, the *P. cryptogea/drechsleri* complex and the *P. citricola* complex (Carstairs & Stukely, 1996). With the continued use of molecular techniques for characterisation of isolates of *Phytophthora*, it seems likely that other currently unrecognised taxa will be identified from natural ecosystems in the SWLD.

Most species of *Phytophthora* that are generally pathogenic on woody host plants were described between 1916 and 1935 (Figure 1). No new taxa in this category have

been described since then and it appears unlikely that many non-host-specific pathogens like *P. cinnamomi*, *P. nicotianae* or *P. palmivora* remain undiscovered. As to the other categories of *Phytophthora*, an average of 3-4 new taxa are described per decade, but it is unlikely that these will pose a threat to the biota of the SWLD.

**Figure 1. Numbers of descriptions of new *Phytophthora* taxa published since 1876**




## 4.2 DETECTION OF *PHYTOPHTHORA* TAXA THAT ARE GENERALLY PATHOGENIC ON WOODY PLANTS

Apple has been reported as a satisfactory bait for detecting 11 of the 15 generally pathogenic *Phytophthora* spp. of woody plants. Other satisfactory baits include lupine radicles (10 species), *Eucalyptus* cotyledons and pear fruit (8 species), and cedar or pine needles (7 species) (Appendix 1). Between 1965 and the late 1970's, the lupine baiting technique (Chee & Newhook, 1965), or modifications of that, were most commonly used for isolation of *P. cinnamomi*. The results of quantitative tests in which the relative effectiveness of lupine radicle and eucalypt cotyledon baits were compared, showed that the latter were most sensitive for detection of *P. cinnamomi* (Marks & Kassaby, 1974). Although these are now used frequently in Western Australia, consideration of the methods summarised in Appendix 1 suggests that the best approach is to employ a combination of baits including pear fruit with eucalypt cotyledons or conifer needles (cedar or pine).

In the current study, pear baits were used successfully (in March, 1997) to recover 91 isolates of *Phytophthora* from 9 of 12 sample sites including the Gairdner River and 11 roadside ponds in the FRNP. In August, a further 160 isolates were recovered from 7 out of 8 roadside ponds in the same area. The 251 isolates were identified by

isoenzyme analysis as belonging to one or other of 10 allozyme taxa. Four of the taxa were isolated from samples collected on both dates (Table 3) and 3 of these were particularly abundant and widespread. Taxon 2(b).3 was recovered from 6 of the 13 sites that yielded *Phytophthora* and it comprised 48% of all isolations. Corresponding values for taxa 2(a).2 and 2(b).2 were 13% (5 sites) and 10% (4 sites), respectively.

When a Contingency Chi Square test was used to compare the proportional representation of the three abundant taxa in March with the equivalent values for August, the seasonal differences were found to be significant ($\chi^2_{obs.}=44.7>\chi^2_{0.05}=5.99$; 2 d.f.). Taxon 2(b).2 showed least response to season with its representation decreasing from 16% in autumn to 12% in early spring. Taxon 2(b).3 increased from 39% to 85% in the same period while 2(a).2 showed the greatest response. It was the most abundant taxon in autumn with 45% representation, but this declined to only 3% in spring.

**Table 3. Allozyme taxon affinity and sporangium morphology of stated numbers of isolates of *Phytophthora* recovered from roadside pond sites in the Fitzgerald River National Park on two dates in 1997 (numbers of sites positive for each taxon are shown in parentheses)**

| Allozyme Taxon[1] | Morphology | Numbers of Isolates (+'ve sites) March | | August | |
|---|---|---|---|---|---|
| 1(a).1 | Semi-papillate sporangia | 3 | (1) | 0 | (0) |
| 2(a).1 | Non-papillate sporangia | 0 | (0) | 18 | (2) |
| 2(a).2 | " | 30 | (4) | 3 | (2) |
| 2(a).3 | " | 12 | (1) | 0 | (0) |
| 2(b).1 | " | 0 | (0) | 26 | (3) |
| 2(b).2 | " | 11 | (3) | 13 | (1) |
| 2(b).3 | " | 26 | (2) | 94 | (5) |
| 2(b).4 | " | 4 | (1) | 0 | (0) |
| 2(b).5 | " | 5 | (2) | 2 | (2) |
| *Phytophthora* spp. | Not determined | 0 | (0) | 4 | (1) |

[1] Taxa including (a) or (b) in their appellations are homothallic or heterothallic, respectively.

Although chance may play an important role in recovery of taxa that are not widespread or abundant, season appears to influence the likelihood of detecting some taxa of *Phytophthora* in roadside ponds. Of the ten allozyme taxa identified here, taxon 2(a).1 (*P.* aff. *megasperma* Black Point) is most often associated with root-rot diseases of Proteaceae in the FRNP or on the sand plains north of Perth. It was only recovered in spring from two of the 13 (15.4%). roadside ponds positive for *Phytophthora* in the

FRNP. Based on a proportional representation of 15.4% of all isolates in sites positive for *Phytophthora*, the calculated number of sites requiring assessment for 80% confidence in recovering *P.* aff. *megasperma* Black Point would be 9.63. In autumn, nine sites that were positive for *Phytophthora* were assessed and yet we did not recover *P.* aff. *megasperma* Black Point. Taxon 2(b).1 is another *Phytophthora* that was not recovered in autumn but was relatively common in spring (Table 3).

Of 71 samples from which *P. cinnamomi* was isolated, either directly from root tissue or indirectly from baits that had been incubated in the soil or from both, only three samples were deemed to be negative using baits, while root tissue in 18 samples failed to yield the fungus (Table 4.). The results of Chi Square analysis indicated that the observed difference in isolation rates between baits and roots was significant ($\chi^2_{obs.}=10.71>\chi^2_{0.05}=3.84$; 1 d.f.).

**Table 4. Isolation outcomes (+ / -) for *P. cinnamomi* and *P.* aff. *megasperma* Black Point (BP) from substrate baits incubated in soil samples, and/or directly from root tissues that were present in the same soil samples**

| Baits and Roots | *P. cinnamomi* | *P.* aff. *megasperma* BP |
|---|---|---|
| Both negative (-) | 22 | 25 |
| Bait - : Root + | 3 | 48 |
| Bait + : Root - | 18 | 3 |
| Both positive (+) | 50 | 10 |
| Total No. of soil samples | 93 | 86 |

A contrasting result was obtained for *P.* aff. *megasperma* Black Point. Out of 61 soil samples in which the pathogen was found to be present, the baiting method produced a negative result on 48 occasions, while only three failures to isolate the fungus from root tissue were recorded (Table 4). Chi Square analysis revealed that the failure rate for baits was significantly greater than that for root tissue ($\chi^2_{obs.}=43.32>\chi^2_{0.05}=3.84$; 1 d.f.).

The results of this work suggest that *P. cinnamomi* is the superior saprophyte and that it may persist well in old, diseased tissues or soils from which it is readily isolated by baiting. On the other hand, *P.* aff. *megasperma* Black Point appears to be less able in that regard. It is clear that detection methods that are suitable for one species of *Phytophthora* might be not be ideal for another.

## 4.3 IDENTIFICATION OF PATHOGENIC *PHYTOPHTHORA* TAXA AND QUANTIFICATION OF THE DAMAGE THEY CAUSE

Six allozyme taxa of *Phytophthora* were recovered from the Point Anne Road site. Five taxa were isolated from ponded water and two of these were unique to the water column. Taxon 2(a).1 (*P.* aff. *megasperma* Black Point) was recovered from diseased plants and from the soil profile but not from the water column. Both the water column and soil profile were relatively rich in *Phytophthora* species by comparison with plant tissues (Table 5).

Application of the Fisher-Irwin Exact Test showed that (out of 6 possibilities), recovery of 5 taxa in the water column and 1 not detected, with 4 taxa in the soil profile and 2 undetected, was very likely on the basis of chance ($p = 0.41$). However, the same test indicated that chance recovery of 5 taxa from the water column (1 not detected) and only 1 in plant tissue was unlikely ($p = 0.04$). Similarly, chance detection of 4 taxa in soil and one in plant tissues was improbable ($p = 0.07$).

Thus, the finding that *P.* aff. *megasperma* Black Point (taxon 2(a).1) was the only taxon of *Phytophthora* to be recovered from diseased plants (Table 5) is not explicable on the basis of chance alone. This is consistent with the suggestion that, of the six taxa assessed, only *P.* aff. *megasperma* Black Point has a pathogenic role on the hosts examined here.

**Table 5. Numbers of isolates of six allozyme taxa of *Phytophthora* recovered from ponded water, soil or plant samples collected in Point Anne Road**

| Taxon | Water Column | Soil Profile | Plant Tissue | Totals |
|---|---|---|---|---|
| 2(a).1 | 0 | 18 | 7 | 25 |
| 2(a).2 | 2 | 14 | 0 | 16 |
| 2(b).1 | 12 | 12 | 0 | 24 |
| 2(b).2 | 13 | 0 | 0 | 13 |
| 2(b).3 | 72 | 60 | 0 | 132 |
| *Phytophthora* spp.1 | 4 | 0 | 0 | 4 |
| Totals | 103 | 104 | 7 | 214 |

The host list for *P.* aff. *megasperma* Black Point is short by comparison with the host range associated with some general pathogens of woody plants, eg. *P.cinnamomi* or *P. nicotianae*. However, the latter species have been recognised for more than half a century. So far, 22 Western Australian species belonging in eight genera of the Proteaceae have been recorded as hosts of *P.* aff. *megasperma* Black Point. Pinaceae is the only other family that includes known hosts of the pathogen (Table 6).

**Table 6. Plant species from which *P.* aff. *megasperma* Black Point has been isolated in five CALM Districts**

| District | Plant Family | Species | Isolations (No.) |
|---|---|---|---|
| Albany | Proteaceae | *Adenanthos cuneatus* | 2 |
| | | *Banksia attenuata* | 6 |
| | | *Banksia baxteri* | 8 |
| | | *Banksia gardneri* | 1 |
| | | *Banksia lemanniana* | 4 |
| | | *Banksia media* | 3 |
| | | *Conospermum distichum* | 1 |
| | | *Daviesia* sp. | 1 |
| | | *Dryandra circioides* | 1 |
| | | *Dryandra cuneata* | 4 |
| | | *Dryandra falcata* | 3 |
| | | *Dryandra plumosa* | 3 |
| | | *Dryandra quercifolia* | 2 |
| | | *Dryandra tenuifolia* | 2 |
| | | *Hakea varia* | 1 |
| | | *Isopogon formosus* | 1 |
| | | *Lambertia inermis* | 1 |
| Esperance | Proteaceae | *Banksia speciosa* | 1 |
| | | *Dryandra sessilis* | 1 |
| Moora | Proteaceae | *Banksia attenuata* | 9 |
| | | *Banksia ilicifolia* | 1 |
| | | *Hakea prostrata* | 1 |
| | | *Hakea* sp. | 2 |
| Pemberton | Proteaceae | *Banksia occidentalis* | 1 |
| SW Capes | Pinaceae | *Pinus radiata* | 3 |

In Section 3.1, taxa of *Phytophthora* which parasitise species in three or more families of woody plants were classified as generally pathogenic on that category of host. Since *P.* aff. *megasperma* Black Point has been recorded on just two host families, its present status is currently that of a specific pathogen. This makes it unique among other species of *Phytophthora* in Australia as it is the only one included in that class. We believe that as *P.* aff. *megasperma* Black Point receives increasing attention it will be found on a broader spectrum of hosts and eventually it will be recognised as a general pathogen of woody plants.

*P.* aff. *megasperma* Black Point has a regional distribution in the south-west of the State where it is mostly confined to heath and woodlands of the coastal plain. Sites infested with the fungus were assessed in the FRNP and on the sand plain north of Perth to determine whether observed disease was in an early or advanced stage of progression (Table 7). Approximately equal numbers of sites were recorded for each of the two stages. It appeared that plant populations on the different sites had either been recently infected, or the epidemic had run its course. This supports the conclusion that progress of disease associated with infestations of *P.* aff. *megasperma* Black Point fits a CCI epidemic progress curve

**Table 7. Progress of disease associated with infestations of *P.* aff. *megasperma* Black Point at locations in the Fitzgerald River National Park (FRNP) and on the sand plains north of Perth (NSP)**

| Region | Disease Progress | Location | Dominant Plant Species |
|---|---|---|---|
| FRNP | Advanced[1] | Collett Road | *Banksia attenuata* |
| | | North of Quaalup Road | *B. baxteri* |
| | | North of West Mt. Barren | *B. attenuata* |
| | Early[2] | West of Point Anne Road | *B. attenuata, B. baxteri, B. coccinea* |
| | | East of West Mt. Barren | *B. baxteri* |
| | | West of West Mt. Barren | *B. attenuata* |
| NSP | Advanced | Cervantes Road | *B. attenuata* |
| | | Jurien Rd 1 | *B. attenuata* |
| | | | *B. prionotes* |
| | | Moora-Badgingarra Road | *B. attenuata, B.menziesii, B. prionotes* |
| | | Namegarra Road | *B. attenuata* |
| | | Yerrumullah Road | *B. attenuata, B. menziesii* |
| | Early | Bibby Road | *B. attenuata* |
| | | Cataby South | *B. attenuata* |
| | | Jurien Road 2 | *B. attenuata* |
| | | Mimegarra Road | *B. attenuata* |
| | | Munbinea Road | *B. attenuata* |
| | | Yerrumullah Road | *B. attenuata, B. menziesii* |

[1] Advanced: the site has been denuded of dominant plant species or most are dead.
[2] Early: the frequencies of dead or diseased, dominant plant species are low.

Podger & Carstairs (unpubl.) have conducted pathogenicity tests with allozyme taxa of *Phytophthora* and with *P. cinnamomi* on *B. grandis* in the southern karri forest. Preliminary results of this work indicate that development of lesions in *B. grandis* proceeds at a much greater rate in plants inoculated with *P. cinnamomi* than in treatments with other taxa of *Phytophthora.* In one trial, the stems of 18 *Banksia* plants that had been inoculated with *P. cinnamomi* were girdled by lesions and all the trees soon died. Although some plants inoculated with other taxa of *Phytophthora* also died, the mortality rates for those treatments did not differ significantly from the control in which *Banksia* stems were wounded but not inoculated with test fungi.

Trials of this type test the capacity of fungal mycelium to grow in live stem tissues, but not the ability of infective zoospores to invade healthy plants and cause disease. Experimentation comparing the infective abilities of several taxa of *Phytophthora* with that of *P. cinnamomi* (soil inoculation with zoospores) is the subject of another trial by Podger & Carstairs (in progress). Definitive experimentation of this type is extremely labour intensive and likely to require long term maintenance and monitoring of trial plots.

# 5 OUTCOMES

## 5.1 IDENTIFICATION OF *PHYTOPHTHORA* TAXA THAT POSE THREATS TO NATIVE FLORA

- Of the 62 taxa included in *Phytophthora*, those that are generally pathogenic to woody plants and are non-caducous, pose the greatest threat to the sclerophyllous flora of the SWLD. These are the first ten species listed in Table 2 (Section 4.1). All have been recorded both in Australia and overseas. Apart from *P. cambivora*, *P. citrophthora*, and *P. syringae*, the other seven species have been recovered from natural ecosystems in the SWLD. While it is unlikely that many new taxa in this class will be discovered elsewhere, we expect that the other described members of the group will be found in Western Australia.

- Molecular techniques for differentiating between taxa of *Phytophthora* are establishing an excellent track record for accuracy and efficiency in Western Australia and in other parts of Australia. However, many mycologists are reluctant to embrace the new techniques on the grounds that adequate diagnostic tools are already available for the characterisation of fungi based on microscopic examination of morphological features.

## 5.2 DETECTION OF *PHYTOPHTHORA* TAXA THAT ARE GENERALLY PATHOGENIC ON WOODY PLANTS

- The results of this study should provide researchers in Western Australia with a better appreciation of the degree to which species of *Phytophthora* other than *P. cinnamomi* pose a threat to native plants in the SWLD. Historically, there is little

doubt that taxa isolated from infested plant communities have not always been correctly identified and that difficulties in detection of important pathogens are experienced from time to time. A flexible methodology for the detection of potentially important, pathogenic species such as *P.* aff. *megasperma* Black Point is outlined in this report.

## 5.3 IDENTIFICATION OF PATHOGENIC *PHYTOPHTHORA* TAXA AND QUANTIFICATION OF THE DAMAGE THEY CAUSE

- Of six allozyme taxa recovered from Point Anne in the FRNP, only one (*P.* aff. *megasperma* Black Point) was considered likely to be pathogenic. *P.* aff. *megasperma* Black Point has a regional distribution and seems to be confined to heath or woodland in coastal plain ecosystems where it has eroded conservation values in the Shannon-D'Entrecasteaux National Park, the FRNP, Cape Arid National Park, and conservation reserves on the sand plain north of Perth.

- The host list of *P.* aff. *megasperma* Black Point suggests that it is specifically pathogenic on woody plant species. However, its current status in that regard should be viewed with caution until more comprehensive studies of the fungus are undertaken.

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## Appendix 1. Baiting techniques for the detection and isolation of generally pathogenic *Phytophthora* species of woody plants

| Species | Bait Material | Conditions of Baiting and Remarks | References |
|---|---|---|---|
| *P. boehmeriae* | Apple slices, lupine radicles. | Immersed in 200 ml of water added to 25 g of soil; 4-10 days. | Gerretson-Cornell (1976). |
| | *E. sieberi* cotyledons. | Detached cotyledons floated on water 5-10 cm over about 850 g soil. | D'Souza *et al.* (1997). |
| *P. cactorum* | Apple fruit. | Buried partly in wet soil in large flat; 24°C, 90-100% RH, 5-6 days. | Schwinn (1961). |
| | Pear fruit. | Immersed partly in 50 cc of soil plus 11 cc of water; 6-7 days; pear better than apple, peach, and apricot fruit as baits. | McIntosh (1964). |
| | Apple fruit, pear fruit. | Buried partly in wet soil at 20°C until rot occurs; isolation from apple & strawberry soils; pear better than apple as bait. | van der Scheer (1971). |
| | Apple fruit. | Immersed partly in 20 mm soil layer with 5 mm of water above soil; optimum 20°C, 14 days; *P. citricola* and *P. syringae* also recovered from apple soils at lower incubation temperatures. | Sewell *et al.* (1974). |
| | Safflower seedlings. | Planted in steamed soil before adding 15 mm layer of test soil in contact with hypocotyl or directly to test soil; optimum 28°C, 3-4 wks; better than apple fruit; used also in quantitative assays of apple soils. | Banihashemi & Mitchell (1975). |
| | Strawberry fruit. | Immature fruit inoculated with soil in small hole; 22°C, 3-6 days; used for isolation from strawberry soils; also susceptible to 5 other *Phytophthora* species. | Molot & Beyries (1976). |
| | Apple seedlings, cotyledons. | Soil suspensions were diluted in up to 72-fold dilutions; seedlings and cotyledons incubated in 15-20 ml of suspension in glass vials; 14 days at 20°C in 16-hour photoperiod. | Harris & Bielenin (1986). |
| | Apple seedlings, cotyledons | Extended baiting (soil air dried, moistened for 3 days, and then flooded). | Jeffers & Aldwinckle (1987). |
| *P. cambivora* | | See Dance *et al.* (1975) under *P. cinnamomi*.<br>See Mircetich & Matheron (1976) under *P. megasperma*. | |
| *P. cinnamomi* | Apple fruit. | Holes made in fruit are filled with soil; incubated at 15-27°C, 5-10 days; suitable also for many other but not all *Phytophthora* species; however, *Pythium* and other soil fungi also colonise fruit. | W.A. Campbell (1949). |
| | Pineapple leaves & rooted crowns. | Immersed in 400-800 ml of water added to about 10-20 cc of soil; 21-24°C, 4 days. | Anderson (1951). |
| | Avocado fruit, *Persea indica* seedlings. | Fruit partly embedded in flooded soil; 27°C, 2-4 days; seedlings planted in wet soil, 2-3 days. | Zentmyer *et al.* (1960); Zentmyer (1980). |

**Appendix 1.** (Continued)

| Species | Bait Material | Conditions of Baiting and Remarks | References |
|---|---|---|---|
| *P. cinnamomi* | Pineapple leaves & rooted crowns. | Leaf base or root tips immersed in water-soil mixture at least 5 cm above the container bottom; 18-27°C. | Klemmer & Nakano (1962). |
| | Lupine radicles, excised. | Root tips immersed in large volume of water over a 20 mm soil layer; 2 days; isolation from pine soils; better than apple fruit; also susceptible to four other *Phytophthora* species. | Chee & Newhook (1965). |
| | Lupine radicles, excised. | Pieces 1-2 cm long floated on water 2-3 mm over 30 cc of soil in petri plate; 2-3 days; used for quantitative assays of eucalyptus soils. | Marks *et al.* (1972). |
| | Lupine radicles. | Immersed in 150 ml of water added to 26 g of soil; 17-24°C; 5 days; lesions distinguishable from those caused by eight other *Phytophthora* species except *P. drechsleri*. | Pratt & Heather (1972). |
| | Apple slices, lupine radicles. | Immersed in 200 cc of water added to 25 g of soil; 4-10 days; both baits were satisfactory. | Gerrettson-Cornell (1974). |
| | Eucalyptus cotyledons. | Detached cotyledons floated on water 5-10 mm over 50-60 cc of soil; 22-24°C, within 60 h; two to four times more sensitive than lupine radicles; used for quantitative assays of eucalyptus soils. | Marks & Kassaby (1974). |
| | Cedar needles, pine needles, lupine radicles. | Floated on or immersed partly in water added to soil; both 16 and 22°C, 3 days; conifer needles also good for *P. cambivora*, *P. citricola*, *P. cryptogea*, *P. drechsleri*, *P. hibernalis*, and a few other *Phytophthora* species. | Dance *et al.* (1975). |
| | Eucalyptus leaf disks. | Floated on water added to soil or roots; 20°C, 24 h; *Pythium* species interfere with results. | Linderman & Zeitoun (1977). |
| | Avocado leaf pieces. | Floated on water added to soil; 4 days; recovery up to 89%. | Pegg (1977). |
| | *Eucalyptus* cotyledons, lupine seedlings, pear fruit. | Floated on water added to soil at various depths; 25°C under continuous light, 3-7 days; all baits successful but more frequently with pear; used in quantitative assays. | Greenhalgh (1978). |
| | *Persea indica* seedlings. | Immersed partly in large volume of water added to 1.2-2.5 cm soil layer; 5-7 days; isolation from avocado soils. | Zentmyer & Ohr (1978). |
| | Azalea leaf disks, cedar needles, fir seedlings, lupine radicles. | Floated on or immersed in 100 ml of water added to 5-50 g of soil; 20°C, 1-2 days; recovery was best with azalea leaf disks and least with lupine radicles. | Shew *et al.* (1979). |
| | Pear fruit. | Pear baits were suspended in streams for 4 days; or were immersed in water 2.5 cm over about 1 kg soil for 5 days then lesions were plated onto selective antibiotic agar plates. | Carstairs & Carstairs (Section 2.2 Methods). |
| *P. citricola* | | See Dance *et al.* (1975) under *P. cinnamomi*. | |
| | | See Sewell *et al.* (1974) under *P. cactorum*. | |
| | *E. sieberi* cotyledons | Detached cotyledons floated on water 5-10 cm over about 850 g soil. | Stukely *et al.* (1997). |
| | | See Carstairs & Carstairs (Section 2.2 of Methods) under *P. cinnamomi*. | |

**Appendix 1.** (Continued)

| Species | Bait Material | Conditions of Baiting and Remarks | References |
|---|---|---|---|
| *P. citrophthora* | | See Klotz & DeWolfe (1958) and Tsao (1960)under *P. nicotianae*. | |
| *P. cryptogea* | | See Dance *et al.* (1975); Carstairs & Carstairs (Section 2.2 of Methods) under *P. cinnamomi*.<br>See Stukely *et al.* (1997) under *P. citricola.* | |
| *P. drechsleri* | Cantaloupe seed & seedlings. | Planted in wet soil mixed with diseased tissues of *Cucumis* species; 20-35°C, 7 days.<br>See Stukely *et al.* (1997) under *P. citricola.*<br>See Carstairs & Carstairs (Section 2.2 of Methods) under *P. cinnamomi*. | Alavi and Strange (1979). |
| *P. gonapodyides* | Apple fruit, pear fruit, citrus leaf pieces, *Begonia* petioles, lupine seedlings. | Apples and pear baits were suspended in reservoirs for 9 days; *Begonia* petioles, citrus leaf pieces, lupine seedlings were suspended in water from reservoirs for 1-4 days and plated on $P_{10}$ARP. | Robertson (1975). |
| | Lupine radicles, *E. sieberi* cotyledons. | Detached radicles and cotyledons were floated on water 2-5 cm over *circa* 1 kg of soil for 5 days then plated onto a selective antibiotic agar plate. | Carstairs & Podger (in prep.) |
| *P. heveae* | | See Lee & Varghese (1974) under *P. nicotianae.*<br>See Gerrettson-Cornell (1976) under *P. boehmeriae.* | |
| *P. hibernalis* | | See Dance *et al.* (1975) under *P. cinnamomi*. | |
| *P. megasperma* | Pear fruit. | Partly immersed in water 1 cm deep over 500 cc of soil; 20°C for 3 days; for isolation from cherry soils; also good for *P. cambivora.* | Mircetich & Matheron (1976). |
| | Pine needles, lupine seedlings, apple fruit, pear fruit. | Needles or seedlings placed in water 5 mm deep over 100 g of soil; 16 and 22°C, 3 days; fruit buried in larger amounts of soil; 22°C, 10 days; for isolation from strawberry and raspberry soils.<br>See Stukely *et al.* (1997) under *P. citricola.*<br>See Carstairs & Carstairs (Section 2.2 of Methods) under *P. cinnamomi*. | Hargreaves & Duncan (1978). |
| *P. nicotianae* *(P. parasitica)* | Apple fruit, lemon fruit, orange fruit. | Soil or citrus tissues inserted into apple the same as W.A. Campbell's (1949) method. Lemon or orange placed on surface of saturated soil; 20°C, 4 or more days; also good for *P. citrophthora* and *P. syringae* from citrus soils. | Klotz & DeWolfe (1958). |

**Appendix 1.** (Continued)

| Species | Bait Material | Conditions of Baiting and Remarks | References |
|---|---|---|---|
| *P. nicotianae* | Lemon fruit. | Immersed partly in 150 ml of water added to 25 cc of soil; 25°C, 6 days; also good for *P. citrophthora*; used for quantitative assays of citrus soils. | Tsao (1960). |
| | Tobacco leaves. | Petiole end immersed in water-soil mixture as in Tsao (1960); used for quantitative assays of black shank fungus in tobacco soils. | Jenkins (1962). |
| | Castor bean seed. | Buried in soil; 63 h; for isolation from betel vine soils or irrigation water. | Narasimham & Ramakrishnan (1969). |
| | Carnation petals. | Immature petals from buds floated on soil suspension in petri plate, 24-35°C, 2-4 days; used for quantitative assays of carnation soils; also detects *P. capsici*. | Ponchet *et al.* (1972), Ricci (1972). |
| | Citrus leaf pieces. | Small leaf pieces from various *Citrus* species, floated on water 1-2 cm above 100 cc of soil; 22-28°C, 3-4 days; calamondin fruit equally effective as bait. | Grimm & Alexander (1973). |
| | Apple fruit, eggplant fruit. | Methods not given. Both fruits also good for *P. capsici*. Cocoa pod also allows isolation of *P. palmivora* and *P. heveae*. | Lee & Varghese (1974). |
| | Tomato fruit. | Green fruit placed in flooded soil from tomato fields after premoistening for 1 week. See Stukely *et al.* (1997) under *P. citricola*. | Ioannou & Grogan (1977). |
| *P. palmivora* | Cocoa pods. | Placed on or in soil inoculated with a loopful of soil suspension on the pod surface and incubated in a moist box; 1-4 days; for isolation from cocoa soils. | Orellana (1954). |
| | Apple fruit, black pepper leaves. | Soil inserted into apple the same as in W.A. Campbell's (1949) method. Black pepper leaves immersed partly in water-soil mixture; for isolation (of MF4) from black pepper soils. Black pepper leaf disks used successfully by P.H. Tsao (unpublished). | Holliday & Mowat (1963). |
| | Cocoa pods. | Soil inserted beneath flaps of endocarp tissue of unripe pods; for isolation from cocoa soils. | P.D. Turner (1965). |
| | Cocoa pods. | Soil or diseased *Hevea* tissues inserted into unripe green pods as in W.A. Campbell's (1949) apple method; 26-30°C, 4-5 days; also good for *P. meadii* and *P. parasitica*). | Chee & Foong (1968). |
| | Cocoa pod tissues. | Small blocks of tissue placed in wet soil; 25°C, 4 days; premoistened for 5 days if soil samples were dry. | Okaisabor (1971a,b). |
| | Cocoa pods and tissues | Four methods: pod on soil, soil in pod, pod tissue in flooded soil (the best), and pod tissue on soil; used in quantitative assays of cocoa soils. | Newhook & Jackson (1977). |
| | *Colocasia esculenta* roots about 2.5 cm long. | Roots autoclaved and incubated in moistened soil (50 g/ Petri plate) at 15°C for 1 week. Roots washed and plated on oatmeal agar. | Satyaprasad & Romarao (1980). |
| *P. syringae* | | See Klotz & DeWolfe (1958) under *P. nicotianae*: Sewell *et al.* (1974) under *P. cactorum*. | |