VERTICORDIA (6) INTERIM RECOVERY PLANS (IMPLEMENTATION) ESU 00006465

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1. GENERAL INTRODUCTION

The South Western Botanical Province of Australia, which spans from Shark Bay in the north to Israelite Bay on the south coast is renowned for its plant species diversity and high level of endemism (Marchant 1973, Hopper 1979, James and Hopper 1981, Hopper 1992). At the species level an accurate census of the flora is still incomplete but it is estimated that at least 8000 species occur with 75% found nowhere else (Hopper 1992). This flora is dominated by speciose genera of woody perennials in families such as Myrtaceae, Proteaceae, Fabaceae, Epacridaceae and Mimosaceae.

For the purpose of understanding patterns of plant species richness and diversity in south-western Australia Hopper (1979, 1992) has recognised three biogeographical zones based on rainfall. These are the High-Rainfall Zone (800- 1500 mm annual rainfall), the Transitional-Rainfall zone (300- 800 mm annual rainfall) and the Arid Zone (less than 300 mm annual rainfall). Speciation in the flora has been most extensive in the Transitional-Rainfall zone. Moreover, species turnover across this zone is very high with similar vegetation types within 0.5 km of one another having 40% of species in common (Griffin et al. 1990). As a consequence many plant species have restricted ranges and it is uncommon to find species that occur throughout much of the region. The Transitional Rainfall Zone has also been the focus for agricultural development in south-western Australia with 93% of the original vegetation cleared (Saunders and Hobbs 1992). Because of the high level of endemism, the large numbers of species with restricted ranges and extensive clearing of native vegetation for agriculture, Western Australia has a large number of rare and threatened plants. For many taxa little is known about the relative importance of the biological and ecological factors constraining population growth. This information is urgently needed for the development of informed management and recovery programs.

The genus *Verticordia* occurs predominantly in the South West Botanical Province where there are 90 species. with the majority of taxa occurring in the Transitional-Rainfall Zone (George 1991). Currently within *Verticordia* there are 6 taxa ranked as critically endangered, 9 taxa ranked as endangered and 3 taxa ranked as vulnerable.

The research described in this report provides information on the biology and ecology of four critically endangered taxa *Verticordia albida*, *Verticordia fimbrilepis ssp. fimbrilepis*, *Verticordia staminosa ssp. staminosa*, and *Verticordia spicata ssp. squamosa*. The research was undertaken with support from the Natural Heritage Trust Endangered Species Program Grant ESU. 00006465. The research program initially aimed to investigate the biology and ecology of six *Verticordia* taxa and was designed on the understanding that there would be a three-year commitment to funding the project but, unfortunately, funding did not continue beyond the second year. Consequently the research and data sets are incomplete and investigations into the biology and ecology of two taxa *Verticordia plumosa var. ananeotes* and *Verticordia plumosa var. pleiobotrya* which were planned for two flowering seasons at the end of the second and third years, were not undertaken. Moreover, because data sets are incomplete it is not possible to interpret results or draw conclusions. The report therefore describes the research and summarises the results of investigations in four taxa for the period of the funding.

2. THE CONSERVATION BIOLOGY OF *VERTICORDIA ALBIDA*

2.1 Background

2.1.1 Current conservation status

Verticordia albida is currently known from 4 populations. The species is currently ranked as critically endangered under World Conservation Union IUCN Red List Criterion B1 + 2c (World Conservation Union 1994).

2.1.2 Description

Verticordia albida is a tall shrub to 2.6 m high with rounded leaves 2-4.5 mm wide. The otherwise white flowers have a pink centre and are held in dense spikes.

2.1.3 Distribution, landscape context and habitat

The four populations of *V. albida* vary in size and landscape context occurring across a range of 40 km in the north central wheatbelt region of south-western Australia near Three Springs approximately 300 km north-east of Perth. The area has been extensively cleared for agriculture with remnants of the original native vegetation occurring in patches of varying size shape and health. Two of the populations occur in highly disturbed road verges, one population occurs in a relatively small (32 ha) remnant of undisturbed vegetation and one population in a disturbed rail reserve. The remaining plants are all found growing in scrub heath on yellow sands.

The area has a Dry Mediterranean climate with cool wet winters and dry hot summers. Mean annual rainfall at the nearest weather station in Carnamah is 389 mm with rain falling predominantly between May and September; summer rainfall does occur but is unpredictable. The mean maximum and minimum temperatures in the warmest month January are 35.9° C and 18.3° C and in the coolest month July are 17.8° C and 7.3° C respectively.

Table 2.1. The status and approximate size of V. fimbrilepis ssp. fimbrilepis populations and sub-populations

Population	Land status	Approx. population size
Wilton Wells Rd	Private property remnant	1000 +
Three Springs-Eneabba Rd	Road verge	100 +
Sweetman Rd	Road verge	13
Lynch Rd	Rail reserve	7

2.1.4 Research objectives

The aim of the project was to undertake research on the biology and ecology as outlined in the Draft Interim Recovery Plan. The research was undertaken with support from the Natural Heritage Trust Endangered Species Program Grant ESU 00006465. The research program was designed on the understanding that there would be a three-year commitment to funding the project but, unfortunately, funding did not continue beyond the second

year. Consequently, the research and data sets are incomplete and it is not possible to interpret results or draw conclusions. This report therefore limits itself to describing the research and summarising the results within scope items for the period of funding.

2.2 Action 01. Investigate and report on the soil seed bank dynamics and the role of disturbance, competition and rainfall on recruitment and seedling survival

2.2.1. Soil seed bank

The presence of seed in the soil was investigated for *V. albida* in the Wilton Wells and Three Springs-Eneabba Rd populations. There is no information on the dispersal of *Verticordia* fruits but observations of the accumulation of fruit beneath plants suggest that the highest density of seeds in the soil is likely to occur under or adjacent to the canopies of existing plants. Consequently soil samples were collected under the drip line of individual plants. The number of plants sampled varied between populations with soil cores being collected from beneath five plants in the Wilton Wells population and two plants in the Three Springs-Eneabba Rd population.

Soil samples were collected at 30 cm intervals beneath the canopy drip line of each plant using a 10-cm diameter metal cylinder pushed 3 cm into the soil. Samples were stored in calico bags and transported to the laboratories where differential sieving separated fruits of both species from the soil. The presence of a viable seed in each fruit was determined by sectioning the fruit with a sharp blade. Seeds were considered viable if they contained a fleshy embryo. The results are presented in Table 2.2.

Table 2.2. Density and viability of Verticordia albida fruit in the soil seed bank along the drip-line of individual plants

Site	Number of plants sampled	Mean density of fruit per m^2 (± SE)	Mean density of fruit with a viable seed per m^2 (± SE)
Wilton Wells	5	184.7 ± 58.3	12.8 ± 3.2
Three Springs-Eneabba Rd.	2	45.9 ± 14.0	0.0 ± 0.0

The mean density of fruits and fruits with a viable seed varied across the two populations. Further research is needed to determine whether the variation is population specific or reflected temporal variation.

2.2.2 The influence of smoke in stimulating germination of soil stored seed

The influence of smoke in stimulating germination of *V. albida* seeds stored in the soil was investigated in the field. In July 1998 aerosol smoke was applied to soil around two clumps of plants and an individual plant at the Wilton Wells population and around two individual plants at the Three Springs - Eneabba road population. Plots were marked out around each clump with a 3m x 1m plot or a combination of 1m x 1m and 2m x 1m plots depending on the density of the vegetation surrounding the *Verticordia* plants. Wooden stakes, 900 mm long, were hammered in to the ground, creating 1m x 1m, 2m x 1m or 3m x 1m plots. A plastic tent was draped over

the marked plots and the sides held down to the ground using sand-filled 'snakes' or plain sand to provide a relatively 'smoke tight' chamber. Into each tent was placed an outlet pipe, which led back to the smoke drum. The smoke drum consisted of a 60 L drum with a lid and small (25mm) metal pipe welded to a hole on the lower portion of the drum, creating an inlet. A larger (45mm) pipe was welded to a hole near the top creating an outlet pipe. The outlet pipe split into three smaller outlets approximately 50 cm from the drum. One small outlet was sealed and two metal pipes were attached to the two remaining outlets. Each pipe was then run to a tent, under the sand 'snakes' and into the tent cavity. A fan was inserted into the inlet pipe and air blown into the drum. A 12V car battery powered the fan and speed was controlled using a voltage regulator. A fire was lit in the drum and stoked with leaves and other green material to produce a thick black smoke. The lid was placed over the top, forcing all of the smoke out through the outlet pipes into the smoke tents. Soil was smoked for an hour, the fire being stoked periodically during that time. After this time the fan was turned off, the fire extinguished and the tents removed, leaving only the stakes. A brown or dark discolouration remained on the soil showing which areas had been smoked.

Germinants were recorded in late November 1998 and survival of seedlings noted throughout 1999. Unsmoked soil adjacent to smoke-treated soil was used as a control and was also examined for the presence of seedlings. The procedure was repeated again in late May 1999 and seedling emergence monitored in October 1999. The results are presented in Tables 2.3 and 2.4.

Table 2.3. The emergence of V. albida seedlings from the soil seed bank following exposure to smoke and subsequent survival of seedlings. Results of July 1998 smoke trial

Population and treatment	Area treated	No. seedlings 12/11/1998	No. seedlings surviving 11/2/1999	No. seedlings surviving 30/3/1999
Wilton Wells *smoke*	9 m^2	8	5	0
Wilton Wells *control*	9 m^2	0	0	0
Three Springs-Eneabba Rd *smoke*	6 m^2	0	0	0
Three Springs-Eneabba Rd *control*	6 m^2	0	0	0

Table 2.4. The emergence of V. albida seedlings from the soil seed bank following exposure to smoke and subsequent survival of seedlings. Results of May 1999 smoke trial

Population and treatment	Area treated	No. seedlings 1/10/99
Wilton Wells *smoke*	9 m^2	35
Wilton Wells *control*	9 m^2	0
Three Springs-Eneabba Rd *smoke*	6 m^2	0
Three Springs-Eneabba Rd *control*	6 m^2	0

The application of aerosol smoke stimulated the germination of soil stored *V. albida* seed in both years. No seedlings were observed in the plots that were not exposed to smoke.

2.3 Action 02. Identify and report on reproductive strategies, phenology and seasonal growth of the species

2.3.1 Reproductive phenology and seed:flower ratios

Landscape context and population size may effect population processes that underlie the development of viable seeds. Investigations on individual plants of reproductive phenology and seed to flower ratios were initiated in two populations to:

1. compare rates of seed production across populations;
2. assess the relative importance of seed production as a factor constraining population growth; and,
3. provide baseline rates from wild plants for comparing and assessing the reproductive success of translocated plants.

All species of *Verticordia* have solitary, pedunculate axillary flowers. There is, however, great variation in the length of the peduncle and in the length of the flower bearing part of the branchlet (George 1991). This gives rise to the appearance of large inflorescences which may be corymb-like, rounded or spike-like racemes (George 1991). *Verticordia albida* has spike-like racemes on the ends of branchlets.

Investigations of flowering in *V. albida* were undertaken during peak flowering in 1998 on five plants in the Wilton Wells population and two plants in the Three Springs-Eneabba Rd population. In each plant the total number of flowering spikes and the number of flowers on 10 randomly chosen spikes were counted. The number of flowers per plant was subsequently calculated using the formula: *Total number of flowers per plant = number of 'flower spikes' per plant x the mean number of flowers per 'spike'*. The results are presented in Table 2.5.

Table 2.5. The mean number of flowers per spike, total number of flower spikes and estimates of the total number of flowers per plant in two V. albida populations

Site	Plant	Mean no. of flowers per spike	Total no. of spikes per plant	Total no. of flowers per plant
Wilton Wells	1	12.8 ± 1.381	141	1804.8 ± 104.348
	2	6.9 ± 1.303	260	1794.0 ± 338.888
	3	13.5 ± 1.827	110	1485.0 ± 200.999
	4	8.6 ± 1.778	105	903.0 ± 186.652
	5	9.6 ± 1.733	196	1881.6 ± 339.733
Three Springs-Eneabba Rd	1	9.0 ± 0.715	50	450.0 ± 35.746
	2	11.2 ± 1.073	62	694.4 ± 66.520

The total number of flowers on plants in the Three Springs-Eneabba Rd population were considerably less than in the Wilton Wells population despite plants being on average taller in the Three-Springs Eneabba Rd population.

Investigations of seed production were undertaken on five plants across four populations in December 1998. *Verticordia* fruit is dry and nut like, enclosed within the faded perianth. It usually contains a single seed and occasionally two. Externally it is difficult to distinguish from a faded flower containing no seed (George 1991). As a consequence fruit with seed cannot be distinguished from empty flowers unless they are dissected. For the purposes of this report all faded flowers are described as 'fruit'. In December 1998 when fruits had matured but before they were dispersed 30 fruits were collected from around the canopy of each plant. In *Verticordia* there is no endosperm in the mature seed, it is absorbed by the embryo into a swollen hypocotyl which has at one end a small root tip and at the other tiny cotyledons. Therefore fruit which showed a full, white, moist embryo were regarded as containing viable seed and fruit with no embryo or which possessed a dark brown, shriveled or dry embryo were regarded as not containing a viable seed. The proportion of fruits containing a viable seed was calculated and the mean seed:flower ratio for each population calculated. The results are presented in Table 2.6.

Table 2.6. The proportion (%) of V. albida fruits containing a viable seed across populations in 1998. Values are means ± SE

Population	Approximate population size	Proportion (%) of fruit containing a viable seed
Wilton Wells	1000 +	32.3 ± 2.2
Three Springs-Eneabba Rd	100 +	27.0 ± 4.9
Sweetman Rd	13	37.6 ± 7.0
Lynch Rd	7	25.8 ± 4.9

The four *V. albida* populations studied all produced viable seeds but the mean proportion of fruit that contained a seed (seed:flower ratio) varied between populations. Unfortunately because the research is incomplete it is not possible to determine whether variation in seed:flower ratios were population specific or reflected temporal variation. The initial data suggests that population size does not affect the proportion of flowers that produce a seed but further replication of the study across at least one more flowering season is needed. However, total seed production may be quite different between populations due to variation between populations in the number of flowers produced (Table 2.5). Consequently seed production may be more limiting in some populations than in others but further research is needed.

2.4 Action 3. Investigate and report on the mating system and pollination biology of the species

Pollination biology studies were planned for the third year of the project but were not undertaken due to project funding not continuing beyond the second year.

Preliminary mating system studies for three populations of *V. albida* have been completed (Table 2.7). These data indicate that population size may be a critical factor influencing the level of inbreeding in populations of this species. There was a significant decrease in the level of outcrossing, based on both the multi-locus (t_m) and single locus (t_s) estimates, associated with a decrease in population size. The smaller population 3 also showed a marked increase in correlated paternity (r_p), the probability that outcrossed sibs share the same father. Although increased correlated paternity may not initially lead to inbreeding, mating in the next generation between adjacent plants that share the same paternal genotype will inevitably result in increased inbreeding effects.

Table 2.7. Mating system estimates for V. albida

Population	t_m (SE)	t_s (SE)	r_p (SE)
1	0.93 (0.04)	0.89 (0.030	0.44 (0.11)
2	0.91 (0.05)	0.81 (0.06)	0.54 (0.13)
3	0.43 (0.25)	0.43 (0.200	0.75 (0.15)

t_m = multilocus estimate of population outcrossing rate. t_s = single locus estimate of outcrossing rate (averaged for all loci). r_p = correlation of paternity estimate. S/D Sparse plant

2.5 Action 04. Investigate and report on the population genetic structures, levels of genetic diversity and minimum viable population size of the species

Seed collections for population genetic structure and genetic diversity studies have been completed from the known populations. This work was planned for 2000 and with no available funding will now be deferred until resources are available

2.6 Action 05. Identify and report on the impact and control of diseases and invasive weeds

Two *V. albida* populations occur in highly disturbed narrow linear strips of remnant native vegetation on road reserves adjacent to agricultural land. The other two populations are less disturbed but occur in a small linear rail reserve and in a relatively small remnant on private property surrounded by agricultural land. The potential for weed invasion is therefore high in all populations. Surveys were undertaken in each population to determine the relative importance of weeds in the remaining vegetation. In each population 20 1m x 1m quadrats were laid out and the percentage cover of litter, woody perennials <1.3 m high and weeds were estimated by eye. In addition the % cover of woody perennials > 1.3 m high was measured with a convex spherical densiometer and the height of the tallest stratum (cm).

Table 2.8. Plant community structure in the remnants where the V. albida populations occur. Values are means ± SE from 20 1 m x 1 m quadrats at each site

Population	Site type	Woody perennial cover < 1.3 m high (%)	Litter cover (%)	Weed cover (%)	Tallest stratum (cm)	Canopy cover > 1.3 m high (%)
Wilton Wells	Remnant vegetation	24.2 ± 3.5	20.7 ± 2.4	0.3 ± 0.1	85.0 ± 3.7	1.9 ± 0.6
Three-Springs Eneabba Road	Road Verge	31.1 ± 5.3	88.0 ± 4.6	6.8 ± 3.6	558.5 ± 24.6	66.4 ± 5.4
Sweetman Road	Road Verge	68.9 ± 6.1	70.5 ± 3.9	21.9 ± 5.0	207.5 ± 13.5	36.6 ± 5.3
Lynch Road	Rail Reserve	60.7 ± 6.6	80.0 ± 5.0	7.5 ± 1.5	224.6 ± 23.6	52.4 ± 5.7

The composition and structural characteristics of the plant communities in the four remnants where *V. albida* occurs varied. Vegetation cover and the height of the tallest stratum were considerably greater in the road verge populations than in the Wilton Wells population. Invasive weeds were recorded in all sites but were most abundant in the three populations that occur on highly disturbed linear road reserves. The effects of removing weeds to reduce competition, applying aqueous smoke solution to stimulate germination of the *V. albida* soil seed bank and their interaction requires further research.

2.7 Action 06. Identify and develop ex situ conservation strategies such as propagation protocols and germplasm storage techniques

Optimal germination protocols for *V. albida* have been identified and developed. Like many other *Verticordia* species, *V. albida* displays innate dormancy imposed by a requirement for after-ripening and the removal of seed from the hypanthium to permit the uptake of water and oxygen. The most successful technique for overcoming seed dormancy and stimulating maximal germination involves complete removal of the hypanthium (seed coat), soaking seed in a smoke water solution for 24 hours and incubation in a 25mg/L solution of Gibberellic Acid (as GA_3). Seeds are manually removed from the hypanthium with a sharp scalpel knife and placed on a 1% w/v agar solution with the addition of GA_3 in covered Petrie dishes and incubated at 15°C with 12 hours of alternating light and darkness. Up to 69% germination can be achieved under these conditions in the laboratory.

Germplasm storage techniques have been identified and developed for *V. albida*. Standard genebank practice recommends the storage of orthodox seeds (those able to withstand desiccation without adverse effects) at low temperatures and low moisture content (IPGRI 1994). These methods involve the reduction of seed moisture content to between 4-7% using a dehumidification process and the freezing of the seed in hermetically sealed containers (laminated aluminium foil) at –20°C. A total of 13,460 seeds representing four populations have been placed in long term storage at CALM's Threatened Flora Seed Centre under these conditions with viability after one year at low temperatures and low moisture conditions at 69%.

2.8. Action 07. Develop the initial stages of a program of experimental translocations and population establishment experiments

A program of experimental translocations and population experiments informed by experiments determining factors which limit seed germination and seedling establishment were planned for the third year of the project but were not undertaken due to project funding not continuing beyond the second year.

2.9 Action 08. Undertake initial development of recovery strategies and provide a report on progress to date

The research will be completed when resources become available and will rank the constraints to the growth of existing populations and inform management and recovery of the species.

3. THE CONSERVATION BIOLOGY OF *VERTICORDIA FIMBRILEPIS SSP. FIMBRILEPIS*

3.1 Background

3.1.1 Current conservation status

Verticordia fimbrilepis ssp. fimbrilepis is currently known from 15 populations and sub-populations, five of which have been recently discovered. The sub-species is currently ranked as critically endangered under World Conservation Union IUCN Red List Criterion B1 + 2c (World Conservation Union 1994).

3.1.2 Description

Verticordia fimbrilepis ssp. fimbrilepis is a small erect shrub to 60 cm high with slender branches and clusters of purplish pink flowers. It has open branches with one basal stem. The stem leaves are 2-5 mm long, linear and slightly tapering with a pointed tip. Floral leaves are oblong to narrowly oval, but otherwise similar to stem leaves. Erect flowers are in rounded flat clusters. The bracteoles are obscurely ridged and remain on the plant. The 1.4-1.6 mm floral tube is a broad top shape with 10 ribs and is slightly warty. The pink petals are 2.5-3 mm long, coarsely fringed along the margins and spreading. The pink sepals are 3-3.5 mm long, spreading and have 5-7 fringed lobes.

3.1.3 Distribution, landscape context and habitat

The 15 populations and sub-populations of *V. fimbrilepis ssp. fimbrilepis* vary in size and landscape context (Table 3.1) occurring across a range of 45 km west and 25 km east of Brookton and approximately 170 km south to the Woodanilling area. The northern populations west of Brookton occur in State Forest on the eastern edge of the jarrah forest mosaic. The plants are growing in low heath growing on sandy clay soils derived from granite and are surrounded by marri-jarrah open woodland. The populations east of Brookton and south to Woodanilling occur in low open heath growing on sandy loam soils in the western wheatbelt region of south-western Australia. These areas have been extensively cleared for agriculture with remnants of the original native vegetation occurring in patches of varying size, shape and health. Ten of the populations and sub-populations occur on linear road verges six of which are highly disturbed. One population occurs in a relatively small nature reserve and one population in a relatively large privately owned remnant which is managed for nature conservation.

3.1.4 Research objectives

The aim of the project was to undertake research on the biology and ecology as outlined in the Draft Interim Recovery Plan. The research was undertaken with support from the Natural Heritage Trust Endangered Species Program Grant ESU 00006465. The research program was designed on the understanding that there would be a three-year commitment to funding the project but, unfortunately, funding did not continue beyond the second year. Consequently, the research and data sets are incomplete and it is not possible to interpret results or draw conclusions. This report therefore limits itself to describing the research and summarising the results within scope items for the period of funding.

Table 3.1. The status and approximate size of V. fimbrilepis ssp. fimbrilepis populations and sub-populations

Population number	Land status	Approx. population size
1	Shire Road Verge	6
3	Shire Road Verge	2
4	Shire Road Verge	90
6	Shire Road Verge	2
7a	Shire Road Verge	800
7b	Nature Reserve	300
8b	Shire Road Reserve	2
8c	Shire Park Land C Class Reserve	27
8d	Shire Road Reserve	7
10	State Forest	5000 +
11a	State Forest	908
11b	State Forest	757
12	Recreation Reserve	43
13	Shire Road Reserve	185
14	Private Remnant	200 +

3.2 Action 01. Investigate and report on the soil seed bank dynamics and the role of disturbance, competition and rainfall on recruitment and seedling survival

3.2.1 Soil seed bank

Investigations of the density of *V. fimbrilepis ssp. fimbrilepis* fruit on the soil surface and in the soil were undertaken in the Jingaring Nature Reserve population in March 1999 after fruit from the previous years crop were dispersed. One soil core (10 cm x 10 cm x 2 cm) was taken at a randomly chosen point beneath the canopies of 30 reproductive adult plants. In each core the surface litter and soil fractions were sampled separately. The soil samples were air dried and passed through a series of differential sieves. Larger pieces of organic matter were fractioned off, removed, and soil aggregations broken up until two fractions remained above and below the normal size range of *V. fimbrilepis ssp. fimbrilepis* fruits. The fraction below the size range of the fruits was discarded; the remaining fraction was examined under a binocular microscope for *V. fimbrilepis ssp. fimbrilepis* fruits. The presence of a viable seed in each fruit was determined by sectioning the fruit with a sharp blade. Seeds were considered viable if they contained a full, white, moist embryo. The results are presented in Table 3.2.

Table 3.2. Abundance of V. fimbrilepis ssp. fimbrilepis fruits on the soil surface, and within the top 2cm of soil, within 10cm x 10cm quadrats beneath plants. Values are means of 30 samples

Fruit characteristics	Mean ± SE
No. fresh fruits on soil surface	28.00 ± 4.52
No. fresh fruits on soil surface with viable seed	2.07 ± .038
No. weathered fruits on soil surface	15.50 ± 5.23
No. weathered fruits on soil surface with viable seed	0.90 ± 0.31
No. fruits in soil (0-2cm)	44.60 ± 8.87
No. fruits in soil (0-2 cm) with viable seed	1.40 ± 0.38

Verticordia fimbrilepis ssp. fimbrilepis fruits containing viable seeds were found on the soil surface (297 seeds m^{-2}) and in the soil (140 seeds m^{-2}) in relatively high densities.

Investigations of the dynamics and longevity of the soil seed reserve were undertaken in the Jingaring Nature Reserve population with a seed burial retrieval experiment. The experiment aimed to determine:

1. How long *V. fimbrilepis ssp. fimbrilepis* seeds remain viable in the soil;
2. Whether *V. fimbrilepis ssp. fimbrilepis* seeds remain dormant in the soil until they are exposed to smoke; and
3. Whether dormancy and germinability are effected by the length of time fruit have been buried.

In March 1999 a 12 x 10 grid consisting of 120 points spaced 1m apart was laid out and 105 nylon mesh bags (10 cm x 10 cm) each containing 100 *V. fimbrilepis ssp. fimbrilepis* fruits and washed river sand buried at individual points on the grid. Following burial 21 bags were randomly allocated for retrieval after 6 months, 12 month, 18 months, 24 months and 36 months. The viability and germinability of seeds following retrievals were assessed by randomly allocating three treatments to the 21 bags. Firstly, in 7 bags the presence of a viable seed in each fruit was determined by sectioning the fruit with a sharp blade; seeds were considered viable if they contained a full, white, moist embryo. Secondly, the contents of 7 bags (fruit and washed river sand) were spread thinly on the surface of punnets containing peat/sand mix and exposed to aerosol smoke for 60 minutes then placed in the glasshouse, watered regularly and subsequent germination monitored. Finally, the contents of 7 bags (fruit and washed river sand) were spread thinly on the surface of punnets containing a peat/sand mix and placed in the glasshouse, watered regularly and subsequent germination monitored.

Table 3.3. The viability and germinability following exposure to smoke of V. fimbrilepis ssp. fimbrilepis seeds which had been buried under field conditions for 6 months

Burial time	Mean % seed viability (cut test)	Mean % seedling emergence (aerosol smoke)	Mean % seedling emergence (control)
6 months	14.85 ± 1.08	4.14 ± 1.58	0.42 ± 0.30

The experiment is incomplete and therefore no conclusions can be drawn about the longevity and dynamics of the soil seed bank. The limited data indicates that seeds remain dormant until they are exposed to smoke whereupon germination begins.

3.2.2 Impact of fire – adult response and seedling recruitment and survival

The impact of fire on adult plants and seedling recruitment is being investigated in the newly discovered *V. fimbrilepis ssp. fimbrilepis* populations which occur in State Forest and have been burnt recently.

3.3 Action 02. Identify and report on reproductive strategies, phenology and seasonal growth of the species

3.3.1 Reproductive phenology and seed:flower ratios

Landscape context and population size may effect population processes that underlie the development of viable seeds. Investigations on individual plants of reproductive phenology and seed to flower ratios were initiated in 11 populations to:

1. compare rates of seed production across populations;
2. assess the relative importance of seed production as a factor constraining population growth; and,
3. provide baseline rates from wild plants for comparing and assessing the reproductive success of translocated plants.

Investigations of flowering and seed production in *V. fimbrilepis ssp. fimbrilepis* were undertaken across nine populations in 1999 and 11 populations in 2000. The number of plants sampled varied depending on the size of the population (see Table 3.4 for sample sizes). In late January 1999 and 2000 when fruits had matured but before they were dispersed 20 fruits were collected from around the canopy of each plant. The presence of a viable seed in each fruit was determined by sectioning the fruit with a sharp blade. Seeds were considered viable if they contained a full, white, moist embryo. The proportion of fruits containing a viable seed was calculated and the mean seed:flower ratio for each population calculated. The results are presented in Table 3.4.

The eleven *V. fimbrilepis spp. fimbrilepis* populations studied all produced viable seeds but the mean proportion of fruit which contained a seed (seed:flower ratio) varied between populations. Unfortunately because the research is incomplete it is not possible to determine whether variation in seed:flower ratios were population specific or reflected temporal variation. The initial data suggests that population size does not affect the proportion of flowers which produce a seed but further replication of the study across at least one more flowering season is needed.

Table 3.4. The proportion (%) of V. fimbrilepis ssp. fimbrilepis fruits containing a viable seed across populations in 1999 and 2000. In each population 20 fruit were collected from a designated number of plants which varied across populations. Values are means ± SE

Population	Approx. population size	No. of plants sampled	Mean % of fruit containing a seed (1999)	Mean % of fruit containing a seed (2000)
Jingaring Road	800	30	27.17 ± 2.45	30.67 ± 3.11
Jingaring Nature Reserve	300	30	13.33 ± 1.92	14.33 ± 1.45
Australian Bush Heritage Reserve	>200	30	Not sampled	29.00 ± 3.08
Douglas Road	180	20	Not sampled	32.50 ± 4.14
Jingaring Road	90	10	15.00 ± 3.09	24.67 ± 3.29
Aldersyde Reserve	29	10	19.00 ± 6.27	14.00 ± 3.56
Aldersyde Reserve	7	7	13.57 ± 2.83	37.14 ± 9.18
Robinson Road	6	6	21.67 ± 9.10	17.50 ± 7.93
Beaufort Bridge Nature Reserve	2	2	5.00 ± 2.89	1.67 ± 1.67
Harrismith Road	1	1	0.00	22.50

3.4 Action 3. Investigate and report on the mating system and pollination biology of the species

3.4.1 Pollination biology

Landscape context and population size may effect the diversity and abundance of pollinators, rates of pollination and subsequent development of viable seeds. Investigations of *V. fimbrilepis ssp. fimbrilepis's* pollination biology were initiated in 11 populations to:

1. compare the diversity and abundance of flower visitors across populations;
2. compare rates of pollination across populations;
3. assess the relative importance of pollination as a factor constraining population growth; and,
4. provide baseline rates of pollinator abundance and pollination in wild plants for comparing and assessing rates in translocated plants.

3.4.1.1 The diversity, abundance and behaviour of insects visiting flowers

Observations of flower visitors were made in a subset of the 11 populations used in the pollination study. The six populations chosen encompassed a range of population sizes and contexts (see Table 3.1 for descriptions). In all populations except the Kweda Rd population eight flowering adults were each observed for intervals lasting 15 minutes. In the Kweda Rd population the single plant in the population was observed for a 15 minute period on six occasions. All observations were made in the middle of the day between November 30 and December 13 1999 during peak flowering. For each 15 minute observation period the identity of flower visitors

(morphospecies), the number of flowers they visited, whether they contacted anthers and stigmas; whether they foraged for nectar and whether they were seen visiting flowers on another plant were recorded. Collections were made of all insects flower visitors for identification (Table 3.5). For all populations the mean number of insect flower visitors, mean insect flower visitor species richness and the mean number of flowers visited during the 15 minute observation periods were calculated. The results are presented in Table 3.6. In addition the percentage of flower visits where insects were observed foraging for nectar or pollen and the percentage of flower visits which resulted in contact with anthers or stigmas were calculated. The results are presented in Table 3.7.

Table 3.5. The diversity of insects observed visiting V. fimbrilepis ssp. fimbrilepis flowers

Insect order	Hymenoptera	Diptera	Lepidoptera	Coleoptera	Hemiptera
No. of species	17	8	3	1	1

Table 3.6. Mean number of insect flower visitors, mean insect flower visitor species richness and mean number of flowers visited during 15 minute observation periods across V. fimbrilepis ssp. fimbrilepis populations. Values are means ± SE

Population	Population size	Mean no. of insects	Mean insect species richness	Mean no. of flowers visited
Jingaring Road	800	5.50 ± 1.40	2.08 ± 0.26	28.08 ± 10.35
Jingaring Nature Reserve	300	2.29 ± 0.56	1.43 ± 0.27	24.21 ± 13.85
Australian Bush Heritage Reserve	>200	1.92 ± 0.60	1.42 ± 0.40	35.67 ± 13.86
Jingaring Road	90	6.21 ± 1.43	2.36 ± 0.43	62.07 ± 26.35
Aldersyde Reserve	29	3.57 ± 1.75	1.36 ± 0.39	43.07 ± 32.78
Kweda Road	1	2.00 ± 0.68	1.38 ± 0.38	8.63 ± 3.08

Table 3.7. Behaviour of insect flower visitors across V. fimbrilepis ssp. fimbrilepis populations

Population	Pop. size	% of visits insects foraged for nectar	% of visits insects contacted anthers	% of visits insects contacted stigmas	% of visits insects moved between plants
Jingaring Road	800	89.39	86.36	86.36	27.27
Jingaring Nature Reserve	300	63.64	51.52	54.55	9.09
Australian Bush Heritage Reserve	>200	86.96	86.96	86.96	43.48
Jingaring Road	90	90.91	87.50	85.23	31.82
Aldersyde Reserve	29	88.00	88.00	88.00	2.00
Kweda Road	1	70.59	52.94	52.94	0.00

Thirty species of insects across five orders were observed visiting *V. fimbrilepis ssp. fimbrilepis* flowers. The mean number of insect flower visitors, mean insect flower visitor species richness and the mean number of flowers visited during the 15 minute observation periods varied across populations. . Unfortunately because the research is incomplete it is not possible to determine whether variation in rates of insect flower visitor abundance and diversity and number of flowers visited is population specific or reflected temporal variation. The initial data suggests that population size does not affect the abundance of insect flower visitors.

3.4.1.2 Rates of pollination

Rates of pollination were measured in 11 populations varying in size and landscape context. The number of plants and number of flowers on which measurements were made varied across populations. In eight of the populations five plants were randomly chosen for measuring pollination rates. In the other three populations sampling was restricted to the plants which were available (Beaufort River 2 plants; Harrismith Rd 2 plants and Kweda Rd 1 plant). In each plant five flowers styles were harvested from around the plant and immediately fixed in FAA fixative. Styles were subsequently dissected from the flowers, examined under a fluorescing microscope and the number of pollen grains per stigma and number of pollen tubes counted. The number of stigmas with pollen grains and the mean number of pollen grains per stigma were calculated across populations. The results are presented in Table 3.8. Similarly the number of styles with pollen tubes at their base and the mean number pollen tubes a t the base of styles was calculated for each population. The results are presented in Table 3.9.

Table 3.8. The number of flower stigmas with pollen grains and the mean(± SE) number of pollen grains per stigma, across V. fimbrilepis ssp. fimbrilepis populations

Population	Population size	n	No. of stigmas with pollen grains	Mean no. of pollen grains per stigma
Jingaring Road	800	25	25	18.68 ± 4.31
Jingaring Nature Reserve	300	25	24	18.16 ± 5.10
Australian Bush Heritage	>200?	25	24	17.92 ± 5.85
Douglas Road	180	25	24	19.2 ± 4.00
Jingaring Road	90	25	25	18.36 ± 4.36
Aldersyde Reserve	29	25	22	15.48 ± 7.78
Aldersyde Reserve	7	25	24	17.88 ± 5.69
Robinson Road	6	25	23	14.88 ± 7.84
Beaufort Bridge Nature Reserve	2	10	10	18.4 ± 2.84
Harrismith Road	2	10	10	20
Kweda Road	1	5	5	14.8 ± 7.95

Table 3.9. The number of flower styles with pollen tubes at their base and the mean(± SE) number of pollen tubes at the style base across V. fimbrilepis ssp. fimbrilepis populations

Population	Approximate Population Size	n	No. of styles with pollen tube at base	Mean no. of pollen tubes at style base
Jingaring Road	800	25	20	8.72 ± 6.34
Jingaring Nature Reserve	300	25	23	9.32 ± 6.22
Australian Bush Heritage	>200?	25	23	10.8 ± 6.09
Douglas Road	180	25	24	8.84 ± 4.64
Jingaring Road	90	25	24	8.8 ± 6.03
Aldersyde Reserve	29	25	15	5.36 ± 5.45
Aldersyde Reserve	7	25	21	9.48 ± 6.96
Robinson Road	6	25	21	7.96 ± 5.56
Beaufort Bridge Nature Reserve	2	10	9	4.9 ± 3.73
Harrismith Road	1	5	10	13.5 ± 5.80
Kweda Road	1	5	4	7.6 ± 4.51
Translocation Site		25	12	3.56 ± 5.32

The number of flower stigmas with pollen grains and the mean number of pollen grains per stigma varied across populations. Similarly, the number of flower styles with pollen tubes at their base and the mean number of pollen tubes per style varied across populations. Unfortunately, because the research is incomplete it is not possible to determine whether variation in rates of pollination are population specific or reflected temporal variation. The initial data suggests firstly, pollination is not a factor constraining population growth and secondly, that a populations size does not affect rates of pollination.

3.4.1.3 Mating Systems

Seed collections for mating system studies and preliminary allozyme studies have been completed to determine appropriate polymorphic loci. Mating system analyses were planned for the third year of the project but were not undertaken due to project funding not continuing beyond the second year

3.5 Action 04. Investigate and report on the population genetic structures, levels of genetic diversity and minimum viable population size of the species

Allozyme studies based on nine isozyme loci have been completed for five populations of *V. fimbrilepis* ssp. *fimbrilepis*and one population of *V. fimbrilepis* ssp *australis*. Preliminary analysis of these data, to investigate population genetic structure and genetic diversity levels, are currently underway. Following the discovery of a number of new populations of this taxon it was planned to complete this study in the third year of the project and include those new populations. Seed material has now been collected from the new populations but with no funding for the third year of the project this work will be deferred until resources are available.

3.6 Action 05. Identify and report on the impact and control of diseases and invasive weeds

Invasive weeds are a threat in the six populations that occur on highly disturbed linear road reserves. The effects of removing weeds to reduce competition, applying aqueous smoke solution to stimulate germination of soil stored *V. fimbrilepis ssp. fimbrilepis* soil seed bank and their interaction are currently being investigated.

3.7 Action 06. Identify and develop ex situ conservation strategies such as propagation protocols and germplasm storage techniques

Optimal germination protocols for *V. fimbrilepis* ssp. *fimbrilepis* have been identified and developed. Like many other *Verticordia* species, *V. fimbrilepis* ssp. *fimbrilepis* displays innate dormancy imposed by a requirement for after-ripening and the removal of seed from the hypanthium to permit the uptake of water and oxygen. The most successful technique for overcoming seed dormancy and stimulating maximal germination involves complete removal of the hypanthium (seed coat), soaking in a smoke water solution for 24 hours and incubation in a 25mg/L solution of Gibberellic Acid (as GA_3). Seeds are manually removed from the hypanthium with a sharp scalpel knife and placed on a 1% w/v agar solution with the addition of GA_3 in covered Petrie dishes and incubated at 15°C with 12 hours of alternating light and darkness. Up to 86% germination can be achieved under these conditions in the laboratory.

Germplasm storage techniques have been identified and developed for *V. fimbrilepis* ssp. *fimbrilepis*. Standard genebank practice recommends the storage of orthodox seeds (those able to withstand desiccation without adverse effects) at low temperatures and low moisture content (IPGRI 1994). These methods involve the reduction of seed moisture content to between 4-7% using a dehumidification process and the freezing of the seed in hermetically sealed containers (laminated aluminium foil) at -20^0C. A total of 27,840 seeds representing eight populations have been placed in long term storage at CALM's Threatened Flora Seed Centre under these conditions between 1996 and 2000, with the viability of at least one population at 86% after one year at low temperature and low moisture conditions.

3.8. Action 07. Develop the initial stages of a program of experimental translocations and population establishment experiments

A program of experimental translocations and population experiments informed by experiments determining factors which limit seed germination and seedling establishment (see sections 3.2.3 and 3.7) were planned for the third year of the project but were not undertaken due to project funding not continuing beyond the second year.

3.9 Action 08. Undertake initial development of recovery strategies and provide a report on progress to date

The research will be completed when resources become available and will rank the constraints to the growth of existing populations and inform management and recovery of the species.

4 THE CONSERVATION BIOLOGY OF *VERTICORDIA STAMINOSA SSP. STAMINOSA* (WONGAN FEATHER FLOWER)

4.1 Background

4.1.1 Current conservation status

Verticordia staminosa ssp. staminosa is currently known from a single wild population of approximately 1200 plants and is currently ranked as critically endangered under World Conservation Union IUCN Red List Criterion C2b, B1 + 2c (World Conservation Union 1994).

4.1.2 Description

Verticordia staminosa ssp. staminosa is a shrub to 30 cm tall with widely spreading branches forming a dense canopy up to 1 m in diameter. Flowers are born in the axils and hang beneath branchlets. The subspecies is distinguished by its long (9-12 mm) bright red stamens (united for 2-3 mm) with yellow anthers and yellow sepals subtended by red persistent bracts.

4.1.3 Distribution, landscape context and habitat

Verticordia staminosa ssp. staminosa has an extremely restricted distribution in the central wheatbelt region of south-western Australia near Wongan Hills approximately 182 km north-east of Perth. The area has been extensively cleared for agriculture with remnants of the original native vegetation occurring in patches of varying size shape and health. The *V. staminosa ssp. staminosa* population occurs in a relatively large remnant of native vegetation containing a granite rock complex and fringing vegetation. Plants grow in areas where there are cracks within the granite pavement and are distributed patchily in 40 more or less isolated sub-populations which range in size from 1 to 200 plants.

The area has a Dry Mediterranean climate with cool wet winters and dry hot summers. Mean annual rainfall at Wongan Hills is 390 mm with rain falling predominantly between May and September; summer rainfall does occur but is unpredictable. The mean maximum and minimum temperatures in the warmest month January are 34.4° C and 17.9° C and in the coolest month July are 16.9° C and 6.7° C respectively.

4.1.4 Research objectives

The aim of the project was to undertake research on the biology and ecology as outlined in the Draft Interim Recovery Plan. The research was undertaken with support from the Natural Heritage Trust Endangered Species Program Grant ESU 00006465. The research program was designed on the understanding that there would be a three-year commitment to funding the project but, unfortunately, funding did not continue beyond the second year. Consequently, the research and data sets are incomplete and it is not possible to interpret results or draw conclusions. This report therefore limits itself to describing the research and summarising the results within scope items for the period of funding.

4.2 Action 01. Investigate and report on the soil seed bank dynamics and the role of disturbance, competition and rainfall on recruitment and seedling survival

4.2.1 Life stage and size class structure of the population

Investigations of the biological status of the population (whether it is declining, stable or increasing) were initiated in 1999. The life stage, size and canopy characteristics of individual plants in 6 patches (sub-populations) ranging in size from 2 plants to 196 plants were described during peak flowering in August 1999. Careful searches were made for *V. staminosa ssp. staminosa* across the site. When a plant was encountered it was numbered with a permanent aluminium tag attached to a branchlet, its life stage recorded (juvenile or reproductive) and canopy diameter at the widest point recorded. Individuals were organised into combined stage size/classes: non-reproductive plants with canopy diameter size classes 2-4 cm, 4-8 cm, 8-12 cm, 12-16 cm; and reproductive plants with canopy diameter size classes 10-30 cm; 30-50 cm; 50-70 cm; 70-90 cm; 90-110 cm and >110 cm). The distribution of plants in life stage/size classes are presented for the largest sub-population surveyed (196 plants) in Table 4.1.

Table 4.1. The life stage and size class structure of a sub-population of V. staminosa ssp. staminosa

Life-stage class	Size classes–canopy diameter at widest point (cm)									
	0-1	2-4	4-6	6-10	10-20	20-40	40-60	60-80	80-120	>120
Seedlings	185									
Juveniles (non-reproductive)		2	1	5	8					
Senescing adults (non-reproductive)				1	4					
Reproductive					8	46	41	25	17	2

In November 1999 at the end of the winter wet season searches were made for seedlings in each sub-population. Seedlings were beginning to die with the onset of the summer drought but both dead and living seedlings were easily recognisable and the counts probably reflect maximum estimates of emergence. When a seedling was encountered its status (living or dead) and habitat position (rock crack or moss mat) were recorded. Living seedlings were marked with plastic cocktail sticks. The results are presented in Table 4.2.

Table 4.2. The number of dead and living seedlings at the end of winter on two substrates a shallow soiled moss covered surface, and cracks in rocks. Values in parenthesis indicate percentages (%) of the total number of seedlings within each sub-population

	Mossmat		Rock crack		
Population	No. of dead seedlings	No. of living seedlings	No. of dead seedlings	No. of living seedlings	Total no. of seedlings
A	8 (9.88)	11 (13.58)	17 (20.99)	45 (55.56)	81
B	33 (16.34)	52 (25.74)	21 (10.40)	93 (46.04)	202
C	2 (7.41)	6 (22.22)	2 (7.41)	17 (62.96)	27
D	17 (62.96)	10 (37.04)	0	0	27
G	12 (20.34)	2 (3.39)	8 (13.56)	37 (62.71)	59
H	13 (37.14)	19 (54.29)	0	9 (25.71)	35
W	32 (17.30)	38 (20.54)	13 (7.03)	102 (55.14)	185
Mean	16.71±4.44	19.71±6.99	10.17±3.15	50.50±14.65	88.00±28.26

Plants were observed across all life stage/size classes. The initial data suggests that seedlings emerge annually in the winter wet season on moss mats and herb fields on shallow soils and in soil accumulated in cracks. Seedling survivorship appears to be highest in soil accumulated on rock cracks. The initial data suggests that the population is stable but further monitoring of tagged individuals is needed to determine the rates of transition of plants between life stage/size classes.

4.3 Action 02. Identify and report on reproductive strategies, phenology and seasonal growth of the species

4.3.1 Reproductive phenology and seed:flower ratios

Population size and density may effect population processes that underlie the development of viable seeds. Investigations on individual plants of reproductive phenology and seed to flower ratios were initiated in nine sub-populations to:

1. compare rates of seed production across populations;
2. assess the relative importance of seed production as a factor constraining population growth; and,
3. provide baseline rates from wild plants for comparing and assessing the reproductive success of translocated plants.

Investigations of flowering and seed production in *V. staminosa ssp. staminosa* were undertaken across nine sub-populations (three large; three medium and three small sub-populations; see Table 4.3 for actual sizes). In the 'large' and 'medium' sized sub-populations surveys were undertaken on five randomly chosen adult plants. In the small sub-populations surveys were restricted to three plants in each of two sub-populations and 2 plants in the other. In each plant a branchlet sub-tending flowers and buds was tagged and labeled. In June 1999 during peak flowering the number of flowers and buds distal to each tag was were counted and recorded. In addition the number of branchlets similar in size to the tagged branchlet were counted on each plant. In November 1999

when fruits had matured but before they were dispersed 50 fruit were randomly harvested from each tagged branchlet . The presence of a viable seed in each fruit was determined by sectioning the fruit with a sharp blade. Seeds were considered viable if they contained a full, white, moist embryo. The proportion of fruits containing a viable seed and the mean seed:flower were calculated for each sub-population. The results are presented in Table 4.3. Estimates of seed production for individual plants in each sub-population were subsequently calculated using the formula: *Total number of seeds produced per plant = number of branchlets x number of inflorescences per branchlet x proportion of fruit which contain a seed.* The results are presented in Table 4.4.

Table 4.3. The proportion (%) of V. staminosa ssp. staminosa fruits containing a viable seed across 9 sub-populations in 1999. In each population 50 fruit were collected from a designated number of plants which varied across populations. Values are means ± SE

Sub-population	Sub-population size	No. of plants sampled	Mean % of fruit containing a seed
W	160	5	35.40 ± 8.98
G	100	5	38.60 ± 9.57
H	89	5	37.50 ± 2.44
B	61	5	50.10 ± 5.58
A	46	5	41.30 ± 5.19
F	20	5	37.20 ± 4.34
D	3	3	44.40 ± 2.41
I	3	3	29.2 ± 9.75
C	2	2	45.60 ± 6.05

Table 4.4. Estimates of the total number of seeds produced per plant across sub-populations

Seeds/plant		Seeds/plant		Seeds/plant		Seeds/plant		Seeds /plant		Seeds/plant		Seeds/plant	
W		G		H		B		A		C and D		I	
W1	84	G1	1 593	H1	872	B1	56	A1	104	C1	1 290	I1	176
W2	826	G2	3 188	H2		B2	111	A2	300	C2	945	I2	250
W3	240	G3	683	H3	295	B3	353	A3	179	D1	82	I3	50
W4	574	G4	704	H4	642	B4	848	A4	1 088	D2	990		
W5	168	G5	185	H5	787	B5	366	A5	395	D3	2 186		

The nine *V. staminosa ssp. staminosa* sub-populations studied all produced viable seeds but the mean proportion of fruit which contained a seed (seed:flower ratio) varied between sub-populations. Unfortunately because the research is incomplete it is not possible to determine whether variation in seed:flower ratios were population specific or reflected temporal variation. The initial data suggests that sub-population size does not affect the proportion of flowers which produce a seed but further replication of the study across at least one more flowering season is needed. The number of seeds produced by individual plants varied between 50 seeds and

3588 seeds. The initial results suggest that the production of viable seeds is unlikely to be limiting population growth.

4.4 Action 03. Investigate and report on the mating system and pollination biology of the species

4.4.1 Pollination biology

Population size may effect the diversity and abundance of pollinators, rates of pollination and subsequent development of viable seeds. Investigations of *V. staminosa ssp. staminosa's* pollination biology were initiated in 9 sub-populations to:

1. compare the diversity and abundance of flower visitors across sub-populations;
2. compare rates of pollination across sub-populations;
3. assess the relative importance of pollination as a factor constraining population growth; and,
4. provide baseline rates of pollinator abundance and pollination in wild plants for comparing and assessing rates in translocated plants.

4.4.1.1 The diversity and abundance of insects visiting flowers

Observations of flower visitors were made in the nine sub-populations used in studies of seed production. In each population an individual plant was observed for a period of fifteen minutes on each of four days. All observations were made in the middle of the day and were restricted to sunny days during peak flowering in July 1999. For each 15 minute observation period the identity and number of insects observed visiting flowers were recorded. For all sub-populations the mean number of insect flower visitors during the 15 minute observation periods were calculated. The results are presented in Table 4.5.

Table 4.5. The mean number of insect flower visitors observed during 15 minute intervals across nine V. staminosa ssp. staminosa sub-populations. Values are means ± SE

Sub-population	Sub-population size	Mean no. of insect flower visitors
W	160	2.01 ± 0.27
G	100	2.82 ± 0.33
H	89	2.86 ± 0.32
B	61	2.68 ± 0.11
A	46	2.84 ± 0.25
F	20	1.81 ± 0.39
D	3	0
I	3	0.96 ± 0.33
C	2	1.29 ± 0.18

Feral honey bees (*Apis mellifera*) were the only insect observed visiting *V. staminosa ssp. staminosa* flowers. The mean number of honeybees observed visiting flowers varied across sub-populations. Unfortunately because the research is incomplete it is not possible to determine whether variation in visitation rates were population specific or reflected temporal variation. The initial data suggests that rates of visitation are lower in the smallest sub-populations but further replication of the study across at least one more flowering season is needed.

4.4.1.2 Rates of pollination

Rates of pollination were measured in the nine sub- populations. In the 'large' (G, H and W) and 'medium' sized (A, B and F) sub-populations surveys were undertaken on five randomly chosen adult plants. In the small sub-populations surveys were restricted to three plants in each of two sub-populations (D and I) and 2 plants in the other (C). In each plant five flowers styles were harvested from around the plant and immediately fixed in FAA fixative. Styles were subsequently dissected from the flowers, examined under a fluorescing microscope and the number of pollen tubes per stigma and number of pollen tubes counted. The number of stigmas with pollen tubes and the mean number of pollen tubes per stigma were calculated across populations. The results are presented in Table 4.6. Similarly the number of styles with pollen tubes at their base and the mean number pollen tubes at the base of styles was calculated for each population. The results are presented in Table 4.7.

Table 4.6. The number of flower styles with pollen tubes on the stigma and the mean(± SE) number of pollen tubes on the stigma across V. staminosa ssp. staminosa populations

Sub-population	Sub-population size	Number of stigmas with pollen tubes	Mean no. pollen tubes per stigma
W	160	88	4.44 ± 0.64
G	100	96	5.36 ± 0.68
H	89	96	4.6 ± 0.93
B	61	100	6.72 ± 0.89
A	46	92	3.40 ± 0.46
F	20	92	3.68 ± 0.55
D	3	73	3.13 ± 0.86
I	3	66	4.47 ± 1.38
C	2	80	5.80 ± 1.93

Table 4.7. The number of flower styles with pollen tubes at their base and the mean (± SE) number of pollen tubes at the style base across V. staminosa ssp. staminosa populations

Sub-population	Sub-population size	Number of styles with pollen tubes at base	Mean no. of pollen tubes at style base
W	160	80	2.12 ± 0.40
G	100	92	2.12 ± 0.26
H	89	72	1.72 ± 0.33
B	61	92	2.72 ± 0.29
A	46	92	1.96 ± 0.24
F	20	80	1.60 ± 0.28
D	3	60	1.33 ± 0.36
I		60	1.60 ± 0.41
C	2	80	1.70 ± 0.42

The number of flower stigmas with pollen tubes and the mean number of pollen tubes per stigma varied across populations, Similarly the number of flower styles with pollen tubes at their base and the mean number of pollen tubes per style varied across populations. Unfortunately because the research is incomplete it is not possible to determine whether variation in rates of pollination are population specific or reflected temporal variation. The initial data suggests firstly, pollination is not a factor constraining population growth and secondly, that a population's size does not affect rates of pollination.

4.4.1.3 Mating Systems

Seed collections for mating system studies and preliminary allozyme studies have been completed to determine appropriate polymorphic loci. Mating system analyses were planned for the third year of the project but were not undertaken due to project funding not continuing beyond the second year.

Action 04. Investigate and report on the population genetic structures, levels of genetic diversity and minimum viable population size of the species

Seed collections for population genetic structure and genetic diversity studies have been completed from the known population of *V. staminosa ssp. staminosa* and related subspecies. This work was planned for the third year of the project but with no funding will now be deferred until resources are available.

4.6 Action 05. Identify and report on the impact and control of diseases and invasive weeds

Invasive weeds are present in *V. staminosa ssp. staminosa's* habitat but in relatively low abundances. Investigations of the impact of weeds on seedling recruitment were planned for the third year of the project but were not undertaken due to project funding not continuing beyond the second year. The initial observations of

seedling emergence and population structure suggest that current weed densities are not restricting seedling recruitment in *V. staminosa ssp. staminosa.*

4.7 Action 06. Identify and develop ex situ conservation strategies such as propagation protocols and germplasm storage techniques

Optimal germination protocols for *V. staminosa* ssp. *staminosa* have been identified and developed. Like many other *Verticordia* species, *V. staminosa* ssp. *staminosa* displays innate dormancy imposed by a requirement for after-ripening and the removal of seed from the hypanthium to permit the uptake of water and oxygen. The most successful technique for overcoming seed dormancy and stimulating maximal germination involves complete removal of the hypanthium (seed coat), soaking seed in a smoke water solution for 24 hours and incubation in a 25mg/L solution of Gibberellic Acid (as GA_3). Seeds are manually removed from the hypanthium with a sharp scalpel knife and placed on a 1% w/v agar solution with the addition of GA_3 in covered Petrie dishes and incubated at 15°C with 12 hours of alternating light and darkness. Up to 100% germination can be achieved under these conditions in the laboratory.

Germplasm storage techniques have been identified and developed for *V. staminosa* ssp. *staminosa.* Standard genebank practice recommends the storage of orthodox seeds (those able to withstand desiccation without adverse effects) at low temperatures and low moisture content (IPGRI 1994). These methods involve the reduction of seed moisture content to between 4-7% using a dehumidification process and the freezing of the seed in hermetically sealed containers (laminated aluminium foil) at –20°C. A total of 2957 seeds of this taxon have been placed in long term storage at CALM's Threatened Flora Seed Centre under these conditions, with viability after one year at low temperature and low moisture conditions at 100%.

4.8. Action 07. Develop the initial stages of a program of experimental translocations and population establishment experiments

Further monitoring of the transition of seedlings and tagged plants between life stage/size classes and modeling of the populations dynamics is required before considering a translocation program which could be technically difficult in the plant's habitat of cracks within the granite pavement.

4.9 Action 08. Undertake initial development of recovery strategies and provide a report on progress to date

The research will be completed when resources become available and will rank the constraints to the growth of existing populations and inform management and recovery of the species.

5. THE CONSERVATION BIOLOGY OF *VERTICORDIA SPICATA SSP. SQUAMOSA*

5.1 Background

5.1.1 Current conservation status

Verticordia spicata ssp. squamosa is currently known from 6 populations with a total of 25 individuals. The species is currently ranked as critically endangered under World Conservation Union IUCN Red List Criterion A1c, B1 + 2c, C1, D (World Conservation Union 1994).

5.1.2 Description

Verticordia spicata ssp. squamosa is a compact shrub to 80 cm tall by 1 m wide with rounded elliptic leaves, 1.5-2 mm long. The leaves have prominent oil glands, closely overlap, and are pressed to the stem providing the scaly appearance from which this subspecies derives its name. The mauve pink flowers are closely packed forming dense spikes on the ends of branches.

5.1.3 Distribution, landscape context and habitat

The six populations of *V. spicata ssp. squamosa* occur across a range of 17 km in the north central wheatbelt region of south-western Australia near Three Springs-Mingenew approximately 300 km north-east of Perth. The area has been extensively cleared for agriculture with remnants of the original native vegetation occurring in patches of varying size shape and health. Most of the populations occur in highly disturbed linear road verges or remnant vegetation on adjacent private property. One population occurs within a gravel quarry in a highly disturbed remnant. The plants are found in open mallee over scrub in deep yellow sands.

The area has a Dry Mediterranean climate with cool wet winters and dry hot summers. Mean annual rainfall at the nearest weather station in Carnamah is 389 mm with rain falling predominantly between May and September; summer rainfall does occur but is unpredictable. The mean maximum and minimum temperatures in the warmest month January are 35.9° C and 18.3° C and in the coolest month July are 17.8° C and 7.3° C respectively.

Table 5.1. The status and approximate size of V. spicata ssp. squamosa populations and sub-populations

Population	Land status	Approx. population size
3	Shire road reserve	1
4b	Private property	4
5	Shire reserve (quarry)	2
6a	Shire road reserve	10
6b	Private property	7
7	Shire road reserve	1

5.1.4 Research objectives

The aim of the project was to undertake research on the biology and ecology as outlined in the Interim Recovery Plan (Phillimore and English 2000). The research was undertaken with support from the Natural Heritage Trust Endangered Species Program Grant ESU 00006465. The research program was designed on the understanding that there would be a three-year commitment to funding the project but, unfortunately, funding did not continue beyond the second year. Consequently, the research and data sets are incomplete and it is not possible to interpret results or draw conclusions. This report therefore limits itself to describing the research and summarising the results within scope items for the period of funding.

5.2 Action 01. Investigate and report on the soil seed bank dynamics and the role of disturbance, competition and rainfall on recruitment and seedling survival

5.2.1 Soil seed bank

The presence of seed in the soil was investigated for *V. squamosa ssp. spicata* in populations 4b and 6b. There is no information on the dispersal of *Verticordia* fruits but observations of the accumulation of fruit beneath plants suggest that the highest density of seeds in the soil is likely to occur under or adjacent to the canopies of existing plants. Consequently soil samples were collected under the drip line of individual plants. The number of plants sampled varied between populations with soil cores being collected from beneath three plants in population 4b and four plants in population 6b.

Soil samples were collected at 30 cm intervals along the canopy drip line of each plant using a 10-cm diameter metal cylinder pushed 3 cm into the soil. Samples were stored in calico bags and transported to the laboratories where differential sieving separated fruits of both species from the soil. The presence of a viable seed in each fruit was determined by sectioning the fruit with a sharp blade. Seeds were considered viable if they contained fleshy embryo. The results are presented in Table 5.2.

Table 5.2. Density and viability of V. spicata ssp. squamosa fruit in the soil seed bank along the drip-line of individual plants

Population	Number of plants sampled	Mean density of fruit per m^2 (± SE)	Mean density of fruit with a viable seed per m^2 (± SE)
4b	3	649.0 ± 250.6	3.2 ± 1.9
6b	4	462.4 ± 91.4	4.4 ± 6.2

5.2.2 The influence of smoke in stimulating germination of soil stored seed

The influence of smoke in stimulating germination of *V. spicata ssp. squamosa* seeds stored in the soil was investigated in the field. In July 1998 aerosol smoke was applied to soil around two clumps of plants in each of populations 4b and 6b. Plots were marked out around each clump with a 3m x 1m plot or a combination of 1m x 1m and 2m x 1m plots depending on the density of the vegetation surrounding the *Verticordia* plants. Bare or

mostly bare soil was chosen preferentially to be smoked, small plants were removed from plots to be smoked as necessary. Wooden stakes, 900 mm long, were hammered in to the ground, creating 1m x 1m, 2m x 1m or 3m x 1m plots. A plastic tent was draped over the marked plots and the sides held down to the ground using sand-filled 'snakes' or plain sand to provide a relatively 'smoke tight' chamber. Into each tent was placed an outlet pipe, which led back to the smoke drum. The smoke drum consisted of a 60 Litre drum with a lid and small (25mm) metal pipe welded to a hole on the lower portion of the drum, creating an inlet. A larger (45mm) pipe was welded to a hole near the top creating an outlet pipe. The outlet pipe split into three smaller outlets approximately 50 cm from the drum. One small outlet was sealed and two metal pipes were attached to the two remaining outlets. Each pipe was then run to a tent, under the sand 'snakes' and into the tent cavity. A fan was inserted in to the inlet pipe and air blown into the drum. A 12V car battery powered the fan and speed was controlled using a voltage regulator. A fire was lit in the drum and stoked with leaves and other green material to produce a thick black smoke. The lid was placed over the top, forcing all of the smoke out through the outlet pipes into the smoke tents. Soil was smoked for an hour, the fire being stoked periodically during that time. After this time the fan was turned off, the fire extinguished and the tents removed, leaving only the stakes. A brown or dark discolouration remained on the soil showing which areas had been smoked.

Germinants were recorded in late November 1998 and survival of seedlings noted throughout 1999. Unsmoked soil adjacent to smoke-treated soil was used as a control and was also examined for the presence of seedlings. The procedure was repeated again in late May 1999 and seedling emergence monitored in October 1999. The results are presented in Tables 5.3 and 5.4.

Table 5.3. The emergence of V. spicata ssp. squamosa seedlings from the soil seed bank following exposure to smoke and subsequent survival of seedlings. Results of July 1998 smoke trial

Population and treatment	Area treated	No. seedlings 12/11/1998	No. seedlings surviving 11/2/1999	No. seedlings surviving 30/3/1999	No. seedlings surviving 12/6/1999
4b *smoke*	6 m^2	1	0	0	0
4b *control*	6 m^2	0	0	0	0
6b *smoke*	6 m^2	17	8	5	0
6b *control*	6 m^2	0	0	0	0

Table 5.4. The emergence of V. spicata ssp. squamosa seedlings from the soil seed bank following exposure to smoke and subsequent survival of seedlings. Results of May 1999 smoke trial

Population and treatment	Area treated	No. seedlings 1/10/99
4b *smoke*	6 m^2	35
4b *control*	6 m^2	0
6b *smoke*	6 m^2	0
6b *control*	6 m^2	0

The application of aerosol smoke stimulated the germination of soil stored seed in both years. No seedlings were observed in control plots which were not exposed to smoke.

5.3 Action 02. Identify and report on reproductive strategies, phenology and seasonal growth of the species

5.3.1 Reproductive phenology and seed:flower ratios

Landscape context and population size may effect population processes that underlie the development of viable seeds. Investigations on individual plants of reproductive phenology and seed to flower ratios were initiated in two populations to:

1. compare rates of seed production across populations;
2. assess the relative importance of seed production as a factor constraining population growth; and,
3. provide baseline rates from wild plants for comparing and assessing the reproductive success of translocated plants.

All species of *Verticordia* have solitary, pedunculate axillary flowers. There is, however, great variation in the length of the peduncle and in the length of the flower bearing part of the branchlet (George 1991). This gives rise to the appearance of large inflorescences which may be corymb-like, rounded or spike-like racemes (George 1991). *Verticordia spicata ssp. squamosa* has spike-like racemes on the ends of branchlets.

Investigations of flowering in *V. spicata ssp. squamosa* were undertaken during peak flowering in 1998 on five plants in both populations 4b and 6b. In each plant the total number of flowering spikes and the number of flowers on 10 randomly chosen spikes were counted. The number of flowers per plant was subsequently calculated using the formula: *Total number of flowers per plant = number of 'flower spikes' per plant x the mean number of flowers per 'spike'*. The results are presented in Table 5.5.

Table 5.5. The mean number of flowers per spike, total number of flower spikes and estimates of the total number of flowers per plant in two V. spicata ssp. squamosa populations

Population	Plant	Mean no. of flowers per spike	Total no. of spikes per plant	Total no. of flowers per plant
4b	1	23.4 ± 2.5	3200	74880 ± 7884
	2	25.0 ± 2.7	1800	45000 ± 1933
	3	22.4 ± 2.3	1500	33600 ± 3407
	4	31.1 ± 1.6	1700	52870 ± 2734
	5	16.5 ± 1.9	1800	29700 ± 3490
6b	1	22.7 ± 1.8	2630	59701 ± 4705
	2	18.5 ± 0.8	2020	37370 ± 1683
	3	20.2 ± 2.1	1920	38784 ± 3963
	4	22.7 ± 2.3	720	16344 ± 1649
	5	30.9 ± 3.1	2740	83844 ± 8432

Verticordia spicata ssp. squamosa produces large numbers of flowers. The total number of flowers on plants ranged between 16 344 and 83 844.

Investigations of seed production were undertaken on plants across four populations in December 1998. *Verticordia* fruit is dry and nut like, enclosed within the faded perianth. It usually contains a single seed and occasionally two. Externally it is difficult to distinguish from a faded flower containing no seed (George 1991). As a consequence fruit with seed cannot be distinguished from empty flowers unless they are dissected. For the purposes of this report all faded flowers are described as 'fruit'. In December 1998 when fruits had matured but before they were dispersed 30 fruits were collected from around the canopy of each plant. In *Verticordia* there is no endosperm in the mature seed, it is absorbed by the embryo into a swollen hypocotyl which has at one end a small root tip and at the other tiny cotyledons. Therefore fruit which showed a full, white, moist embryo were regarded as containing viable seed and fruit with no embryo or which possessed a dark brown, shriveled or dry embryo were regarded as not containing a viable seed. The proportion of fruits containing a viable seed was calculated and the mean seed:flower ratio for each population calculated. The results are presented in Table 5.6.

Table 5.6. The proportion (%) of V. spicata ssp. squamosa fruits containing a viable seed across populations in 1998. Values are means ± SE

Population	Approximate population size	Proportion (%) of fruit containing a viable seed
6a	10	9.6 ± 2.8
6b	7	7.3 ± 2.4
4b	4	21.7 ± 4.6
7	1	0

The mean proportion of fruits which contained a seed (seed:flower ratio) varied between populations. Unfortunately because the research is incomplete it is not possible to determine whether variation in seed:flower ratios were population specific or reflected temporal variation. The initial data suggests that seed production in population 7 is impaired.

5.4 Action 03. Investigate and report on the mating system and pollination biology of the species

Pollination biology and mating system studies were planned for the third year of the project but were not undertaken due to project funding not continuing beyond the second year.

5.5 Action 04. Investigate and report on the population genetic structures, levels of genetic diversity and minimum viable population size of the species

Seed collections for population genetic structure and genetic diversity studies have been completed for populations of *V. spicata* ssp. *squamosa* and related subspecies. This work was planned for the third year of the project but with no funding will now be deferred until resources are available

5.6 Action 05. Identify and report on the impact and control of diseases and invasive weeds

The *V. spicata ssp. squamosa* populations occur in highly disturbed narrow linear strips of remnant native vegetation on road reserves or adjacent private property and are surrounded by agricultural land. The potential for weed invasion is therefore high in all populations. Surveys were undertaken in each population to determine the relative importance of weeds in the remaining vegetation. The structure of the plant communities where *V. spicata ssp. squamosa* occurs were described in 20 1m x 1m quadrats. In each quadrat the percentage cover of litter, woody perennials <1.3 m high and weeds were estimated by eye. In addition the % cover of woody perennials > 1.3 m high was measured with a convex spherical densiometer and the height of the tallest stratum (cm).

Table 5.7. Plant community structure in the remnants where the V. spicata ssp. squamosa populations occur. Values are means ± SE from 20 1 m x 1 m quadrats at each site

Population	Woody perennial cover < 1.3 m high (%)	Litter cover (%)	Weed cover (%)	Tallest stratum (cm)	Canopy cover > 1.3 m high (%)
4b	41.7 ± 6.4	40.2 ± 7.5	11.3 ± 2.8	153.0 ± 15.4	46.2 ± 8.7
6a	61.8 ± 5.9	54.5 ± 7.1	4.52 ± 1.3	207.5 ± 40.7	6.35 ± 2.0
6b	33.7 ± 7.6	64.5 ± 4.7	35.6 ± 7.1	395.5 ± 45.6	56.7 ± 9.1
7	21.5 ± 6.3	34.3 ± 6.2	49.2 ± 6.1	197.5 ± 37.1	32.9 ± 10.1

The structural characteristics of the plant communities in the four remnants where *V. spicata ssp. squamosa* occurs varied. Invasive weeds were recorded in all populations and in very high abundances (> 30 % cover) in two populations. Invasive weeds are a threat in all populations. The effects of removing weeds to reduce competition, applying aqueous smoke solution to stimulate germination of the soil seed bank and their interaction are currently being investigated.

5.7 Action 06. Identify and develop ex situ conservation strategies such as propagation protocols and germplasm storage techniques

Optimal germination protocols for *V. spicata* ssp. *squamosa* have been identified and developed. Like many other *Verticordia* species, *V. spicata* ssp. *squamosa* displays innate dormancy imposed by a requirement for after-ripening and the removal of seed from the hypanthium to permit the uptake of water and oxygen. The most successful technique for overcoming seed dormancy and stimulating maximal germination involves complete removal of the hypanthium (seed coat), soaking seed in a smoke water solution for 24 hours and incubation in a 25mg/L solution of Gibberellic Acid (as GA_3). Seeds are manually removed from the hypanthium with a sharp scalpel knife and placed on a 1% w/v agar solution with the addition of GA_3 in covered Petrie dishes and

incubated at 15°C with 12 hours of alternating light and darkness. Up to 86% germination can be achieved under these conditions in the laboratory.

Germplasm storage techniques have been identified and developed for *V. spicata* ssp. *squamosa*. Standard genebank practice recommends the storage of orthodox seeds (those able to withstand desiccation without adverse effects) at low temperatures and low moisture content (IPGRI 1994). These methods involve the reduction of seed moisture content to between 4-7% using a dehumidification process and the freezing of the seed in hermetically sealed containers (laminated aluminium foil) at -20^0C. A total of 4,4130 seeds representing three populations have been placed in long term storage at CALM's Threatened Flora Seed Centre under these conditions with the viability of at least one population at 60% after one year at low temperature and low moisture conditions.

5.8. Action 07. Develop the initial stages of a program of experimental translocations and population establishment experiments

A program of experimental translocations and population experiments informed by experiments determining factors which limit seed germination and seedling establishment were planned for the third year of the project but were not undertaken due to project funding not continuing beyond the second year.

5.9 Action 08. Undertake initial development of recovery strategies and provide a report on progress to date

The research will be completed when resources become available and will rank the constraints to the growth of existing populations and inform management and recovery of the species.

6. THE CONSERVATION BIOLOGY OF *VERTICORDIA PLUMOSA VAR. ANANEOTES* AND *VERTICORDIA PLUMOSA VAR. PLEIOBOTRYA*

Initial population surveys have been carried out and monitoring sites were chosen for demographic studies on populations of both sub species. Seed material for mating system, population genetic structure and genetic diversity and seed biology studies has been collected from both taxa. Data collection for demographic and genetic studies was planned to start during the 1999 flowering season and continue to the end of the 2000 flowering season. Because notification for the cessation of funding for this project was received in late 1999 all planned work on theses two taxa was cancelled.

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