PROJECT 5

IDENTIFICATION, GERMPLASM STORAGE AND *IN VITRO* PROPAGATION OF *PHYTOPHTHORA- AND CANKER-* THREATENED TAXA

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1 INTRODUCTION

Threatening processes, particularly loss of habitat, weed invasion, dieback (*Phytophthora*) and canker disease have, and continue to be, major factors in the local extinction of native plant species in Western Australia. Loss of populations and substantial reduction in population size may not necessarily lead to immediate extinction but usually results in loss of genetic diversity. Where population extinction in the wild cannot be prevented, genebanks or germplasm storage facilities can be utilised as an interim solution to prevent loss of genetic diversity within a species or, as a last resort, to prevent extinction of the species. One of the most cost-effective methods for genebanking in plants is long term storage (i.e., a 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, and low temperature seed storage is more economical than maintaining collections of living plants in botanic gardens. The success of germplasm conservation depends on the longevity of the seed under the storage conditions used and the ability to regenerate adequate quantities of high quality seed without genetic change when the viability declines (Morse *et al*, 1993).

In late 1992, the Threatened Flora Seed Centre (TFSC) was established for this project by the Western Australian Department of Conservation and Land Management (CALM) as the

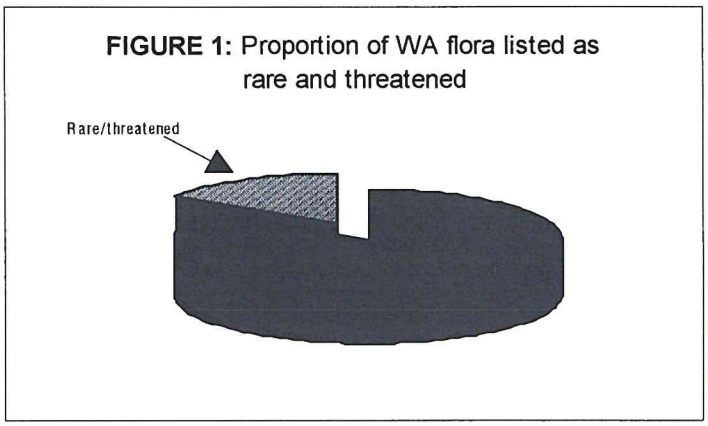
State's principal long term seed storage facility for the conservation of rare and threatened taxa. At the onset, the project encompassed five scope items:

1. Identification of rare and threatened flora at risk from dieback disease
2. *In vitro* propagation
3. Cryostorage
4. Seed collection
5. Seed storage, viability testing and inventory system

Items 2 and 3 were discontinued in the first year (Department of Conservation and Land Management, 1993).

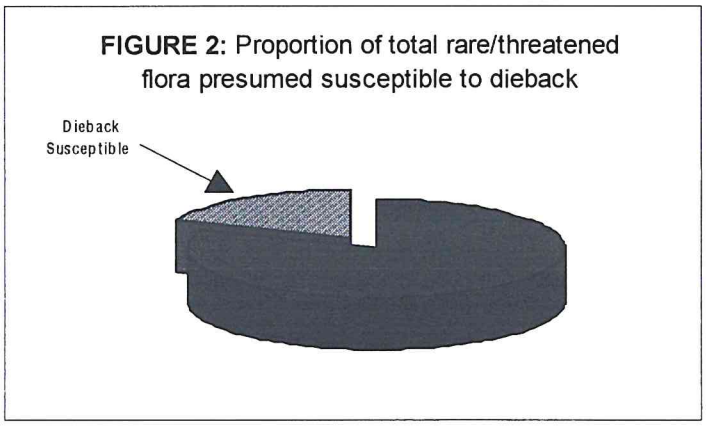
Prior to the establishment of the TFSC, there was no long term seed storage facility in the state that was directed towards the preservation of germplasm of rare and threatened flora. The focus of the Kings Park and Botanic Gardens seed store has been primarily on plants of horticultural potential using bulked collections, usually with a narrow genetic base. CALM's Manjimup Seed Centre is an active seed storage facility involved in forestry silviculture and the supply of seed to nurseries.

Priority listings of taxa under immediate threat of dieback have been derived from laboratory research and field observations by CALM staff and consultants and have been constantly revised over the past five years. At the onset of the project a list of 66 taxa from the Southern Forest and South Coast Regions were targeted for collection. By mid 1993 an updated list of 198 taxa from the South Coast, Wheatbelt, Central Forest, Metropolitan, Northern Forest and Greenough regions was used. This represented about 10% of flora listed on CALM's then current *Declared Rare and Priority Flora List* for Western Australia (Ken Atkins 02/02/94). Taxa were predominantly from the families Proteaceae, Epacridaceae, Fabaceae, and Myrtaceae.



Continued consultation with CALM dieback interpreters and other field or research staff has since provided additional information on the range of threatened species thought to be susceptible to diseases caused by canker pathogens and *Phytophthora*. Considerable intrageneric variation in host response to infection had also become apparent thus seed collections were made on a species by species basis, dependent on site characteristics as well as host susceptibility. Particular areas were targeted, including the species-rich heathlands of the Stirling Range, Fitzgerald River and Mt. Leseur National Parks.

It is now thought that approximately 25% of the declared rare and priority flora of the south-west of the State may be susceptible to dieback disease and many such species face at least localised extinction in the field within the next 10 to 20 years.



Most plant species targeted for collection belong in the Proteaceae, Epacridaceae, Fabaceae, and Myrtaceae. Taxa selected in other families including Dilleniaceae, Casuarinaceae and Xanthorrhoeaceae have been recorded as variously susceptible to dieback infection depending upon site conditions.

Recently, collecting has been concentrated on critically endangered taxa as well as continued acquisition of *Phytophthora*- and canker-dieback susceptible species in the south-west of the State. Consultation with Kings Park and Botanic Gardens has highlighted the gaps in the *ex-situ* conservation of critically endangered taxa and additional effort is being made to secure germplasm of these species in the face of declining populations and possible extinction. CALM's *Declared Rare and Priority Flora List* for Western Australia (Ken Atkins 06/10/96) has been used for the most recent collections.

2 OBJECTIVES

The objective of the TFSC is to ensure the maintenance of genetically representative seed collections of Western Australian threatened flora under long term storage conditions as an interim solution for the prevention of genetic degradation or local extinction of endangered populations. A broad sample of genetic variation is essential if stored material is to be effectively used for long term re-establishment of a species in the wild following removal of any threats. To achieve that goal, the determination of optimum storage conditions is critical.

The objective of seed collection at the TFSC has been to systematically capture 90-95% of the common alleles from each threatened taxon on a representative, range-wide basis. However, many threatened taxa are not well known either taxonomically, geographically or phenologically. Poorly known seed biology and ecology also contribute to make effective systematic sampling difficult. This is often exacerbated by small population size or low and sporadic seed production or high seed predation levels. Moreover, all sampling strategies are subject to bias derived from temporal fluctuations in the genetic contribution of individuals to the genepool due to disturbances such as disease, fire or drought. Therefore, obtaining seed samples that have a wide genetic base (routine for common taxa) is often difficult for rare taxa.

3 METHODS

3.1 SEED COLLECTION

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

3.1.1 Sampling Strategy

For any given species, the number of populations to be sampled depends on the magnitude of genetic variation between individual populations but for most threatened taxa this information is unknown. Sampling protocols should be standardised within limits that allow flexibility and adjustment according to known distribution patterns of target populations and the resources available to make seed collections. As a guideline, all populations should be sampled if their known number is less than five and in all other cases, samples should be limited to a maximum of five populations.

To capture 90-95% of the common alleles the optimum sample size for a population is between 10 and 50 individuals (Brown and Briggs, 1991). Brown and Briggs point out that the capture of greater than 90-95% of the common alleles may become an exponential drain on resources requiring a sample of hundreds of individuals to achieve marginal genetic gains. Optimal sampling is dependent on factors affecting population genetic structure, particularly the breeding system. For the majority of target TFSC taxa, population genetic structure is unknown.

Sampling unrelated, individual parent plants within a given population is prerequisite for all family accessions. This may be achieved by spacing source individuals according to the seed dispersal mechanisms involved, root suckering ability or extent of any lignotubers, and the type and movement patterns of pollination vectors. Each individual sampled from a population should contribute seed in equal quantities to avoid biasing the collection. The

maintenance of family accessions has top priority for the TFSC during all seed collections. This will allow the widest range of end uses including attribution of morphological variation to a specific family, genetic studies to assess breeding systems, and bulking of highly representative sub-samples using viability figures. Field bulked accessions are justified when seeding events produce sparse and erratic seed crops that yield inadequate quantities for the maintenance of family accessions in long term storage. In cases where population size is such that collection from unrelated source plants is impossible, collection of seed is warranted from as widely spaced individual plants as possible.

For any population, 1000 seeds is the minimum number required to adequately represent and conserve a genepool under long term storage conditions (Brown and Briggs, 1991). If a 10 individual accession is collected this amounts to 100 seeds per source plant. Fewer seeds are required from source plants if the number of families sampled increases. Ideally, well in excess of 1000 viable seed per accession should be collected to enable extraction of representative sub-samples for germination and viability testing, determination of moisture content and subsequent long term monitoring of viability at intervals of three to five years (over at least 50 years). Invertebrate predation can affect seed yields and this must be taken into account during collections.

The above objectives have been difficult to achieve when sampling most rare and threatened flora populations. Quantities adequate for long term storage have been accessed by implementing a repetitive, seasonal sampling regime until an adequate collection was obtained. Depletion of the population associated with the contribution of seed from any source individual was avoided. However, exceptions to this were necessary at sites harbouring critical levels of pathogen infestation.

3.1.2 Population Reconnaissance

To obtain a genetically representative seed collection, population reconnaissance is necessary. The status of each plant needs to be carefully assessed with respect to

- the number of breeding plants in the target seed crop;
- the seed production status of each plant;
- maturity of seed crops;

- the population spatial structure.

Population reconnaissance facilitates evaluation of the optimum number of families from which uniform quantities of seed should be collected, as well as the systematic spacing of source plants to minimise the possibility of sampling closely related progeny.

3.2 SEED STORAGE, VIABILITY TESTING AND INVENTORY SYSTEM

The TFSC laboratory protocols involve registration, cleaning, fumigation, testing, storage and monitoring of all seed collections (see Appendix 1). The design of genebanks and protocols recommended for use in genebanks have been formulated by the International Board for Plant Genetic Resources (IBPGR) (Cromarty *et al* 1985, Ellis *et al* 1985a,1985b).

3.2.1 Documentation

Documentation of all details of the collection, testing, storage and monitoring of seed is critical if resampling and regeneration programs are to be successfully implemented. Voucher specimens may require identification after drying and freezing prior to incorporation into the main collection. The registration of relevant details such as location of species and number of seed collected, as well as data relating to population phenology and site characteristics, has been made on the WASEED database. WASEED is specifically designed for the TFSC but could readily be applied to other genebanks. It utilises the Microsoft ACCESS database program with Microsoft EXCEL for graphing purposes. All facets of the seed collection process are entered into the database which will be linked to CALM's Western Australian Herbarium WAHERB specimen database. Each collection is allocated a chronologically sequential accession number with a suffix appended which indicates priority ranking. The TFSC accession number is linked to the unique field collection number (recorded on a provenance information data sheet) and to a voucher specimen lodged at the Western Australian Herbarium (entered into the WAHERB database). The TFSC numbers for each accession are always appended to relevant sample packaging. All data pertaining to laboratory procedures are also incorporated into the WASEED database as an ongoing process.

Prior to the development of WASEED, a number of small, inhouse databases had been designed to facilitate data transfer. A Seed-in-Store database was established to facilitate rapid auditing of seed stocks and to ensure correct and timely monitoring of accessions over the long term. A phenology database, which has since been incorporated into WASEED, was established to assist the planning and appropriate timing of future seed collection work according to phenological information. This facet of the database will be updated with information derived from herbarium specimens, field observations, Kings Park and Botanic Gardens collection records, floras, and from the literature. A comprehensive TFSC reference library and reprint collection have also been established.

3.2.2 Cleaning and Fumigation

Field collections of seed received by the TFSC usually require cleaning and, in most cases, seed must be extracted from cones, follicles or capsules prior to testing and storage. Seed is cleaned by hand to attain an acceptable degree of purity. However, for some small-seeded species, attainment of 100% purity may use excessive time or resources and here, a lower standard of purity may be justifiable. Nevertheless, sub-samples extracted from impure accessions for use in germination and viability tests must be further treated to avoid fungal contaminants.

Fumigation is required to eliminate invertebrate predators from seed prior to storage. *Dryandra* and *Banksia* follicles are commonly predated by hemipterans. Certain wasps also affect the developing seed by laying their eggs in the ovule during anthesis thus allowing larvae to feed on the maturing seed. During seed extraction the activity of these wasps becomes apparent when seed coats containing well developed larvae are manifest. Invertebrate predation of collected seed may be arrested by fumigating the accession with carbon dioxide using heat-sealed, laminated plastic bags. Carbon dioxide also has an adverse effect on the growth of fungi and many other microorganisms. This technique is employed by the CSIRO's Australian Tree Seed Centre. Fumigation is complete after two weeks. Accessions stored as woody cones can be fumigated directly.

3.2.3 Quantifying the Accession

The method of quantification varies according to the particular type of fruit and seed. For example, easily extracted small seeds (eg. most Myrtaceae) are efficiently counted by weighing. A small subsample is counted, weighed, and the result extrapolated to the total accession. Seeds of some species must be all individually counted. Detailed quantification of accessions requires accurate assessment and databasing to ensure that monitoring procedures during storage can determine if adequate viable seed is being maintained.

3.2.4 Germination and Viability Testing

After cleaning and fumigation, an accession must undergo testing for germination and viability. In most cases the optimum pre-treatment for germination of rare or threatened taxa is unknown due to poorly understood seed biology. A range of dormancy-breaking techniques such as the use of gibberellic acid, and heat treatment (boiling water) have been trialed to promote germination. Temperature is critical to seed germination for species in the south-west winter rainfall zone, and all accessions derived from this zone are initially tested at a constant 15°C with a 12-hour photoperiod. Consideration of optimal recruitment events in the natural habitat is recommended when dealing with difficult taxa.

Once optimal germination has been attained (ie. > 90% if possible) viability is determined using a representative sub-sample with at least three replicates. The number of seeds per replicate will be influenced by the amount of seed constituting the accession. For accessions comprising 500-1000 seeds, 5% of the seeds are used for testing, while in the case of critically small collections, this may be reduced to 2.5%.

All germinants produced in the TFSC that are not used at the Western Australian Herbarium have been donated to Kings Park and Botanic Gardens for planting in the Rare Flora garden or for experimentation on the biology of rare plants.

3.2.5 Moisture Content Determination, Reduction and Drying of Seed

Two important factors affecting the viability of stored seeds are seed moisture content and temperature. The Karl Fischer Auto-Titrator or the Oven Dry Method (103°C for 17 hrs) is used to measure moisture content. Ideally all analyses should be carried out using the former method as it specifically detects water molecules in the sample whereas results obtained from oven drying are influenced by the presence of volatile components. While the high degree of precision associated with the Auto-Titration method permits testing of very small samples, at least 50 seeds per replicate are included to cover the range of size and maturity of seeds. This degree of replication is fixed by the variation in results obtained during analyses and an acceptable level of discrepancy between replicates is around 0.2%.

Desiccators with silica gel were used for drying seed until the construction of a seed drying room in late 1996. This room is held at a constant 15°C and 15% relative humidity. Seed reaches an equilibrium moisture content of 5 ± 1% over a period of 1 - 8 weeks prior to storage at sub-zero temperatures.

3.2.6 Packaging, Storage and Monitoring Regime

Laminated aluminium foil is used as a packaging medium. This foil consists of three layers: polyethylene on the inside provides a seal, aluminium foil in the middle acts as a barrier to moisture and polyester on the outside protects the foil from damage and oxidation. Laminated foil bags are made up with the use of a heat sealer. Dried germplasm is placed in the bags which are then filled with carbon dioxide and sealed. Shrestha *et al* (1985) found carbon dioxide to be an effective storage medium, second to nitrogen but cheaper and easier to use. During extensive laboratory testing, it was found that germination capability and subsequent seedling vigour were maintained at high levels over periods of time when oxygen was excluded from the storage atmosphere. The maintenance of seed germination vigour and capacity during storage is critical for any genebank (Hanson, 1985). Seed viability and seedling vigour are known to decline with increased length of storage, and seeds stored at ambient temperatures and relative humidity soon lose viability.

Long term storage conditions (-18°C) have been used for base collections when seed is fully extracted. Some seed has been stored on the long term, unextracted in cones or follicles at 4°C, also sealed in foil with carbon dioxide and placed in plastic storage drums for safety. Germination and moisture content testing may take 6-8 weeks and interim storage during this period has been at 4°C if the seed is fully extracted or at room temperature (20-25°C) if seed is enclosed in cones, follicles or bracts. Low temperatures are preferable for all accessions during pre-storage handling. Prior to storage of base collections, sub-samples for retesting have been placed in separate packages, labelled, sealed as usual and stored at -18°C.

The determination of an appropriate monitoring regime to assess seed response to storage at 18°C is dependent on the quantity of seeds collected and their original viability and moisture content. The IBPGR recommends monitoring at least every five years for active collections or those with poor initial viability or storage life. In a newly established genebank it is advisable to begin with a short monitoring interval to assess specific responses to medium and long term storage. Since there is little known about the reaction of Australian flora to storage, a conservative monitoring regime would seem appropriate. The TFSC has considered it prudent to initially check the accessions after one year in storage at -18°C, and thereafter at intervals of five then ten years dependent upon the species and its' response to sub-zero storage temperatures. Any significant decline in seed quality must be countered by implementing a resampling strategy.

4 RESULTS AND DISCUSSION

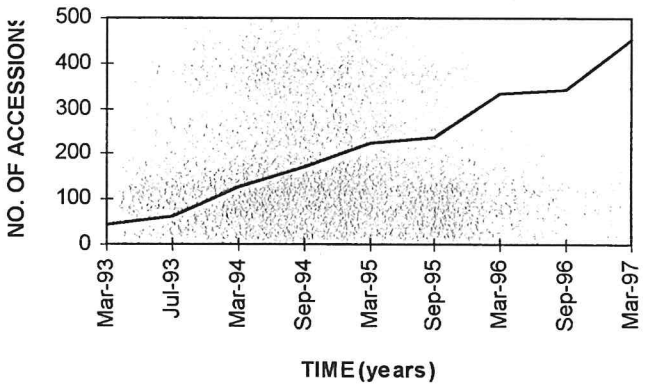
A fully operational long term seed storage facility of international standard has been established at CALM's Western Australian Herbarium. This facility has already been referred to in a report to the Endangered Species Unit of Environment Australia as a model system for the seed-based germplasm storage of threatened species (Morse *et al*, 1993).

4.1 SEED COLLECTION

4.1.1 Threatened Flora Seed Centre Accessions

In July 1993, when the present manager of the TFSC assumed responsibility for the project, 61 accessions had been collected or incorporated into storage in the TFSC. These accessions represented 23 taxa in 2 families, the Proteaceae and Epacridaceae. By March 1997, 452 accessions of rare and threatened taxa had been collected (see Appendix 2). The TFSC now has in store 175 taxa in 43 genera in 15 families. Figure 3 shows the increase in number of accessions incorporated into the TFSC since March 1993. Figure 4 shows the progressive increase in number of taxa stored between March 1993 and March 1997. These collections cover *Phytophthora*- and canker-threatened species present on the south coast, the northern sandplains, the northern and southern wheatbelt and the Swan coastal plain. These accessions represent a total of 353 collecting sites.

FIGURE 3: No. of accessions held in storage, March 1993 to March 1997



Good genetically representative seed collections of most if not all known populations of 23 critically endangered taxa have been made by staff at the TFSC. A further 10 critically endangered taxa have seed from at least some populations held in long term storage. Most of the remaining critically endangered taxa have been visited but no seed was collected due to either timing, or poor fruit set.

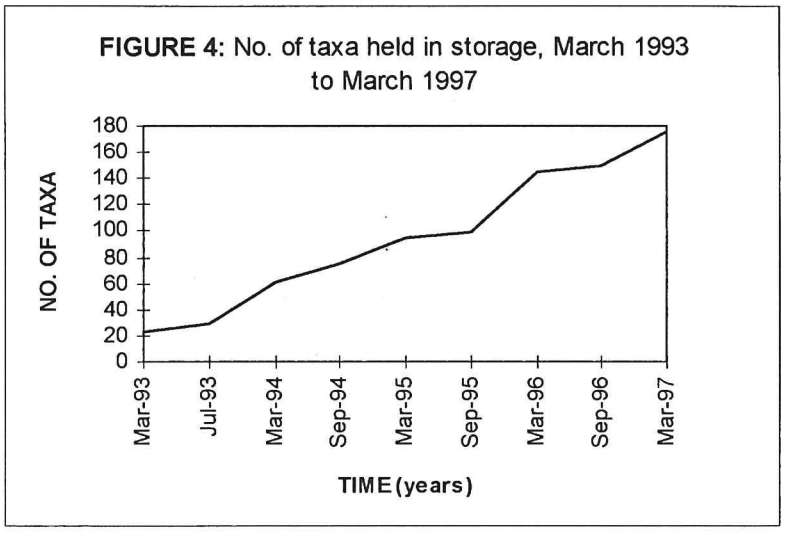


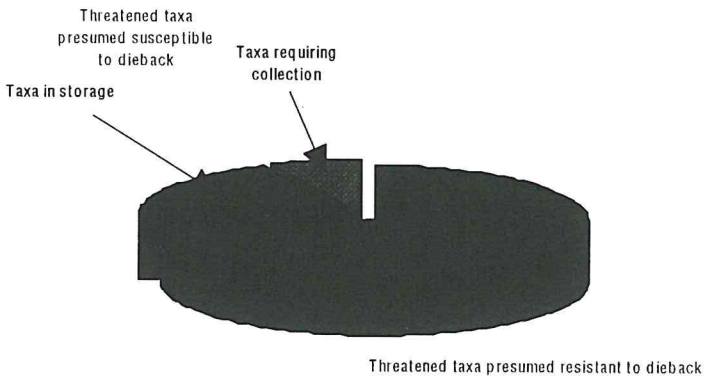
FIGURE 4: No. of taxa held in storage, March 1993 to March 1997

Some taxa have not been dealt with due to known poor seed set, existing projects covered by Kings Park and Botanic Gardens, lack of locality details or doubtful taxonomy. The eight orchid taxa on the critically endangered flora list have not being considered as Kings Park and Botanic Gardens are better equipped to deal with the collection and storage of the associated fungi which are so vital for the propagation of these plants.

Thirty-five endangered and vulnerable taxa are well represented in long term storage and a further 34 have seed from at least some of their populations stored in the TFSC. Over 59% of these taxa are presumed susceptible to *Phytophthora* dieback disease. Sixty-nine taxa on Western Australia's priority flora list (taxa poorly known and in need of further survey) are in storage, with 30 of these well represented, 18 moderately represented and 21 with at least some populations represented. Of these 69 taxa, 12 are currently being recommended for gazettal as Declared Rare Flora. A further 4 geographically restricted or otherwise interesting taxa are also represented in the long term storage facility.

Although only 34% (102 taxa) of the taxa declared rare under the Western Australia Wildlife Conservation Act (302 taxa) have seed represented in the TFSC, this equates to approximately 74% of threatened taxa in dieback- or canker-susceptible genera occurring in known or likely disease prone sites in the State (see Figure 5). Many of the remaining taxa have not been collected due to reasons such as poor or sporadic seed set, immaturity of populations, difficult access to sites or simply due to lack of time and resources.

FIGURE 5: Proportion of presumed dieback susceptible threatened taxa held in storage



During the past five years, collection of seed from a number of rare and threatened taxa has been difficult due to small population sizes, poor and sporadic seed production, high seed predation levels and disturbances such as disease, fire or drought, which cause temporal genetic fluctuations in the genepool. Seed traps were devised and tested in spring and early summer, 1993 on a number of *Adenanthos* spp. on the south coast. Differential fruiting of some taxa has warranted this method of collection. Although partly successful, high levels of seed predation have been noted. Monitoring of the traps is thus required on a regular basis (7-14 days) to prevent excessive seed loss. In 1994 a local farmer was employed to assist in seed collection from established traps. This proved successful, and it is proposed to seek additional funding to involve local community members in other areas where long term continuous seed collections are needed.

4.2 SEED STORAGE, VIABILITY TESTING AND INVENTORY SYSTEM

4.2.1 Monitoring of Accessions

Monitoring of accessions is an integral part of seed storage. As of March 1997 over 150 accessions representing 75 taxa have been tested for viability after one year in storage and 73% of accessions have maintained their initial viability. A small number of accessions have

shown a reduction in germinability after storage (27%). Further testing of these taxa is needed to confirm whether some species suffered a loss of viability *per se*, or actually induced dormancy on drying and freezing. Of the accessions that showed a reduction in viability after storage, 5% of cases were attributable to application of inappropriate sterilisation and pre-treatment techniques resulting in seed mortality. Protocols are now in place to ensure that the sequence of treatments applied prior to germination is standard. Fungal contamination and small sample sizes also render comparison of pre- and post-storage germination results difficult. The narrow environmental tolerances of some wild seeds can also present a potential problem in germination testing. Routine germination tests deplete seed accessions, and duplication of tests under different conditions is rarely possible with small samples.

In some cases storage conditions may have imposed dormancy upon the seed. If so, this further complicates research into the response of seeds to a variety of storage regimes, and might also limit the value of germination tests.

In general, laboratory research into a variety of techniques to promote germination has resulted in a better understanding of the biology of some of Western Australia's rare and threatened taxa. The use of growth hormones, heat treatment and scarification have provided useful information for assessing viability and determining the optimum methods for inducing germination of collected seed. Long term storage is only truly effective if dormant seed can be induced to germinate when required for monitoring or reintroduction purposes. Gibberellic acid has been used successfully to induce germination in otherwise dormant seeds (eg. *Dryandra* spp.). Scarification has been used as a pre-treatment to promote germination of some hard-fruited species and a small lapidary tumbler was utilised to investigate this in species of *Eremophila, Grevillea,* and *Astroloma.* New techniques including the use of smoke for promoting germination in *Verticordia* and *Andersonia* have produced mixed results. In some cases, smoke has stimulated germination, while in others high concentrations of smoke have had the opposite effect. The conflicting results obtained with different concentrations of smoke are unlikely to be explained until the chemical(s) that trigger germination are isolated. Further research into dormancy-breaking mechanisms is urgently required.

Figures 6a and 6b demonstrate the response of *Lambertia fairallii* and *Dryandra nivea ssp. uliginosa* (Proteaceae) to storage at sub-zero temperatures. There was no significant difference between the proportions of seeds germinating before and after storage. Germination results for *Banksia brownii*, a rare species highly susceptible to dieback, did not vary greatly between provenances despite a range in population size and condition (Figure 7). The response of this species to storage was mainly positive with good initial germination of seed.

In some cases, storage appeared to reduce the rate of seed germination, as indicated by a tendency in *Dryandra seneciifolia* for the germination rate of stored seed to lag behind that of unstored seed (Figure 8a).

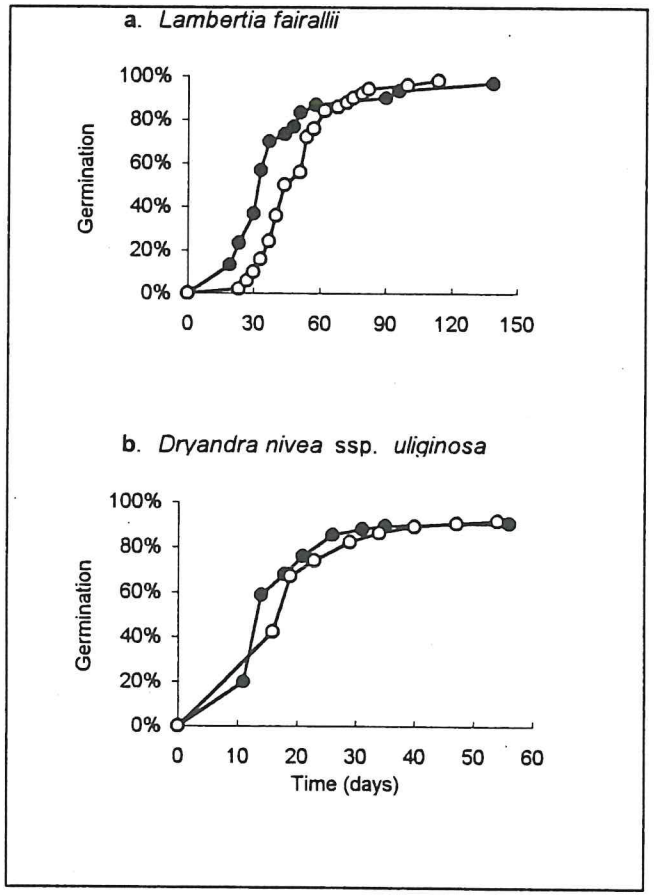


Figure 6: Response of two Proteaceous species to low moisture content and low temperature storage.

(●) initial base collection

(O) germination after one year in storage

Carbon dioxide might be expected to depress the seed germination rate since the gas is known to reduce or halt metabolism of germplasm. It is possible that secondary dormancy is imposed on the seed through the storage process, although storage in carbon dioxide does not appear to reduce the seed's ability to germinate in the long term. On the other hand, samples of *Banksia brownii* stored frozen and at room temperature sealed in carbon dioxide showed no significant difference in percentage germination after one year, but the samples stored at room temperature took much longer to attain maximum germination than those kept at -18°C (Figure 8b). Figure 8c illustrates that seed germination in *Daviesia megacalyx* occurred at a greater rate for samples stored at -18°C for one year than for unstored seeds. This may have been due to a combination of low moisture content and sub-zero temperatures enabling the seed coat to imbibe water at a faster rate, although the pre- and post-storage samples were both subjected to the same heat treatment prior to germination.

The contrasting results obtained in these trials with different species, underscore the importance of testing seed germination in all species before and during long term storage.

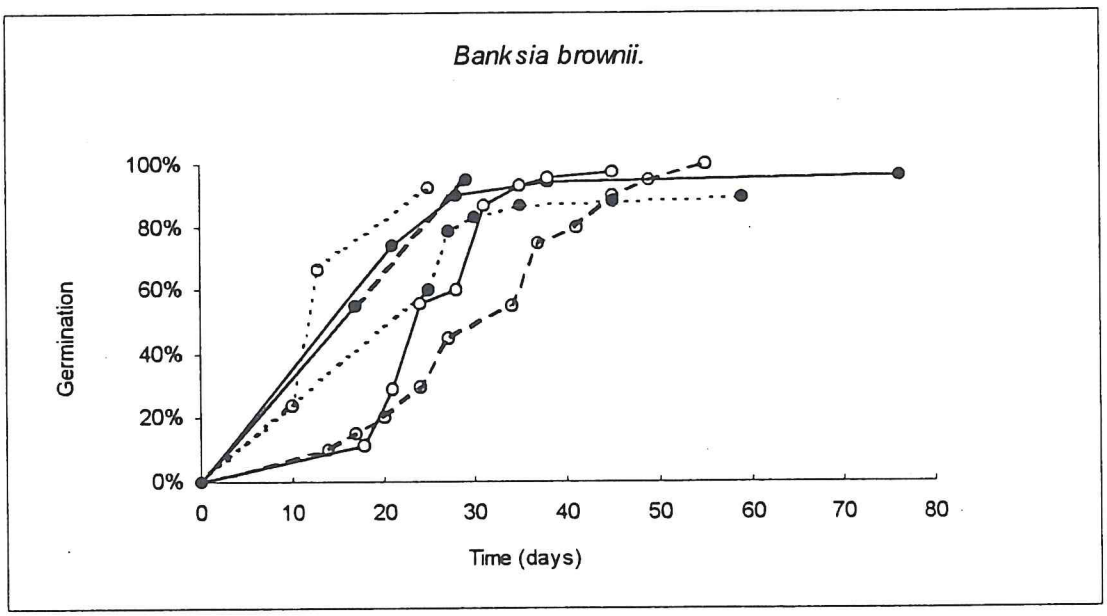


Figure 7: Pre- and post-storage germination results for three populations of *Banksia brownii*

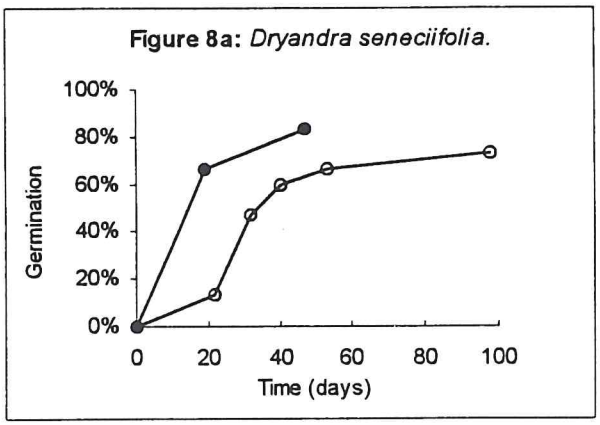
(●) initial base collection
(O) germination after one year in storage

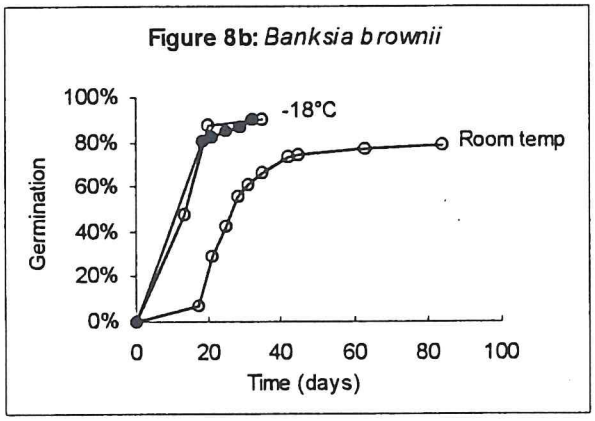
A range of pre-treatments have been used to assist germination. Figure 9a illustrates the differences in germination for *Verticordia staminosa* ssp. *staminosa* when seed is soaked in a

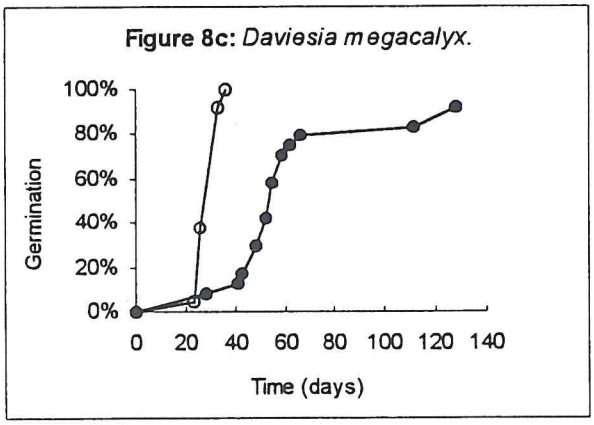
100% solution of smoked water for 24 hours or when full strength smoked water is added to agar on which seeds are incubated. The proportion of seeds germinating in the former treatment was much greater that that noted on agar. The results shown in Figures 9a and 9b tend to support the suggestion that smoke treatment may sometimes inhibit germination. The effects of four different pre-treatments on seed germination in *Andersonia echinocephala* are shown in Figure 9b. Two concentrations of gibberellic acid and two concentrations of smoked water in agar solutions have been compared. Seed germination was enhanced by inclusion of gibberellic acid in the medium and the best result was obtained at the higher concentration of growth hormone. Germination was relatively poor in the smoke treatments particularly at the highest concentration of smoke. The seed from all four treatments was leached in running tap water for four hours prior to commencement of the trial.

Research into seed set and germination in a range of rare and poorly known *Verticordia* has produced some interesting results. Seed set varies both spatially (between provenances) and temporally (within a collecting season and between collecting years). Variations in viability and dormancy between provenances of the same species, as well as between collecting seasons have also been noted for a number of species. A brief period of environmental stress during seed maturation may help to explain differences in the germinability of seed produced in successive years at the same location or in the same year at different locations.

Observations made during the past three years suggest that the timing of collections for some species is very important. Collections of one species of *Verticordia* have been made repeatedly over a period of 6-8 weeks and tested for viability, with results showing that seed viability varied throughout the season (Figure 10). Seed set was not found to differ despite fruits appearing ripe at all times. This is vital information for the planning of field collections. In addition, the quality of the seed collected will ultimately affect seed longevity.





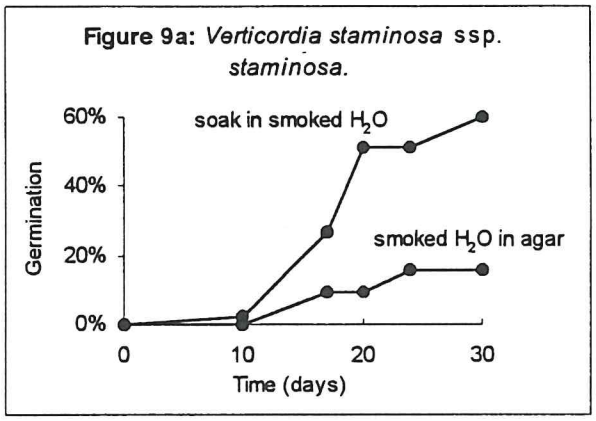


Figures 8a-c: The effect of storage on germination rate.

(●) initial base collection

(o) germination after one year in storage

Preliminary research into the use of liquid nitrogen (-196°C) as a storage medium has produced some interesting results. After storage at ultra-low temperature, seeds of *Banksia brownii* germinated at a slower rate than seed not stored at -196°C, although germinability was comparable within the constraints of the small sample size (Figure 11a). Tests on *Acacia tenuissima* showed that pre-treatment in boiling water prior to plunging into liquid nitrogen was vital to prevent loss of seed due to shattering of the endosperm (Figure 11b).



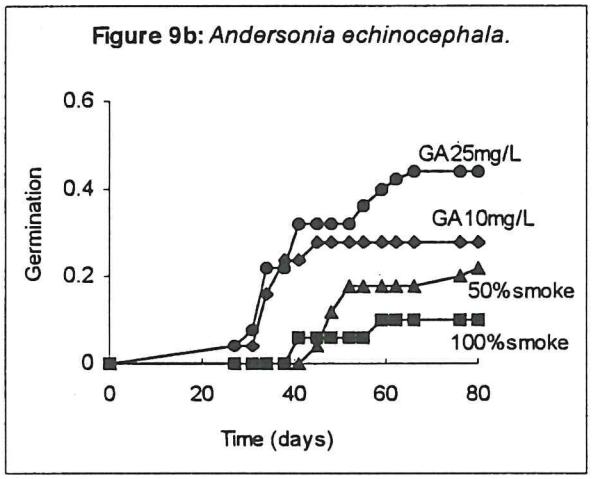


Figure 9a -b: The effect of pre treatment on percentage germination

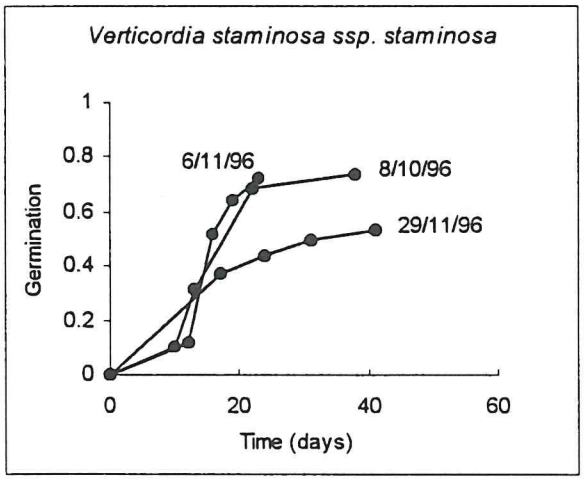
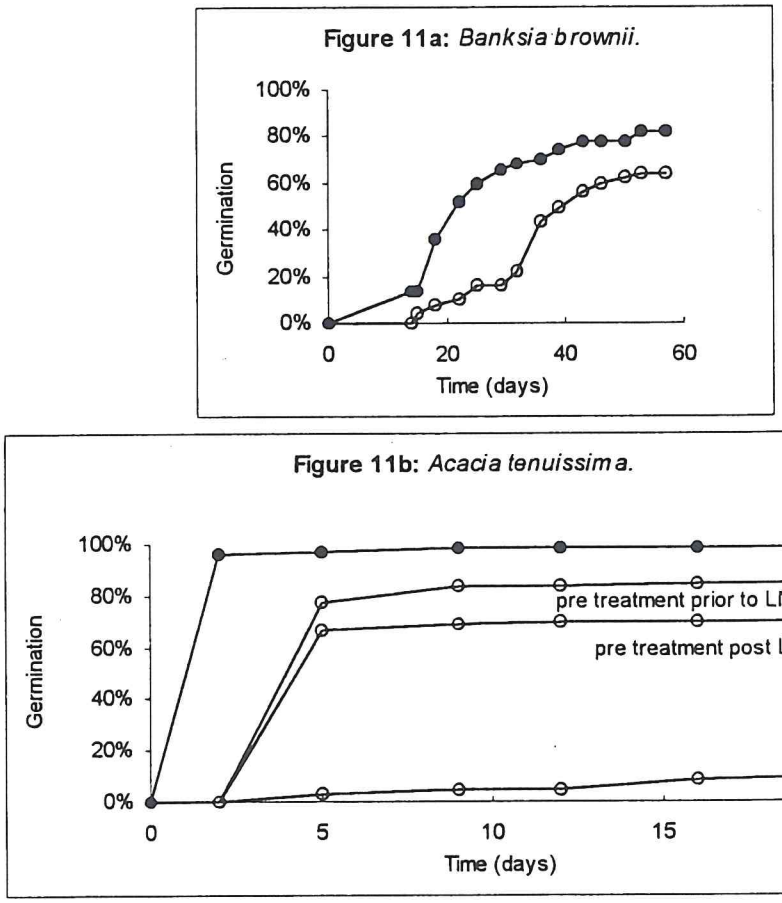


Figure 10: The effect of collection time on percentage germination

Other research into dormancy-breaking mechanisms have included the use of ethylene to emulate dry storage after-ripening in *Dryandra*. Results have been disappointing, and it appears that ethylene at the concentrations tested is no substitute for dry storage. Some preliminary investigations into the effects of different growth media on germinability of a range of taxa have also been conducted during the course of routine viability testing. A range of media have been used including sterile soil, vermiculite and agar.



Figures 11a -b: The effect of storage in liquid nitrogen (-196°C) on percentage germination.

(●) initial base collection

(O) germination after storage in liquid nitrogen (-196°C)

5 OUTCOMES

5.1 SEED COLLECTION

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

- A total of 452 accessions of rare and threatened Western Australian flora representing 175 taxa have been incorporated into the TFSC. A total of 74% of Western Australia's threatened taxa, presumed susceptible to dieback and canker, is represented in low moisture, low temperature storage.

- An ongoing and well coordinated germplasm collection program has been established at the TFSC.

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

- New populations of threatened taxa and extensions of previously known populations continue to be discovered on routine collection trips.

- 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. Collaborative field trips with other CALM staff and collectors have yielded good results, and additional germplasm material from critically endangered and other threatened taxa has often been collected for Kings Park and Botanic Garden.

- A small proportion of the time spent in the field has been devoted to collecting germplasm from a variety of species for research into their population biology and to assess the taxonomic status of some flora.

- An understanding of many of the variables that affect phenology, pollination, seed set, and seed ripeness as well as the identification of seed of a wide variety of taxa has been consolidated during seed collection over the past five years.

5.2 SEED STORAGE, VIABILITY TESTING AND INVENTORY SYSTEM

- In the laboratory, research into a variety of techniques to promote germination have 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 assessing viability and determining the optimum methods for germination of collected seed. Research into seed storage, germination and dormancy-breaking mechanisms for the south-western flora has enhanced the overall effectiveness of the operation of the TFSC.

- Storage data is now available for a wide range of species, and the effects of moisture content reduction and storage of germplasm in carbon dioxide at sub-zero temperatures is now known for many taxa held in the genebank. The maintenance of high viability of stored seed has been demonstrated for 73% of accessions. For those accessions that have failed to maintain high viability, further research will be 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 stringently implemented to enable more accurate comparisons to be made between pre- and post-storage germination.

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

- New equipment has been purchased and facilities upgraded to enable the TFSC to operate to the international standards required for a long term seed storage facility. This will allow greater control over the drying and storage of seed, and will permit more advanced

research into the response of Western Australian species to moisture content reduction and sub-zero storage.

- A comprehensive database has been developed 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.

- Preliminary investigations of the effect of growth media on the germinability of a number of rare and threatened species has commenced, with interesting results.

- 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

- A number of publications have been produced outlining the techniques used for seed collection and storage (eg. Cochrane and Coates, 1994 and Cochrane *et al*, 1996). The results of the past three years work on the response of native seed to low moisture and low temperature storage is being prepared for publication in a refereed journal.

- A Churchill Fellowship has been awarded to the manager of the TFSC for 1997. Visits to the International Plant Genetic Resource Institute (formerly IBPGR) in Rome, genebanks in the USA, the Seed Conservation Section of Wakehurst Place, Kew, UK and to South Africa have been arranged. The increase in knowledge that will be gained from this opportunity to visit other scientists in genebank management overseas, will be invaluable to the operations of the TFSC and assist in efforts to conserve the native flora.

- Staff have attended a number of relevant conferences and symposia over the past few years, and presented posters and papers on genebank issues. A poster paper describing the work of the TFSC and entitled *Western Australia's Threatened Flora Seed Centre,* was presented to the 1994 First National Workshop on Native Seed Biology in Perth. In 1995,

the 4th International Botanic Gardens Congress was attended and two poster papers on germplasm storage and documentation were presented. A training session on Developing a Germplasm Bank was also given at this conference. An oral paper was delivered to the Second National Workshop on Native Seed Biology for Revegetation in Newcastle in 1996 entitled *Seed Germination and Storage of Rare and Threatened Species from Western Australia.* A poster paper on seed biology of the rare *Hemigenia exilis* was also delivered to this workshop.

- Considerable input on technical aspects of seed collection and storage was given to the Australian Network for Plant Conservation for inclusion in *the Germplasm Conservation Guidelines for Australia* (still in draft format).

- Staff are now in the position to offer interested parties a training course in the identification of seed and in collecting techniques and storage methods.

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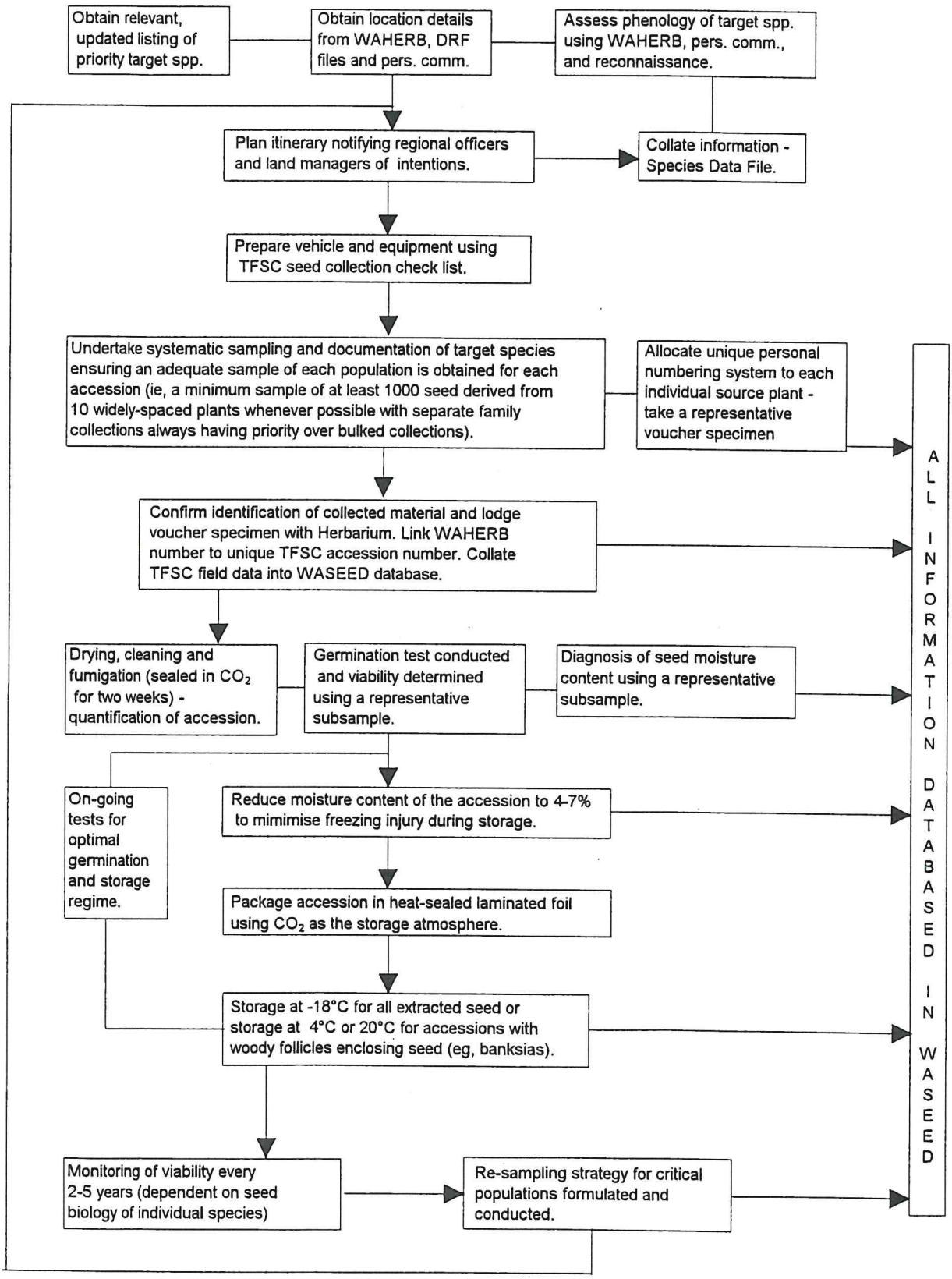
APPENDIX 1: Seed Collection & Storage Protocols for the Threatened Flora Seed Centre



APPENDIX 2: Threatened Flora Seed Centre Accessions to March 1997.

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 P4	30/11/93	*Adenanthos labillardieri*	B/12
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
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	6/01/94	*Adenanthos pungens ssp pungens*	B/12
00118 E	6/01/94	*Adenanthos pungens ssp pungens*	I/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/10
00123 P4	11/01/94	*Adenanthos labillardieri*	I/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* sp Two Peoles Bay	I/12
00133 P2	15/02/94	*Andersonia* sp. Mt. Lindesay	I/10
00134 P2	16/02/94	*Banksia occidentalis ssp. formosa*	I/17
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
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 E	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 aff. varia*	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* sp Two Peoples Bay	B/10
00199 C	14/12/94	*Andersonia* sp Two Peoples Bay	B/10
00200 C	14/12/94, 11/01/95	*Andersonia* sp Two Peoples Bay	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
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/9
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 ss.p 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 ss. 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 P1	22/11/95	*Chamelaucium* sp. Gin Gin	B/15
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
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	*Vert stam 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 P1	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	12/12/95, 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. occidentalis*	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/?
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 harveyii*	B/30
00336 V	18/04/96	*Verticordia carinata*	B/100+
00337 E	18/04/96	*Verticordia harveyii*	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	27/10/95	*Acacia recurvata*	B/50
00345 C	27/10/95	*Daviesia bursarioides*	B/5
00346 C	27/10/95	*Daviesia bursarioides*	B/8
00347 C	27/10/95	*Daviesia bursarioides*	B/9
00348 C	27/10/95	*Daviesia bursarioides*	I/10
00349 P2	28/10/95	*Acacia recurvata*	B/100
00350 P2	28/10/95	*Acacia wilsonii*	I/18
00351 C	28/10/95	*Grevillea humifusa*	B/5, I/6
00352 E	29/10/95	*Chamelaucium* sp. Gin Gin	B/50
00353 E	29/10/95	*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 appressum*	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
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 P2	13/12/96	*Patersonia spirafolia*	B/10
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	14/01/97	*Dryandra montana*	I/4
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	2/01/97	*Adenanthos labillardierii*	I/10
00435 V	3/02/97	*Verticordia fimbrilepis ssp. australis*	B/100
00436 C	4/02/97	*Dryandra anatona*	B/500
00437 C	4/02/97	*Dryandra anatona*	B/25
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 aff. varia*	I/10
00448 P1	20/02/97	*Calothanmus aff. quadrifidus*	B/20
00449 E	20/02/97	*Dryandra nivea ssp. uliginosa*	I/5
00450 P1	21/02/97	*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

I = seedlot comprised of seed from individual parent plants kept separate;

B = seed from individual parent plants bulked as one seedlot due to sporadic or sparse seed production

/number indicates plants represented in the seedlot.

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

FINAL REPORT

TO THE THREATENED SPECIES AND COMMUNITIES UNIT, BIODIVERSITY GROUP

ENVIRONMENT AUSTRALIA

MAY 1997

Department of Conservation and Land Management
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Environment Australia
Biodiversity Group