The Biology and Ecology of Threatened *Daviesia* Species in Western Australia

Final Report

prepared by

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The views and opinions expressed in this report are those of the authors and do not necessarily reflect those of the Commonwealth Government, the Minister for the Arts, Sport, the Environment, Tourism and Territories, or the Chief Executive Officer of the Australian National Parks and Wildlife Service.

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Summary

The aim of this study is to understand the biology and distributional status of *Daviesia* species to facilitate the recovery and management of threatened species. The study defines factors mitigating against species survival. The study focuses on life history attributes, which may explain the biology and conservation status of the species.

Most threatened *Daviesia* species occur in remnant vegetation on road verges in agricultural areas or in gravel pits, still used for gravel extraction and in areas where the natural habitat has been cleared for agricultural purposes. All populations of threatened study species (Table 1) occur on mechanically disturbed areas and all plants grow on areas cleared or shallow graded.

Little information is known about the impact of predators and the effect of diseases on *Daviesia* species. However, no diseases have been recognised in the study species during the two year study period.

Most *Daviesia* species flower in late autumn, winter or spring with seed set approximately three months later but the flower and seed production is markedly constrained by high incidences of flower and fruit abortion.

Plant propagation from seed is the most common method, however pre-treatments are required to increase germination. All study species responded with high germination (80 to 100 %) to nicking as a standard pre-treatment. However, *Daviesia speciosa* ms can only be vegetatively propagated from stem or root cuttings.

Fire is well known as an agent for regeneration of a great number of Australian species and the study indicates that five out of five study species are fire sensitive.
Smoke fumigation did not increase germination, and the influence of smoked water in any dilution had in general a negative influence on germination of the *Daviesia* species studied.

The *Daviesia* species studied grow in soil with a large portion of sand and silt and up to 72 % gravel. The contents of organic carbon, nitrogen, phosphorus and potassium are extremely low and the soils are acidic with a mean pH of 6.2.

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1. Introduction

Because of geological, climatic and evolutionary processes, the flora of Western Australia has attained a high degree of endemic biological diversity with an estimated 8000 species in the South West of Western Australia. Geologically, Western Australia is ancient with relatively infertile soils.

Through adaptation to these conditions the flora has become highly specialised with restricted distribution consequently prone to extinction by broad scale clearance of native vegetation for agriculture and associated development after arrival of Europeans.

The Declared Rare (Threatened) and Priority Flora List (CALM, 1994) includes approximately 1872 taxa as 'Declared Rare Flora - Extant Taxa (R)', or 'Declared Rare Flora - Presumed Extinct Taxa (X)', 'Priority One' - Poorly known Taxa with generally less than five populations which are under threat, 'Priority Two' - Poorly known Taxa with less than five populations which are not believed to be under immediate threat, 'Priority Three' - Poorly known Taxa with several populations which are not believed to be under immediate threat or 'Priority Four' - Rare Taxa which are considered to have been adequately surveyed and which, whilst being rare in Australia are not currently threatened by any identifiable factors.

About 29 of the *Daviesia* species occurring in Western Australia are included on the 'Declared Rare and Priority Flora List'. Nine of these species are on the schedule of Declared Rare Flora and are regarded as being threatened (R). One species of *Daviesia* is incorporated into Priority One, 12 species into Priority Two, three species into Priority Three and four species into Priority Four. All species declared as being rare (R) are included in this project.

Many *Daviesia* species occur in areas which are cleared or disturbed as a result of habitat dissection. Plant biological features such as response to fire may also play a role in the rarity of the species, and will be examined in this report.

Table 1: Threatened species included in this study

Daviesia species	**Conservation Code**	**CALM Region**
D. bursarioides ms*	Threatened	Greenough
D. campephylla ms*	Priority 2	South Coast
D. euphorbioides	Threatened	Wheatbelt
D. megacalyx ms*	Threatened	South Coast
D. microcarpa ms*	Threatened	South Coast
D. oxylobium ms*	Threatened	Wheatbelt
D. pseudaphylla ms*	Threatened	South Coast
D. purpurascens	Threatened	Wheatbelt, Gold Fields
D. speciosa ms*	Threatened	Greenough
D. spiralis	Threatened	Wheatbelt

*: All ms names applied courtesy of M. Crisp (ANU, Canberra)

1.1 Aims and Objectives

The aims of this study are to understand the biology and distributional status of *Daviesia* species to facilitate the recovery and management of threatened species and to define factors mitigating against species survival. To ensure survival of threatened *Daviesia* species this study aims to develop an integrated conservation approach covering aspects of the biology, morphology and phenology of selected indicator species.

Of further interest are the seed biology, disturbance factors and propagation methods, also defined in this study. The study also undertook surveys to determine the nature and extent of the distribution of rare species.

Objectives of these studies are to investigate habitat requirements for the threatened *Daviesia* species and to define common life history attributes, which may help to explain the biology and conservation status of these species. Further objectives have been established for population protection of extant populations and species restoration, which will enhance the situation of threatened species.

Outcomes for this project include recommendations for the recovery and production of a management plan for threatened *Daviesia* species.

2. Background

2.1 Study Species

Fifteen common and ten threatened *Daviesia* species were selected for the research program.

2.1.1 Taxonomy and Relationship

(Taxonomy and relationships of study species)

Class: Dicotyledonea (Magnoliopsida)
Subclass 5: Rosidae
Order 2: Fabales
Family: Fabaceae
Sub-Family: Mirbelieae
Genus: *Daviesia*
Species:

Threatened: *bursarioides* ms, *campephylla* ms, *euphorbioides, megacalyx* ms, *microcarpa* ms, *oxylobium* ms, *purpurascens* , *pseudaphylla, speciosa* ms, *spiralis*

Common: *benthamii, brevifolia, collectioides, cordata, daphnoides, decurrens, divaricata, flexuosa, hakeoides, juncea, nudiflora, oppositifolia, pectinata, triflora, uniflora*

Daviesia species belong to the Family Fabaceae (syn.: Pea Family, Leguminosae (with four Segregates: Mimosaceae, Caesalpiniaceae (syn. Cassiaceae), Krameriaceae and Papilionoideae (syn. Fabaceae)). The Fabaceae are the second largest of the three hundred families of flowering plants and contain about 657 genera with about 16 400 species of cosmopolitan distribution which are predominantly herbs, frequently shrubs and less commonly trees. The leaves are usually pinnate to tripinnate sometimes palmate or simple. Stipules are present, sometimes none or reduced to glands often falling away as the leaf matures. The flowers are bisexual and usually barely perigynous with five sepals and five petals. The five petals are differentiated into a banner, two wings and a keel formed from two petals coalescent by their adjacent margins and enclosing the stamens and the pistil (Plant Classification, Lyman Benson, 1959).

Daviesia species are usually glabrous shrubs. The leaves are often reduced to phyllodes or scales, variable and reducing along branchlets and often pungent. Stipules are minute or absent. Inflorescences of axillary racemes are modified to false umbels, clusters or solitary flowers.

Flowers are articulated on pedicel and bracts present on the basal part of the inflorescence axis. Usually flowers are yellowish with a reddish centre and without bracteoles. The calyx is campanulate with five lobes shorter than the tube. The upper two lobes are often broader and more or less connate. The keel is often beaked. All ten stamens are free and the filaments are occasionally flattened and coherent over most of their length. The anthers are dimorph and alternately large and small. The smaller anthers often have confluent cells. All *Daviesia* species have glabrous ovaries and styles and only two ovules. The fruit pods are compressed, swollen or inflated and obtriangular in outline. Seed dehiscence is elastic and explosive with sudden seed release upon maturity.

2.1.2 Description of Rare Species

(General Description, Habitat, Associated Species and Ecological Relationships; Distribution and Population Summary)

General description of main species studied in this project. All species classified as threatened (Conservation Code = R) by the Department of Conservation and Land Management (CALM).

Daviesia bursarioides ms:

A shrub with a distinctive divaricate branching pattern, spiny branchlet tips and small greyish leaves. This divaricately branched shrub with small narrow obovate phyllodes to 20 mm long and prominently spiny branchlets. In a sterile state it superficially resembles some *Bursaria* species (Pittosporaceae).

There were three known populations of *Daviesia bursarioides* ms restricted to the Three Springs locality, (350 km N of Perth) comprising a total of 45 to 50 plants. One plant is growing on private property. The remainder are growing on road verges. Recently two new populations were discovered around Three Springs by the Department of Conservation and Land Management. One of those populations (Table 2) is growing on private property. No seedling recruitment was observed but this population shows a cross-section through all plant ages.

The landscape is undulating with a red laterite clay soil and is dominated by mallee vegetation of *Eucalyptus gittinsii*, *Allocasuarina* species and *Dryandra* species.

Daviesia bursarioides ms flowers between June and September in peduncles of three to five flowers each with yellow-brown flowers.

Table 2:
Distribution and Population Summary of *Daviesia bursarioides* ms

Daviesia Species	CALM Region	Distribution/ Location	No. of Plants	Condition of Plants
D. bursarioides ms	Midwest	**Popn.1**: Three Springs - Perenjon Rd., on road verge.	5	Very poor
		Popn.2: N of Three Springs on Midland Rd., road verge	> 20	Good, regeneration from seeds
		Popn.3: S SW of Three Springs on Eneabba - Three Springs Rd.; on road verge.	> 20	Moderate
		Popn.4: N of Three Springs on Midland Rd., road verge	Appr. 10	Moderate
		Popn.5: Three Springs, SW off Kings Rd., in gravel pit and adjacent native bushland (private property)	> 100	Moderate - good; old plant stock is dying

Daviesia euphorbioides (Wongan Cactus)

The 'Wongan Cactus' is endemic to the sand plains about 220 km N NE of Perth between Cadoux and Wongan Hills. This species morphologically resembling a cactus, has erect, leafless, pithy stems, is soft wooded and reaches maturity in two to three years.

Leaves are reduced to small prickly conical scales and the yellow to dark red flowers appear in winter between June and October, depending on the prevailing climatic conditions. There are fifteen known populations on road verges around Wongan Hills - Ballidu, Dowerin and Goomalling with approximately 150 plants. The habitat of *Daviesia euphorbioides* is sand-plain with heath dominated by *Allocasuarina*, species, *Actinostrobus* species and shrubs of the myrtle family (Myrtaceae).

In some years *Daviesia euphorbioides* senesces rapidly after hot dry summers. While observing plants in November 1994 for seed collection, no dead plant material were observed. About five months later, 75 % of all plants had died representing a cross-section of all plant ages from two to eight years old. Reasons could be a drop of the water table, unobserved plant diseases, salinity, extreme aridity or an inability to cope with its environment.

Table 3:
Distribution and Population Summary of *Daviesia euphorbioides*

Daviesia Species	CALM Region	Distribution/ Location	No. of Plants	Condition of Plants
D. euphorbioides	Wheatbelt	Shire of Wongan Hills - Ballidu, Dowerin and Goomalling, all 15 popn. on road verges.	~ 150	Poor - depending on popn., aggressive resprouting after mechanical disturbance; approx.
		Popn. for main study (CALM population No. 2: NW of Wongan Hills, both sides of railway line off Wongan-Piawaning Rd.		75% of plant stock died last summer

Daviesia microcarpa ms

One of the most critically threatened *Daviesia* species occurs about 550 km SE of Perth and NE of Norseman. Originally discovered in 1974 by Mr. D. Whibley of the State Herbarium of South Australia, there is now only one extant population containing twelve plants.

This species is a low and sprawling shrub to 40 cm high with many weak tangled stems with prickly, spirally arranged phyllodes 8 to 20 mm long, with small flowers and pods. The flowering season is from August to September. All plants observed were on a highly disturbed road verge on a low range of hills constructed of ultra-basic rock, weathered to a sandy gravelly soil. The natural vegetation is dominated by *Allocasuarina* species with an understorey of *Triodia* species. Most plants were the same size and it is assumed that they are more or less of the same age.

This uniformity of age and plant size is possibly the result of habitat disturbance, such as road maintenance work. No plants of *Daviesia microcarpa* ms were found in undisturbed, adjacent bushland.

The long distances involved in reaching this population made field studies difficult.

Table 4:
Distribution and Population Summary of *Daviesia microcarpa* ms

Daviesia Species	CALM Region	Distribution/ Location	No. of Plants	Condition of Plants
D. microcarpa ms	South Coast	Eyre Hwy, E of railway crossing at Norseman on road verge	12	Moderate to good in Sept. 1993, but under extreme threat, estimated plant age appr. 4-6 years

Daviesia purpurascens

Collections of this species by R. Helms date back to the Elder Exploring Expedition of 1891, which crossed Central Australia. Recent collections were done by M.D. Crisp in 1979.

Daviesia purpurascens usually has a purplish tinge but presents an unattractive appearance because of rigid, thorny, spreading leaves. The flowers are yellow and dark red appearing in winter from August to September and unlike other species *Daviesia purpurascens* has spherical pods.

This species is more widespread than other threatened study species and occurs in the sand plains near Hyden and Kondinin in the wheatbelt, in Cranbrook south of Perth and in the southern Great Victorian Desert. This species grows on plains in sandy soil which varies from white in the south to red in the north. The associated vegetation is always mallee but the dominant *Eucalyptus* species differ between the sites.

Although this species was formerly widespread, it now seems to be reduced to small populations.

Often populations are on road verges in areas undergoing new agricultural development or road construction. Recently discovered new populations containing many plants have reduced the urgency of work on this species.

Table 5:
Distribution and Population Summary of *Daviesia purpurascens*

Daviesia Species	CALM Region	Distribution/ Location	No. of Plants	Condition of Plants
D. purpurascens	Wheatbelt, Goldfields	District of Narrogin, mainly on road verges **Popn.1**: S SW of Coolgardie of Gnarlbine Rock./ Goldfields **Popn.2**: W of Hyden on Hyden-Kondinin Rd. **Popn.3**: E NE of Kondinin. **Popn.4**: N of Bendering **Popn.5**: E of Billericay East Rd. **Popn.6**: E on Billericay E Rd. **Popn.7**: E on Mouritz Rd. **Popn.8**: N of Gorge Rock.	> 500	Average to good.

Daviesia speciosa ms

This species was discovered by the botanist C.A. Gardner near Mingenew in 1958 and C. Chapman, a naturalist, who brought this spectacular plant to notice. Further populations has been discovered in 1969.

Daviesia speciosa ms is one of the most striking species of this study. This taxon has not been recorded with seeds intact and pollination studies have not yielded seed. This study species has masses of grey thorny leaves with stiff, erect prickly stems and large, long keeled, red, pea-shaped flowers, presumably adapted for bird pollination. This

species flowers *in situ* in autumn from April to May and about four to six weeks earlier in *ex situ* propagation (Kings Park Nursery).
The northern populations flower, approximately two to four weeks, later than the southern populations in Tathra National Park.

Daviesia speciosa ms apparently occurs in five populations, two in Tathra National Park about 280 km N of Perth and three in gravel pits next to road verges around Mingenew about 370 km NE of Perth. All plants appear to reproduce clonally by rhizome sprouts. The populations occur in gently undulating laterite country and grow in shallow gravelly sand over gravelly clay. The vegetation is a low heath with *Daviesia daphnoides*, *Dryandra armata* (Proteaceae), *Gastrolobium oxylobioides* (Fabaceae), *Eucalyptus gittinsii* (Myrtaceae) and *Allocasuarina campestris* (Casuarinaceae).

The clonal nature of the root system with aggressive resprouting after mechanical shoot disturbance makes this species even more interesting and is not known for any other *Daviesia* species.

The main threat to this species comes from its small size, apparent lack of genetic diversity, and locations close to roads and in gravel pits.

Table 6:
Distribution and Population Summary of *Daviesia speciosa* ms

Daviesia Species	CALM Region	Distribution/ Location	No. of Plants	Condition of Plants
D. speciosa ms	Midwest	**Popn.1**: Tathra National Park. **Popn.2**: Tathra National Park. **Popn.3**: Yandanooka Rd. on road verge and adj. private property. **Popn.4**: Yandanooka Rd. in gravel pit. **Popn.5**: Yandanooka Rd. in laterite rise.	> 850	Good conditions but threatened by maintenance work and gravel extraction; plants in natural habitat smaller than in gravel pits.

Daviesia spiralis

Daviesia spiralis grows in a gravel pit near Wongan Hills where it is known from a single population of approximately 700 plants. This species has rapid, synchronised seed release. The habitat soil is red gravel with a high content of clay which is rock hard during summer and moist and clayey in winter.

Daviesia spiralis has spiral, ribbed and slightly twisted alternate leaves up to 10 cm long, grows in very dense up to 1.5 m high bushes, has bright yellow pea-flowers from September to January and seed set from December to January. The root system is a single tap root with few lateral roots and hair roots.

This species is threatened by its low number of plants and the existence of only one population in a gravel pit, which is still being used. *Daviesia spiralis* is confined to open situations adjacent to mallee scrubland. The habitat is dominated by *Daviesia spiralis* but also *Acacia* spp. and *Eucalyptus* spp. are found.

Table 7:
Distribution and Population Summary of *Daviesia spiralis*

Daviesia Species	CALM Region	Distribution/ Location	No. of Plants	Condition of Plants
D. spiralis	Wheatbelt	W of Wongan Hills	> 600	Moderate to good; seedling recruitment

2.2 Threats and Impact

Most populations of threatened *Daviesia* species occur on areas subjected to mechanical disturbance, with most plants growing on areas cleared or shallow graded.
Plants or populations growing on road verges, which were graded regularly, are extremely threatened by physical damage and removal. By chance, each grading could be a stimulus for regeneration from seeds, stored in the seed bank (legumes are described as disturbance opportunists) by cracking hard coated or weathered seeds, which increases germination but soil removal also causes an interruption of the ecological balance *in situ* and reduction of habitat.

Two species, *Daviesia speciosa* ms and *D. spiralis*, occur without exception in gravel pits and *Daviesia euphorbioides* occurs partly in gravel pits, road verges or railway

reserves. These gravel pits are disturbed by surface exploration for gravel or the surface sand has been scraped away and the gravel sub-surface disturbed.
Only *Daviesia spiralis* has been located in adjoining deep, permanent gravel but *D. euphorbioides* and *D. speciosa* ms only occur in shallow gravel or on rock with a sparse cover of sand or gravel in open bushland.

Bushfires are one of the most critical natural ecological factors influencing the natural diversity of the Australian vegetation. Repeated fires are likely to have a marked influence on vegetation, eliminating fire-sensitive species, encouraging the spread of resistant species and most probably modifying vegetation structure (Beard, 1990).

Fire in forest or woodland is always dependant on the flammability of the accumulated leaf litter. Fire behaviour is affected by the amount and type of fuel, air temperature, fuel dryness, wind spread, dryness and topography. Different fuel types exist in the bushland and are dependent upon the vegetation type and weed infestation.

The role of fire in stimulation of seed germination has long been recognised. Germination cues associated with fire may be direct, as high temperature or plant-derived smoke, or indirect by modified micro-environments or exposure of seed to the leachate of charred wood (Beard, 1990). Furthermore, ethylene is released during burning of vegetation and is known to stimulate seed germination and flowering of native species (Baxtor, 1993).

Studies on the stimulatory effects of plant-derived smoke on seed germination of legumes have thus far been limited studies of the effect of smoke from burning sclerophyllous vegetation collected in Kings Park and Botanic Garden on germination by using the methods developed by Dixon, Roche and Pate (1993). Results are detailed under 2.3.5.

Little information is available on the impact of predators and the effect of diseases on *Daviesia* species (Brown, 1993). No diseases have been recognised during the study, except a few benign insect galls on *Daviesia divaricata* (common resprouter in Kings Park). The impact of dieback (*Phytophthora* spp.) on the study species is not known, however *Phytophthora* spp. are known to kill many other Kwongan (heathland) plant species. Although dieback has not affected the threatened study species, it may threaten those species and habitats that occur within the susceptible zone in the future.
Leaf and fruit predation by insects occurred but without major damage to the plants. However, quantitative information on reproductive loss due to insect predation is not available.

All study species occur in gravel pits, on road verges or on railway reserves and so far there is no danger of grazing through sheep and cattle. Only young seedlings are threatened by grazing as plants develop prickly or spiny branchlets after six to twelve months.

2.3 Breeding System and Reproductive Biology

2.3.1 Flowering and Seed Biology

The inflorescence of legumes is basically racemose which means the flowers are developed from the axillary meristem. The flowers are bilaterally regular or zygomorphic, basically with five sepals and five petals, with often united sepals. All Fabaceaes have ten stamens. The ovary is superior with a single carpel. Flowering time for most *Daviesia* species is winter or early spring with the exception of *Daviesia spiralis*, which flowers in early summer and *Daviesia speciosa* ms flowering in autumn.

Most species are adapted for bee pollination with an exception of *Daviesia speciosa* ms which is presumably adapted for bird pollination because of the long keel and the bright red colour. Pollination occurs in the early morning, when flowers have ample pollen available and temperatures are high enough for bee activity. Artificial pollination should be finished before the temperature gets too high and bees have collected most of the pollen.

All *Daviesia* species have two ovules in the ovary but in most cases only one develops. The legume fruit is a pod, which is usually dehiscent. It is necessary to collect the seeds just prior to dehiscence or to cover the plants with nylon nets or the ground with paper or plastic.

Daviesia species belong to that group of legumes which include species with ant dispersed seeds. Seed collection is therefore difficult because of high ant activity in late spring or early summer when seed set of *Daviesia* species occurs.

Seed set of *Daviesia* species happens approximately three months after flowering, but can take longer if the season is hot and dry. The seed of common legumes consist of a large embryo with a normally hard seed coat. The cotyledons are usually thick and fleshy and occupy most of the seed's volume which does not contain any endosperm.

All *Daviesia* species and may other legumes have elaiosomes with high starch/ oil contents, which makes the seed attractive to animals.
Upon dehiscence the seed is collected by ants or vertebrates. Seeds collected by ants are most likely stored below the surface to utilise the starchy elaiosome.

The seed bank in the top soil around the plants of the study species contains seeds in small numbers but it is assumed the major part of the seed produced is stored at depth without contributing to the viable seed bank. Seed buried by ants in deeper profiles of the soil may be more immune to predation effects but it is not clear if or how this seed can be drawn up to germination depth (2.3.7 Seed Longevity).

2.3.2 Pollination Study

Materials and Methods

A pollination study of the clonal species *Daviesia speciosa* ms was undertaken at Eneabba and Mingenew in five different populations. Recipient plants were located in all populations. Flowers were hand-pollinated to determine the impact of intra-population selfing and outcrossing from distant populations on fertilisation success and seed set. Three hundred flowers were pollinated, including the control for each population. Per population ten self-pollinations, 40 outcrossing and ten controls were attempted, with a total of 60 flowers per population.

Hand-pollination was undertaken in the morning just prior to pollen release and high bee activity. Donor pollen was collected from distant populations approximately 90 km apart and from within the recipient populations for intra-population selfing. Pollinated flowers were covered with white nylon enclosures and already fertilised flowers were taken off the plants before artificial pollination was undertaken. Flowers were picked twelve hours, 24 hours and three weeks after pollination and were fixed in FAA for two hours and stored in 70 % ethanol before examination of pollen tube growth. Flowers were stained with aniline blue (pH 8.5) or toluidine blue (pH 4.4) for examination of the pistils under a fluorescence microscope. Different procedures of staining pistils and observing pollen tube growth were used, eg. pistils had been soaked in boiling water from 30 seconds up to three minutes in room temperature water, in a solution of one percent sodium hypochlorite or pistils had been soaked from five minutes up to one hour in one percent sodium hypochlorite and treated afterwards with aniline blue or toluidine blue.

Results and Discussion

Hand-pollination, using pollen collected from plants within the recipient population and from plants in a geographically separate population, did not improve seed set in *Daviesia speciosa*. In all hand pollination trials and control flowers, all ovules were aborted and hence all failed to set seed. Microscopic examination of flowers did not show any pollen that had germinated on stigmas to indicate pollen viability. Furthermore, no pollen tube growth could be observed penetrating the stigma and ovary in flowers from all treatments. As observed in earlier years not a single flower, naturally or artificially pollinated, became fertilised and developed seed pods or seed. Therefore, abortion of ovules can be explained by pollen infertility, insufficient fertilisation success or using an inappropriate hand pollination procedure. Further, *Daviesia speciosa* ms is described as a bird pollinated species (pers. comm. Crisp) but field inspections revealed no bird pollinator activity in the vicinity of the plot. Native and European honey bees are normally very active in the early morning, collecting nectar without contracting the stigma because of the anatomy of the long keeled pea-flowers.

Further trials were undertaken to germinate pollen of *Daviesia speciosa* ms on artificial media. Pollen was taken from flowers in early, mid and late flowering season and cultured on artificial media. Components of this media were 15 % sucrose, 100 mg boric acid and 300 mg of calcium chloride. Pollen was directly transferred on filter papers after removal from flowers. Filter papers had been soaked in germination media till saturation. Pollen were then protected with cover slips.

Over a period of ten days pollen germination was observed after staining with aniline blue or toluidine blue.

No pollen germination was observed and pollen infertility is most likely, which does not necessarily threaten the present population structure and dynamics because of the ability of *Daviesia speciosa* ms to reproduce vegetatively. In the future, genetic research could be undertaken to determine the reasons for clonal growth and the lack of seed production.

2.3.3 Plant Reproductive Success

Introduction

Plant reproduction from seeds contains fundamental information about population structure and population biology. To understand the population biology, information should include assessments of seed productivity (seed : ovule ratio), which is a measure of the reproductive success of species; germinability, which is impossible to determine for all species because of often low or no seed set of common and especially of threatened species, and seed dispersal mechanisms or seed longevity in soil.

Legumes bear many more flowers than mature fruits. Furthermore, within flowers of most species, there are many more ovules than those that are matured to seed.
The reproductive success may be limited by a combination of factors including genetic, nutritional, hormonal, predational, seasonal factors, short life span or the availability of pollination vector. Combined, these factors determine the seed quality, including seed viability and germinability, and the seed quantity.

Materials and Methods

Microtoming, by sectioning dead plant material of *Daviesia bursarioides* ms, *D. speciosa* ms and *D. spiralis*, indicated that *Daviesia* species often die after eight to ten years. It is assumed by measuring plants, that after six years the plant crown starts to die, sometimes very rapidly, which affects plant growth, assimilation, flower production and therefore the plant reproductive success.

Assessment of the reproductive success of species is commonly undertaken by determination of the seed : ovule ratio (S:O ratio) in combination with the flower : fruit ratio (F:F ratio).
The S:O ratio may represent the influence of inefficacious pollination, infertility of pollen and/ or fertilisation, resource limitation and/ or lack of genetic resources on the reduction of ovule development and reproductive success. Considered alone, S:O ratios and F:F ratios do not incorporate measures of germinability.

The germinability of seeds of *Daviesia* species will be explained in further detail in sections 2.3.5 and 2.3.6.

In this study S:O ratios and F:F ratios are determined as a measure of the potential reproductive success. The seed : ovule ratio was derived as the total number of potentially viable seeds divided by the initial number of ovules (always 2 ovules for *Daviesia* species).

Seed : ovule ratios were calculated for 20 rare and common *Daviesia* species comprising 13 seeder species, five resprouter species and two species most probably categorised as seeder species.

The fruit : flower ratios could only be estimated for 15 *Daviesia* species, including ten seeder species and five resprouter species.

To determine the ratios, flowers or pods were sampled and examined to quantify the total seed production on a per plant basis. This was done by counting or sampling at least 10 branches, or ten percent of the whole plant to get an estimate for the total seed production by then multiplying this amount of pods or flowers to estimate the total plant reproductivity. The seed : ovule ratio is of special interest to determine the reproductive success.

Results and Discussion

Table 8:
Fruit : Flower (F:F) and Seed : Ovule (S:O) Ratio of threatened and common *Daviesia* species

Daviesia species	Life Form	% Ovules aborted or not developed	No. Flowers or Pods estimated per plant	Fruit:Flower Ratio (F:F) %	No. Ovules estimated per plant	Seed:Ovule Ratio (S:O) %
brevifolia	s	98.9	87 pods, 2 seeds	NA	174	1.1
bursarioides ms*	s	98.1	201079 fl., 7701 pods	3.8	402158	1.9
collectioides	s	98.3	1459 fl., 52 pods	3.5	2918	1.7
daphnoides	s	91.6	1601 fl., 268 pods	16.7	3202	8.3
decurrens	r	99.9	32289 fl., 24 pods	0.1	64578	0.04
divaricata	r	99.8	138945 fl., 340 pods	0.2	277890	0.1
*euphorbioides**	s	99	99253 fl., 2032 pods	2	198506	1
*euphorbioides** (*in cultivar*)	s	96.2	22402 fl., 1295 pods, 1692 seeds	7.5	44804	3.7
hakeoides	NA	95.5	11 pods, 1 seed	NA	22	4.5
juncea	s	95	240 pods, 24 seeds	NA	480	5
nudiflora	r	99.1	912 fl., 17 pods	1.8	1824	0.9
pectinata	NA	98.4	62 pods, 2 seeds	NA	124	1.6
speciosa ms*	clonal r	100	6901 fl., 0 pods	0	13802	0
spiralis[1]*	s	96.1	887 fl., 75 pods	7.8	1924	3.8
spiralis[2]*	s	99.6	1798 fl., 6962 ped., 72 pods	0.8	17664	0.4
spiralis [3]*	s	99.5	1146 fl., 5722 ped., 66 pods	0.9	13868	0.4
*spiralis**	s	99	272731 fl., 5344 pods	1.9	545462	0.9
triflora	r	97.7	53574 fl., 2397 pods	4.4	107148	2.2
uniflora	s	98.9	4328 fl., 93 pods	2.1	8656	1.1

legend:

*: threatened species
fl.: flowers
ped: peduncles
r: resprouter species
s: seeder species
NA: not assessed
[1]: early flowering season
[2]: mid flowering season
[3]: late flowering season

The total number of flowers estimated ranged from 887-138 945 per plant and a total number of pods from 11-7 701. The total number of seeds found or measured of collected plant material or seed production determined for the whole plant vary between zero and 1 692 pods.

Microscopic examination of ovules was then made to assess the relative proportions of fully developed healthy ovules, partially developed aborted ovules and non-developed ovules. Regarding the threatened species in this study, only *Daviesia euphorbioides* and *D. spiralis* partly develop two seeds per pod with about 25 % for *D. euphorbioides* and less than ten percent for *D. spiralis*. All the other species abort at least one of the ovules at an early stage of the fruit developing. Non-developed ovules included both flowers which had not been successfully fertilised or pollinated, and ovules which had been aborted at an early stage after pollination.

Combining all study species, an average S:O ratio of 2.3 % is shown with almost no demarcation between seeder (2.83 %) and resprouter (1.34 %) life forms. The lowest value occurred in the clonal resprouter, *Daviesia speciosa* ms with no ovules maturing to seeds. All ratios are below ten percent and the highest value of 8.36 % occurred in the seeder species, *Daviesia daphnoides* growing in Tathra National Park associated with *D. speciosa ms.*

Of interest are the S:O ratios of *Daviesia euphorbioides* with a S:O ratio of 1.02 % *in situ* and 3.77 % in cultivation. Plants propagated in Kings Park Nursery with adequate watering show higher seed set than plants growing in nature with long and dry summers and no summer rainfall. The seeder species *Daviesia spiralis* showed a S:O ratio of above average of 3.89 % in early flowering season (October), decreased down to 0.47 % in late flowering season (December). This may indicate how hot and dry periods without summer rainfall influence seed set and fertilisation of ovules.

High seed productivity and fertilisation of ovules occur in early flowering season, which means in early spring, and low seed production or fertilisation of ovules in early summer, when fertilised seeds from early flowering season are already close to maturity. This could be a preferential nutrient supply within the plant to develop seed.

Little is known about the impact of seed predators and the effects of diseases on the reproductivity capacity of *Daviesia* species, which are generally regarded to be fairly resistant against pathogens and insect damage (Brown, 1993). Further, no major impact of insect damage was observed during this study influencing the plant reproductivity.

Insect galls at the common resprouter species *Daviesia divaricata* in Kings Park were observed but with no larger decrease in seed production.

2.3.4 Plant Propagation

The basic objective of plant propagation is twofold: to achieve an increase in number and to preserve the essential characteristics of the plant, especially of rare flora. There are two essentially different types of propagation: sexual and asexual.

The three main methods commonly used for the propagation of native Australian plants are seed (sexual) and cuttings or divisions (asexual and *in vitro* cultivation). Methods used for propagation of *Daviesia* species are seed, shoot and rhizome (root) cuttings.

2.3.4.1 Seed Propagation

Seed is the most common means of propagation for plants. It is often the only possible or practical propagation method and is usually the cheapest method. Seeds also offer a convenient method for storing plants, when kept dry and cool. Another advantage is that seed propagation provides a method for propagating disease-free plants. This is especially important with respect to virus diseases. The major disadvantage to seed propagation is the long time required by some plants to reach maturity from seed. Most seed can be sown without treatment but there are some groups, like Fabaceae, which require pre-treatment before germination will commence. These seeds are said to be in a dormant state and this dormancy must be broken.

The easiest type of dormancy to overcome is the physical dormancy possessed by such hard-coated seeds as all *Daviesia* species. Artificially, there are several methods to overcome physical dormancy, like scarifying, nicking or hot water treatment.

These treatments in combination or by themselves tend to breach the hard testa and the seed imbibes water and swells. The swollen seed should then be removed to the sowing punnet for germination.

More detailed studies are mentioned under 2.3.5 'Smoke Experiment' and 2.3.6 'Germination Experiment'.

2.3.4.2 Stem and Root/ Rhizome Cuttings

Materials and Methods

The term 'cutting' refers to any detached vegetative plant part that can be expected to regenerate the missing part or parts to form a complete plant. Cuttings are commonly classified by plant part and in this study only stem and root/ rhizome cuttings were used for propagating for *Daviesia* species. In stem cuttings a new root system must be initiated and in root/ rhizome cuttings a new shoot must be initiated.

Cutting propagation is one of the most important means of vegetative propagation, by separating portions of a plant from the parent plant and getting them to root and so produce new plants, is an important method for multiplying native plants. To reproduce a plant genetically identical to its parent, it must be propagated vegetatively. In this way the progeny are said to belong to the same clone as the parent. Cuttings are a means of clonal reproduction. Some plants do not produce large quantities of viable seed so cutting may be the easiest means of propagating these species.

Stem cuttings were taken from *Daviesia euphorbioides*, *D. speciosa* ms and *D. spiralis* and root/ rhizome cuttings only from the clonal species *D. speciosa* ms. Mainly juvenile cuttings were collected in late winter or early spring by following practical horticultural methods.

Results and Discussions

Table 9:
Asexual plant propagation of threatened *Daviesia* species

Study Species	collected Material	Treatment	Potting Media	Strike Rate
Daviesia euphorbioides	Stem cutting	With Clonex 3[1] and Hormone 20[2]	Hort. mix (quartz sand : peat : perlite/ 2:1:1)	35%
		No further pre-treatment	Hort. mix plus habitat soil (1:1)	1%
Daviesia speciosa ms	Stem cutting	With Clonex 3 and Hormone 20	Hort. mix (quartz sand : peat : perlite/ 2:1:1)	22%
		No further pre-treatment	Hort. mix plus habitat soil (1:1)	0%
Daviesia speciosa ms	Root/ rhizome cutting	No further pre-treatment	Hort. mix plus habitat soil (1:1)	33%
Daviesia spiralis	Stem cutting	No further pre-treatment	Hort. mix plus habitat soil (1:1)	23%

[1] Clonex 3: rooting hormone gel, recommended for softwood and semi-hardwood cuttings; active constituent 3g/l Indole-3-Butyric Acid

[2] Hormone 20: a mixture of hormone growth stimulants for transplanting seedlings and shrubs; regenerating sick and dying trees; promoting growth of indoor plants, orchids and bulbs; Hormone 20 assists in overcoming the shock of transplanting and ensures that plants get away to a quick start. Hormone 20 is generally compatible with all fertilisers and pesticides.

As shown in the table above, propagation of Daviesia species using cuttings is only moderately successful. *Daviesia euphorbioides* is very difficult to propagate from stem cuttings because of the very thick branches with a pithy texture inside. Treatment with Clonex 3 and Hormone 20 does increase propagation success (cutting strike) by 34 % compared to cuttings with no hormone treatment and propagation in clayey habitat soil with one percent strike rate.

Twenty two percent stem cuttings of *Daviesia speciosa* ms rooted after hormonal treatment in horticultural mix compared to zero percent rooting in habitat soil without further pre-treatment.

Root cuttings of *Daviesia speciosa* ms, a clonal resprouter, successfully grew without further pre-treatment in horticultural soil mix for 33 % of cuttings. Young stem cuttings of *Daviesia spiralis* set roots in horticultural soil mix without hormonal pre-treatment for 23 % of cuttings.

Hormonal treatment of stem cutting does increase root growth but habitat soil for propagation should not be used because of the high content of clay which results in a lower success rate for cuttings. In cultivation *Daviesia* species require good drainage and full to reduced sun. They are reasonable hardy plants but occasionally seem to die quickly and without warning from as yet unknown causes. Propagation is easy from scarified seed and growth of most species is reasonably fast.

2.3.5 Smoke Experiment

(Seed germination experiment with common *Daviesia* species focusing on the influence of smoke and pre-treatments)

Introduction

Several external factors in the environment, like water, gases, temperature, light or fire affect germination by determing whether or not germination occurs and the rate at which it does so. Furthermore these factors affect seed viability and life span of seeds.
Internal factors, like dormancy or hard coated seeds, prevent germination even when placed in conditions that are normally regarded as favourable to germination, and may be affected by fire.
This study was designed to determine if smoke is a stimulus for regeneration from seeds in the genus *Daviesia* (Fabaceae) and was set up to determine the most optimal conditions for seed germination.

Aims

The aims of this experiment were to determine the factors influencing germination, namely the influence of smoke, smoked water and fumigated filter papers on germination and the impact of pre-treatments on germination by itself and in combination with smoke treatments.

Three different treatments were used: Experiment 1 will show the influence of smoke fumigation for 60 minutes considering the different pre-treatments; in experiment 2 smoked water in dilutions of 100 % (full strength), 50 % and 25 % were used to compare the effect on germination including the three pre-treatments and the control. Experiment 3 tested the germination of seeds on smoked filter papers watered with sterilised rain water.

Subsequent research by van Perger, B. (unpublished) has determined that germination of a wide range of seeds increase by using an optimal concentration of ten percent smoked water .

Material and Methods

Study species

Five seeder species were examined in this study for recording germination events and to determine the most optimal germination conditions: *Daviesia benthamii, D. cordata, D. flexuosa, D. oppositifolia* and *D. teretifolia.*

Seed Sterilisation

With the exception of *Daviesia cordata*, seeds were sterilised in two percent sodium hypochlorite, vacuum infiltrated twice for five minutes with five minutes rest, and washed in several changes of sterile distilled water. It was noticed that the seeds of *Daviesia cordata* imbibed rapidly during the sterilisation procedure. Therefore, a lower dilution of the sterilent (one percent sodium hypochlorite) was used for this species.

Pre-treatment of Seed

Pre-treatments are necessary for many legumes because of hard coated seeds and resultant seed coat impermeability. Different treatments were tested to determine the most advantageous for *Daviesia* species.

Following the sterilisation, seeds of five species were either nicked under sterile conditions (hereafter referred to as nicking); soaked in sterile hot water for two hours (hot water treatment); soaked in room temperature water for 24 hours (cold water treatment); or seeds were sterilised but otherwise left untreated.

The experiment was divided into three parts:

(1) seeds in punnets kept under greenhouse conditions,
(2) seeds in plastic containers with different dilutions of smoked water, and
(3) seeds in plastic containers on smoked filter papers

Experiment 1:

After pre-treatment and sterilisation, seeds of five species were sown in punnets (9x11x6 cm). The infill of the punnets was a pasteurised soil mixture of sand, peat and perlite in a proportion of 2:1:1. Afterwards seeds were covered with a layer of quartz sand to a depth approximately the diameter of the seed. Sixty punnets representing the three pre-treatments and four replicates were smoke fumigated in the manner of Dixon, Roche and Pate (1994) for a duration of 60 minutes. The remaining punnets (control) also with three pre-treatments were left without fumigation. Punnets were kept under greenhouse conditions and watered with rain water as necessary.

Experiment 2:

The seeds in this section of the study were incubated in eleven cm round plastic containers under controlled incubated conditions at a constant temperature of 18°C in darkness. Four to five one cm square sponges were placed in the base of the plastic containers and filter papers with ten seeds on it were placed upon them. Approximately fifteen ml of smoked water of 25 %, 50 % or full strength (100 %) was added to each container. Control replicates were moistened with sterilised rain water.

Smoked water was generated by bubbling smoke through a 20 litre drum of distilled water for a period of 60 minutes (Dixon, Roche & Pate, 1994). The experiment consisted of five species with three pre-treatments in combination with four treatments (smoked water in different dilutions) including the control without pre-treatment, plus three replicates for each series.

Experiment 3:

Seeds were placed on smoked filter papers produced by fumigation. Control replicates were placed on plain filter papers soaked with distilled water. Sterilised rain water was added to all plastic containers which were incubated as for (two) above. This trial had a total number of 60 plastic containers representing five species with three pre-treatments plus the control, and replicated three times.

All trials were scored twice a week for ten weeks for evidence of germination.

Results and Discussion

Statistical Analysis

All germination data was Arcsine transformed. Prior to treatment of the data, Analysis of Variance (ANOVA), Fisher's PLSD and Scheffe tests were used to determine significant differences between treatments, pre-treatments and a combination of both at a significance level of 95 %.

Summary of significant or non-significant germination responses of common *Daviesia* species after different smoke treatments

Table 10:
Seed germination of common *Daviesia* species after smoke fumigation for 60 minutes

Smoke fumigation for 60 minutes				
Treatment	Fumigation			
Pre-treatment	Nicking	Hot water treatment	Cold water treatment	Without pre-treatment
Daviesia species				
D. benthamii	ns, -	ns, -	ns, -	ns, -
D. cordata	ns, -	s, -	ns, -	ns, +
D. flexuosa	ns, +	s, -	ns, -	ns, -
D. oppositifolia	s, -	ns, +	ns, +	ns, 0
D. teretifolia	ns, -	ns, -	ns, +	ns, +

Table 11:
Seed germination of common *Daviesia* species under incubated conditions with different dilutions of smoked water

Treatment	Smoked water in different dilutions											
	100% (full strength)				50%				25%			
Pre-treatment	Nicking	Hot water treatment	Cold water treatment	Without pre-treatment	Nicking	Hot water treatment	Cold water treatment	No pre-treatment	Nicking	Hot water treatment	Water treatment	Without pre-treatment
Daviesia species												
D. benthamii	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -
D. cordata	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -	NA	s, -	s, -	ns, -
D. flexuosa	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -	ns, -	ns, -	ns, -	s, -
D. oppositifolia	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -	s, -	ns, -	ns, -
D. teretifolia	s, -	s, -	s, -	s, -	s, -	ns, -	NA	ns, -	ns, -	ns, -	NA	ns, -

Table 12:
Seed germination of common *Daviesia* species on smoke fumigated filter papers

Treatment	Germination on fumigated filter papers			
Pre-treatment	Nicking	Hot water treatment	Cold water treatment	Without pre-treatment
Daviesia species				
D. benthamii	ns, -	ns, +	ns, +	ns, -
D. cordata	NA	ns, -	ns, +	ns, +
D. flexuosa	ns, -	ns, -	ns, +	ns, +
D. oppositifolia	s, -	ns, +	ns, -	ns, -
D. teretifolia	ns, 0	ns, 0	NA	ns, +

Tables 9 to 11 showing significance's or non-significance's in germination of smoke treated trials in comparison to the controls for each pre-treatment

legend:

ns: non significant response to smoke treatment
s: significant response to smoke treatment
+: enhancement in germination compared to control
-: decrease in germination compared to control
0: no difference in germination compared to control
NA: not assessed

Smoke fumigation does not increase germination but rather decreases the percentage of germination. The influence of smoked water in any dilutions has in general a negative influence on germination with no germination by using 100 % smoked water and considerable decrease of germination by using 50 % and 25 % smoked water. Depending on the species and pre-treatments germination on filter papers may enhance or decrease germination, and germination results are very similar to germination in the controls on plain sterilised filter papers.

The sterilisation method by using two percent sodium hypochlorite and vacuum infiltration twice for five minutes with five minutes rest in between is applicable for most of the species, except *Daviesia cordata*. This species imbibed sodium hypochlorite during the sterilisation process and cotyledons and seed radicles become damaged, and no objective germination results could be determined. A one percent concentration of sodium hypochlorite achieved better results with no imbibition of any species during sterilisation. Infiltration twice for five minutes with five minutes rest in between might not be necessary. One stage of sterilisation is probably enough to avoid contamination and would achieve the same sterility without damaging or stressing the seeds. An alternative to the use of sodium hypochlorite could be the use of Benlate (fungicide) for *in vitro* seed germination.

Nicking as a standard pre-treatment achieved in general highest germination and is so far the most recommendable pre-treatment for legumes. It is not clear why nicked seeds of *Daviesia cordata* show such a low germination compared to seeds which have been soaked in hot water under the same conditions. Possibly low germination occurs when seeds of *Daviesia cordata* imbibe too quickly after pre-treatment or no imbibition occurs at all without any pre-treatment. Soaking seeds in hot water for two hours or in cold water for 24 hours attains higher germination than the control replicates but germination would be probably higher by soaking seeds only a few minutes in hot water and reduced time in cold water. Some legumes only require five seconds boiling and longer times had decreased germination because the seeds took up boiling water and were cooked (pers. comm. Atkins, 1995).

Stronger dilutions of smoked water (full strength and 50 %) added to the plastic containers depressed germination drastically, while the weaker dilution of 25 % smoked water caused similar germination results to germination on smoked filter papers. Apart from *Daviesia flexuosa* none of the study species germinated by using 50 % smoked water. *Daviesia flexuosa* is obviously more tolerant to smoked water in strength of 50 % and 25 %.

Depending on the species the impact of smoke fumigation, smoked water or germination on smoked filter papers has almost no enhancing effect on germination, it rather depresses germination drastically by using too high concentrations of smoke or smoked water.

Of interest are the different pre-treatments with highest germination after nicking seeds. No after-ripening process is required for legumes but nicking or hot and cold water treatment does increase the permeability of the seed coat which results in higher germination.

Regarding the germination *in situ*, most *Daviesia* species flower in winter with a seed set in spring or early summer before the climax of the summer temperature. The hard seed coat is necessary to prevent the seed from dehydration and for long term storage in the seed bank. Extreme heat or bush fires probably crack the seed coat and increase germination but the influence of smoke (smoke chemical) on germination has obviously no positive effect on seed germination of *Daviesia* species. Scarification (nicking) of the seed coat during mechanised disturbance is also an effective means of breaking the seed dormancy.

2.3.6 Germination Experiments

Germination is a series of events from dormant seed to growing seedling and is dependent upon seed viability, the breaking of dormancy and suitable environmental conditions.

2.3.6.1 Germination experiment of *Daviesia spiralis* under sterile controlled conditions

Materials and Methods

Germination is a series of events from dormant seed to growing seedling and is dependent upon seed viability, the breaking of dormancy and suitable environmental conditions. These external conditions are temperature, moisture and oxygen.
The aim of this experiments was to determine if germination could be influenced under sterile incubated conditions.

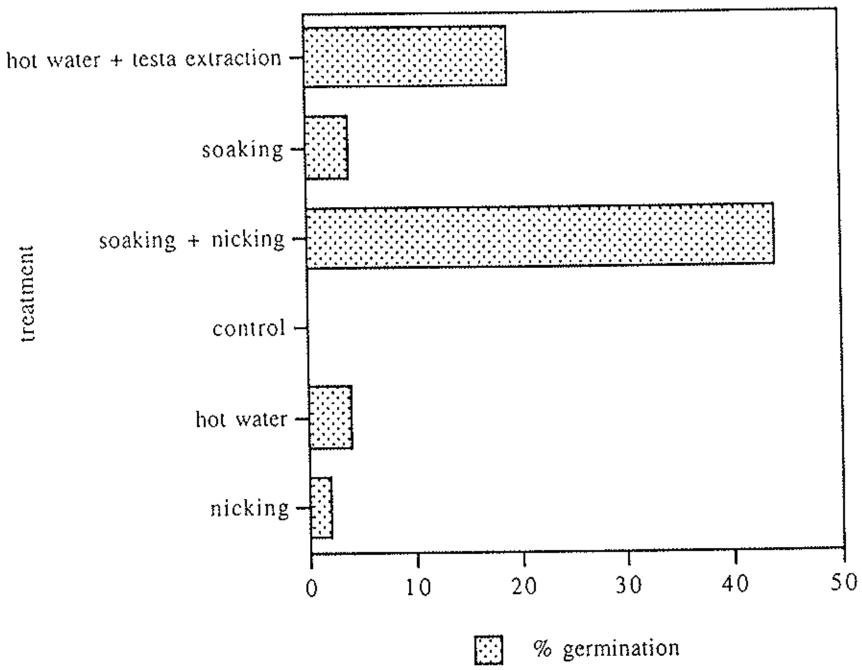
This germination study of *Daviesia spiralis* was set up under sterile controlled conditions in darkness and constant room temperature of about 20°C. Ten trials were set up with ten seeds per replicate, replicated five times. Seeds were sterilised in two percent sodium hypochlorite and twice vacuum infiltrated for five minutes with a five minute rest. All seeds were washed three times with sterile water to avoid contamination. Germination trials were treated with Du Pont Benlate DF Fungicide in a dilution of 0.5 % to avoid fungal diseases and contamination.

Different pre-treatments for seed, like nicking, soaking in hot or room temperature water, testa extraction or combinations of different treatments were used to determine if seed germination could be increased by mechanically breaking of dormancy without the influence of smoke. After pre-treatment seeds were transferred onto filter papers, placed on square sponges, in petri-dishes and watered with sterilised rain water as required.

Germination events were recorded twice a week for a duration of ten weeks.

Results and Discussion

Graph 1:
Seed germination of *Daviesia spiralis* under controlled conditions at 18°C



Daviesia spiralis responded most with a highest germination of 44 % in response to a combination of soaking seed in room temperature water and nicking after soaking. The second highest germination of 19 % occurred after soaking seeds in hot water and allowed to cool, plus testa extraction. Seeds in the control without any treatments did not germinate. All other treatments showed only marginal germination and it is rather surprising, why hot water treatment and nicking had a very low effect on germination. This was probably caused by aggravated water uptake, low humidity or low germinability of seeds.

Fifty three percent germination of *Daviesia spiralis* was achieved, when seeds were 'dry nicked' and than soaked for 1.5 hours in room temperature water. After pre-treatment seeds were transferred in seedling punnets with a soil mix of sand, pit and perlite.

This trial was set up in winter and spring (July-October) with cool night temperatures of about 12°C and short days with maximum temperatures of 22°C.

2.3.6.2 CALM germination trial of threatened *Daviesia* species (by Anne Cochrane, Research Scientist, CALM, Como, Western Australia)

Table 13:
Germination of rare *Daviesia* species under incubated conditions

***Daviesia* species**	**Mean germination**
Daviesia campephylla ms	89%
Daviesia megacalyx ms	92%
Daviesia microcarpa ms	94%
Daviesia oxylobium ms	91%
Daviesia pseudaphylla	94%

The studies undertaken by CALM determined germination of rare *Daviesia* species of the south coast and the wheatbelt. Seeds were pre-treated by pouring boiling water onto it and soaked for two hours. Seed was then placed onto vermiculite and incubated at a constant temperature of 15°C with 12 hours artificial light a day.

CALM achieved extremely high germination between 89 % and 94 % (Table 12). Legume seeds are not sensitive for light during imbibition and germination, so the light factor would not make any difference. Pouring hot water onto seeds as a pre-treatment and incubating seeds achieved successful germination.

Unclear is, why hot water treatments in different germination trials achieved extreme different results. This could be caused by low germinability of seeds or failure in experimental procedure.

2.3.7 Seed Longevity

Materials and Methods

The abundance of seedling recruitment after disturbance events is largely defined by the ability of species to maintain a proportion of each annual seed fall in the soil bank.

Therefore, even with very low annual seed output, species may accumulate sufficient viable seeds in the soil to effect more than adequate seedling : parent replacement after a germination event. No research is available to suggest that the 'weathering effect' has any influence on seed viability and testa impermeability of *Daviesia* species. Consequently, a seed burial study was undertaken for a period of one year.

Seeds from four seeder species, *D. bursarioides* ms (threatened), *D. daphnoides* (common), *D. euphorbioides* (threatened) and *D. spiralis* (threatened) were collected when mature and dehiscing (November to January 1993/94) in the wheatbelt of Western Australia. Seeds were dry-stored under laboratory conditions for two months in darkness and at room temperature. Ten seed replicates per species containing ten seeds of each were placed in flat nylon enclosures (five cm^2) with a mesh opening of 0.5 mm^2. Enclosures were then attached to galvanised nails and placed in habitat soil at Eneabba, Three Springs and Wongan Hills in March 1994. Ten replicates of each species were placed at surface level and ten enclosures were buried in five to ten cm depth, with each replicate spaced several metres apart. Seed samples were collected after six and twelve months and then inspected under a dissecting microscope to separate viable and non-viable seeds.

Results and Discussion

Table 14:
Seed viability* of *Daviesia* species 14 months after harvesting

Daviesia species	Viable Seeds (%)		
	Treatment		
	seeds buried for 6 months	seeds buried for 12 months	seeds for 12 months on surface soil
D. bursarioides ms	100	65	NA[1]
D. daphnoides	NA[1]	75	NA[1]
D. euphorbioides	57	50	75
D. spiralis	40	35	62

NA[1]: not assessed

*viability assessed by microscopic extraction of seed

Seeds excavated after six months did show a slight reduction in size with a totally dried out elaiosome still attached to the seed or partly removed. No changes of the physical dormancy (hard seed coat) were noticed. Seeds were sown in punnets as mentioned in 2.3.5 and 2.3.6 and observed another six months without germination.

All seeds were perfectly 'healthy' looking with no elaiosomes attached to the seed coat, except the seeds of *Daviesia euphorbioides*, which had the elaiosome still attached to the testa.

The seed coat of viable seed became harder while buried in habitat soil (and during the germination trial), and the embryo is green. Seed without a viable embryo has a soft seed coat and the embryo is yellow. It is hard to determine if the seeds were 100 % viable after harvesting but the results of the germination experiments showed, all seeds had a viability of close to 100 % after pre-treatment.

Only *Daviesia bursarioides* ms kept its high viability of 100 % recorded by microscopic extraction. Viability of *Daviesia euphorbioides* and *D. spiralis* deteriorated to more than 40 % for *D. euphorbioides* and 60 % for *D. spiralis*. There was no evidence of predation in any of the deteriorated seeds.

Seed viability of *Daviesia bursarioides* ms buried in habitat soil for twelve months was reduced to 65 % compared to 100 % viability six months earlier. *Daviesia daphnoides* kept a relatively high viability of 75 % and *D. euphorbioides* as well as *D. spiralis* reduced its viability to 50 % and 35 %, which means a loss of viability of five to seven percent. The seed coat of *Daviesia euphorbioides* was very dry and deeply cracked.

Almost no data was determined for seed enclosures, fixed for 12 months on the soil surface in full sun or partly shaded, because of seed predation vertebrates within three weeks after setting up the experiment.

Seeds of *Daviesia euphorbioides* reduced its viability to 25 % compared to 50 % viability for seeds buried in five to ten cm deep. The viability of *Daviesia spiralis* seed is slightly (but not significantly) higher than seed viability of buried seed buried.

The seed of *Daviesia spiralis* is much smaller than for *D. euphorbioides* and the coat is harder to abrade, indicating that the embryo is probably better protected. Furthermore *Daviesia spiralis* seed did not show any changes of its seed coat and size but the seed of *D. euphorbioides* was slightly smaller in size and had developed fine cracks in its testa. Seed predation was only observed in *Daviesia euphorbioides* seed with hollow or partly hollow seeds.

3. Soil Analysis

Materials and Methods

Twelve soil samples (see App. 1: Sample 1-12) were taken from different sites in the wheatbelt and sand plains of Western Australia, where six study species occur.
All samples were collected over a period of nine months and oven dried at a temperature of 100°C. Before taking the soil samples all fresh and dry leaf litter was taken off the top soil, so that the actual content of organic matter in the soil was determined.

Results and Discussion

The pH (H_2O) of all samples varied between 5.2 and 9.1, but most *Daviesia* species rather prefer acidic soils than alkaline. *Daviesia microcarpa* ms is the only study species growing in alkaline soil and with a high salinity.

All samples contain a high portion of sand (75.5 - 98.5 %) and a low amount of clay (0.5 - 16 %)which explains the low content of nutrients.
The amount of silt varies from < 0.5 and 10 % in all soil samples. The large amount of sand and silt with low capacity for binding nutrients and the high proportion of stones (15 - 72 %) in all soil samples with almost no capacity to retain nutrients, explains the poor vegetation cover on road verges and the difficult situation for young seedling recruitment of *Daviesia* species.

Thus, the content of organic carbon (org C), total nitrogen (N),phosphorus (P) and potassium (K) are extremely low in all samples with nitrogen at 0.044 % extremely limited. However, the N-fixing ability of the study species is likely to contribute to a better balance of nitrogen for plant growth.

(Appendix 1: Soil analysis from different sites in the wheatbelt and sand plains of Western Australia)

Discussion and Management Considerations

Daviesia species are so called 'disturbance opportunists'. All threatened study species, including *Daviesia bursarioides* ms, *D. euphorbioides*, *D. microcarpa* ms, *D. purpurascens*, *D. speciosa* ms and *D. spiralis*, occur on road verges, graded areas or in gravel pits. Plant presence at those sites is most likely a result of habitat disturbance, eg. for agricultural purposes, road maintenance work or gravel extraction. Part or whole road verges or reserves were cleared or graded, which has promoted recent germination of *Daviesia bursarioides* ms, *D. euphorbioides*, *D. speciosa* ms and *D. spiralis*.

Daviesia euphorbioides is not just a 'disturbance opportunist', coming up after soil removal, but also aggressively resprouts after mechanical damage with aerial shoots. Problematic is the sudden plant death of *Daviesia euphorbioides* during summer months (summer 1994/ '95), which wiped out almost whole populations, including juvenile and adult plants.

Daviesia speciosa ms resprouts from rhizomes after mechanical disturbance and can increase its number by root suckers, reproducing new plants with masses of grey thorny leaves and covers an area of several square metres. This study species resprouts aggressively with shoot growth up to 25 - 30 cm in the first growing season after disturbance.

Sexual reproduction by seed was never observed for *Daviesia speciosa* ms and pollination studies over a period of two flowering seasons *in situ* and *ex situ* conditions failed. This study species has long keeled pea-flowers, presumably adapted for bird pollination, but no birds were observed pollinating flowers during studies. Native and European honey bees rob nectar in early morning hours, without touching the anthers or stigma. A lack of pollinators might be a reason for the lack of sexual reproduction.

Staining flowers for pistil examination under the fluorescence microscope did not show any pollen tube growth, nor pollen germination. Further no pollen germination was observed on artificial media after pollination was done artificially.

This could be due to an inappropriate hand-pollination procedure, but is most likely caused by pollen infertility or insufficient fertilisation success, because of high flower abortion rates.

Only one population of *Daviesia bursarioides* ms was just recently discovered on private property in natural habitat around Three Springs. This population of about > 100 plants should be fenced and carefully managed in collaboration with the land owner for conservation and further studies, like seed bank analysis in natural habitat soil and seedling recruitment of a partly undisturbed population.

The plant reproductive success of all study species, considering seed : ovule ratios and seed set, is extremely low with early flower abortions. This is most likely caused by a lack of water, but could also be related to a lack of pollinators or insufficient fertilisation success.

Plant propagation from seed is the easiest and cheapest way to propagate legumes, but the physical dormancy has to be broken to achieve reasonable germination results. Nicking or soaking seed in hot water are the most preferable methods increasing germination with even higher results in a combination of both. High temperature under incubated conditions after pre-treatment of seed can increase germination close to 100 percent.

Heat treatment, like controlled burning in habitats of threatened *Daviesia* species, would brake the physical dormancy and increase recruitment from seed. Unknown is the impact of controlled burning in these habitats and the presence of a sufficient seed bank for seedling recruitment.

It could be done in cycles of six to eight years after seed set occurred, using the high viability of young seed and to avoid seeds predation by vertebrates or insects. The risk is of wiping out whole populations by controlled burning at the wrong time of the year or after low seed set. An alternative to fire is habitat disturbance by light 'scarification' of the top soil to brake the physical dormancy of seed. This could also be done in cycles of six to eight years to maintain populations with ongoing seedling recruitment.

The impact of smoke fumigation or 'smoked' water depresses germination drastically at low to high concentrations.

The seed longevity experiment indicates that the viability of *Daviesia* species decreased rapidly within fourteen months. Low seed set combined with a rapid decrease of seed viability in habitat soil within one growing season indicate the limited seed bank and ability of seedling recruitment. The weathering effect on seeds, buried in 5 to 10 cm depths, affected especially seeds of *Daviesia euphorbioides*, with cracks in the seed coat and soft embryos.

Because of nutrient poor and sandy gravelly soils and habitat disturbance, the plant diversity is limited in almost all threatened *Daviesia* populations. All *Daviesia* species prefer an open stand in full sun and no dominance of taller species. *Daviesia* species growing under *Allocasuarina* species., like *Daviesia bursarioides* ms and *D. euphorbioides*, suffer extremely from a lack of light and water with slow growth and even lower seed set.

The short life cycle of *Daviesia* species (plants seem to die after six to eight years) and first sexual reproduction after three years indicate, combined with low seed set and 'short term' viability of seed, the problematic situation of these species.

Annual seed collection and cryostorage is absolutely necessary of setting up a seed resource for future rehabilitation projects. If controlled burning or shallow grading will be done for seedling recruitment, *ex situ* plant propagation of these threatened species should be done to ensure the availability of plant resources, if *in situ* treatment fails.

Suggested Management Considerations:

1. Further survey for populations and annual monitoring of known populations.
2. Annual seed collection and cryostorage to keep seed viability and establish seed resources.
3. Careful management of road verges and gravel pits to stimulate seedling recruitment every six to eight years by controlled burning or shallow grading. *Daviesia speciosa* ms has a clonal root system and resprouts after shoot and root disturbance. Shallow grading every six to eight years could increase the number of plants.
4. Intensive weed control at all populations of *Daviesia euphorbioides.*
5. Remove tall *Allocasuarina* species overcrowding *Daviesia bursarioides* ms.
6. Fence and protect populations of *Daviesia bursarioides* ms and *Daviesia speciosa* ms on private properties from any further disturbance to maintain population dynamic in natural habitat.
7. Stop extracting gravel from gravel pits in Wongan Hills and Tathra National Park to protect *Daviesia speciosa* ms and *D. spiralis.*
8. Genetic research could be undertaken to explain clonal structure of *Daviesia speciosa* ms.
9. *Ex situ* cultivation of all threatened species for future reintroduction.
10. Encourage local communities and conservation groups of taking responsibilities for threatened flora the organisation of public campaign.

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Graph Summary:

Table Summary:

Appendix: Soil analysis from different sites in the wheatbelt and sand plains of Western Australia

Table 15: Legend for Soil Analysis

Sample No.	Species	Location
1	*Daviesia purpurascens*	Corrigin-Hyden Rd., wheatbelt
2	*Daviesia bursarioides*	Midland Rd., Three Springs
3	*Daviesia purpurascens*	Coolgardie, Gnarlbine Rock
4	*Daviesia speciosa*	Tathra National Park, Eneabba
5	*Daviesia spiralis*	Wongan Hills - Piawaning Rd., Wongan Hills
6	*Daviesia speciosa*	Tathra National Park, Eneabba
7	*Daviesia speciosa*	Yandanooka Rd., Mingenew
8	*Daviesia euphorbioides*	Wongan Hills - Piawaning Rd., Wongan Hills
9	*Daviesia bursarioides*	Perenjon Rd., Three Springs
10	*Daviesia speciosa*	Yandanooka Rd., Mingenew
11	*Daviesia bursarioides*	Eneabba-Three Springs Rd., Three Springs
12	*Daviesia microcarpa*	Eyre Hwy., Norseman

all soil samples without organic top soil, depth of score about 5-10 cm
from different places in population

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Kings Park Nursery
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ATTENTION: T. Schwarten

Report on 14 samples of soil from Wheat Belt
received on 26-MAY-1994

28-JUN-1994

LAB NO	SAMPLE	Stones	pH (H2O)	pH (CaCl2)	EC (1:5)	Al (CaCl2)	OrgC (W/B)	N (total)
93A		%			mS/m	ppm	%	%
1662001	1	15	6.3	5.6	9	1	1.40	0.058
1662002	2	44	5.5	4.8	8	2	1.42	0.081
1662003	3		6.8	6.0	2	<1	0.45	0.017
1662004	4		[illegible].0	5.0	3	1	0.77	0.028
1662005	5	36	5.9	5.0	2	<1	1.57	0.050
1662006	6	60	5.5	4.6	1	2	0.35	0.018
1662007	7	45	6.0	5.0	2	<1	0.31	0.020
1662008	8	24	5.2	4.7	3	1	1.40	0.058
1662009	9	59	6.4	5.4	4	<1	0.88	0.053
1662010	10	45	5.7	4.7	5	1	0.92	0.046
1662011	11	72	5.7	5.0	7	1	1.38	0.071
1662012	12	44	9.1	8.2	10	<1	0.42	0.030

Stones		= Stones greater than 2mm
pH	(H2O)	= pH (1:5) in water
pH	(CaCl2)	= pH (1:5) in 0.01M CaCl2
EC	(1:5)	= Electrical Conductivity (1:5) at 25 deg C
Al	(CaCl2)	= Aluminium Al, extracted in 0.01M CaCl2
OrgC	(W/B)	= Organic Carbon C, Walkley and Black method
N	(total)	= Nitrogen N, total
%		= per cent
ppm		= parts per million
mS/m		= milliSiemens per metre

The results apply only to samples as received.

Non-chargeable cost $2174.00

DJ Allen for

N.E. ROTHNIE
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ATTENTION: T. Schwarten

Report on 14 samples of soil from Wheat Belt
received on 26-MAY-1994

28-JUN-1994

LAB NO	SAMPLE	P (total)	P (PRI)	P (HCO3)	K (HCO3)	Sand	Silt	Clay
94SA		ppm	mL/g	ppm	ppm	%	%	%
1662001	1	45	5.0	2	150	88.5	4.0	7.5
1662002	2	98	27	5	160	85.5	5.0	9.5
1662003	3	26	5.0	<2	44	94.5	1.0	4.5
1662004	4	16	0.7	<2	14	98.5	0.5	1.0
1662005	5	76	780	<2	91	82.0	9.5	8.5
1662006	6	34	22	<2	35	91.0	2.5	6.5
1662007	7	41	48	<2	56	81.0	3.0	16.0
1662008	8	42	30	<2	96	85.0	4.5	10.5
1662009	9	94	8.9	2	120	89.0	2.5	8.5
1662010	10	55	19	<2	78	86.5	3.5	10.0
1662011	11	93	40	4	100	78.5	6.5	15.0
1662012	12	33	49	<2	94	75.5	10.0	14.5

P (total) = Phosphorus P, total
P (PRI) = Phosphorus Retention Index
P (HCO3) = Phosphorus P, extracted in 0.5M $NaHCO_3$ (1:100)
K (HCO3) = Potassium K, extracted in 0.5M $NaHCO_3$ (1:100)
Sand = Sand, 2.0 to 0.02mm
Silt = Silt, 0.02 to 0.002mm
Clay = Clay, less than 0.002mm

..../2

%   = per cent
ppm   = parts per million
mL/g   = millilitres per gram

The results apply only to samples as received.

Non-chargeable cost $2174.00

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