

Growth and habitat of *Sebaea ovata* (Gentianaceae) in New Zealand and Australia

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Growth and habitat of *Sebaea ovata* (Gentianaceae) in New Zealand and Australia

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ABSTRACT

Sebaea ovata (Labill.) R.Br., a critically endangered indigenous annual herb, was locally common in coastal lowlands and swampy ground in New Zealand. It is now only present as two small populations (Whitiau and Waitotara) in dune flats in the Wanganui Conservancy. The field population of *S. ovata* at Whitiau Scientific Reserve (New Zealand) and field sites in Australia were investigated. Cultivation and morphological and genetic comparisons of New Zealand and Australian plants were made to guide the management of *S. ovata* in New Zealand. At Whitiau, *S. ovata* is restricted to an area of c. 300 m² with a total population of between 8757 plants (December 1998) and 641 plants (March 1999). Sediment core samples showed no evidence of an extensive seed bank. Soils were very low in available nitrogen compared to Waitotara. *S. ovata* requires open vegetation in which to establish and maintain its population. Winter flooding of the dune habitat excludes the more competitive perennial species. If flood events do not coincide with the life cycle of *S. ovata*, plants may be killed without contributing seed. A series of atypical seasons could lead to extinction, therefore the status of *S. ovata* in New Zealand is considered critical. The extinction of one or both populations is likely unless conservation management is undertaken. In southern mainland Australia, *S. ovata* occupies a different habitat. It occurs in open eucalypt forest amongst a diverse assemblage of native and introduced annual species which grow and reproduce in spring/early summer, influenced by seasonal rainfall. Differences in habitat and plant size have led to suggestions that *S. ovata* from New Zealand and Australia may be different taxa. Glasshouse cultivation of plants from both countries, and morphological comparison (supported by ITS sequence data) indicate they are indeed the same species. Successful cultivation of *S. ovata* has been achieved. Cultivated plants and seed have been returned to the Department of Conservation for replanting and resowing. The maintenance of *S. ovata* in culture, as well as the protection of suitable *S. ovata* habitat are the key to ensuring the survival of the species in New Zealand.

Keywords: endangered, annual herb, loss of habitat, weed encroachment, dune hollows, Whitiau Scientific Reserve, New Zealand, Warrandyte State Park

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1. Introduction

The indigenous *Sebaea ovata* (Labill.) R.Br. (Gentianaceae) is a critically endangered annual herb that was recently thought to be extinct (Ogle 1997). Previously, *S. ovata* was locally common in coastal and lowland boggy and swampy ground, and dune-hollows from c. lat. 35°S southwards (Allan 1961). In 1989 what was considered to be the last remaining population of *S. ovata* was discovered in the Whitiāu Scientific Reserve (Wanganui Conservancy) (Ogle 1998b). At Whitiāu *S. ovata* is restricted to one area of dune flat, where its habitat is declining due to colonisation by tall-growing rushes, sedges and grasses along with introduced weedy herbs (Ogle 1998b). *S. ovata* is classified in the highest class of threat of extinction, 'critically endangered' (Dopson et al. 1999).

The Science and Research Division of the Department of Conservation contracted NIWA to investigate aspects of the ecology and biology of the critically endangered *S. ovata* in 1998/99. These studies involved the assessment of the population size and possible seed bank structure at the last known New Zealand population at the time (Whitiāu Scientific Reserve, Wanganui Conservancy), and also germination and culture requirements for this species. Initial findings of a declining population and limited seed bank suggested that extinction of *S. ovata* was likely within the next five years unless ex situ culture and in situ management intervention was trialled and implemented.

It was vital to the conservation of New Zealand *Sebaea* to ascertain what the taxonomic relationship between New Zealand and Australian *Sebaea* is (Dopson et al. 1999). It has been suggested that New Zealand plants differed sufficiently from Australian material to constitute a separate taxon (Ogle 1989). Additional funding from Science and Research Division of DOC enabled material of *S. ovata* from New Zealand and Australia to be cultured, allowing a comparison of morphological characters and genetic variation to be made. A general enquiry to the Enviroweeds email group (ENVIROWEEDS@nre.vic.gov.au) regarding the location of *S. ovata* in Australia led to correspondence with David van Boekel of Parks Victoria, who knew of several populations of the plant in the Warrandyte State Park. Funding was obtained from the International Science and Technology Linkages Fund of the Royal Society of New Zealand (Bilateral Research Activities Programme) to enable a visit to Victoria and South Australia to collect *Sebaea* species and investigate the ecology of *S. ovata* in Australia. Material of *S. albidiflora* F Muell was also collected from Tasmania by Alex Buchanan (Tasmanian Herbarium) and dried plants were imported to New Zealand to compare with New Zealand sourced *S. ovata*.

This report investigates the ecology of the Whitiāu field population of *S. ovata* in New Zealand and field sites in Australia, attempts to cultivate this plant, and morphological and genetic comparisons of New Zealand and Australian plants for the management of *S. ovata* in New Zealand.

At the time of inception of the present project, *S. ovata* was only known to survive in New Zealand at one field site, Whitiau (nr Wanganui). During the course of the present study, Jim Campbell (DOC Wanganui) discovered a second New Zealand population of *S. ovata*, at the Waitotara River mouth in a similar dune flat habitat. Where time allowed, information from the Waitotara site and plants were also gathered for comparison with the Whitiau field site and Australian *Sebaea*.

2. Field studies

Field investigations were undertaken to estimate the *S. ovata* population and seed bank, to describe the habitat of *S. ovata* (associate vegetation and nutrient profile of the sediments), and the habitat of *S. ovata* in Australia, to allow a better prediction of the long-term viability of *S. ovata* plants in New Zealand.

2.1 METHODS AND RESULTS

2.1.1 Estimate of population size of *Sebaea ovata*

The total area occupied by *S. ovata* at Whitiau was estimated on three field visits, in December 1998, March 1999, and February 2000. Population size of *S. ovata* was estimated using plant numbers from 30 randomly thrown quadrats (310 mm × 310 mm) within the area. Table 1 shows the results of the population estimates of *S. ovata* at Whitiau.

The individual plants of *S. ovata* sampled at Whitiau were invariably small (less than 100 mm tall) and supported one or two flower heads (Fig. 1). In order to assess reproductive output, plants collected by Colin Ogle in 1997/98 were assessed for flower number, total number of seed, and where dehiscence had not occurred, seed number per capsule (Table 2).

TABLE 1. ESTIMATION OF POPULATION SIZE OF *Sebaea ovata* AT WHITIAU SCIENTIFIC RESERVE.

	DECEMBER 1998	MARCH 1999	FEBRUARY 2000
Total area in which <i>S. ovata</i> is found in Whitiau (m ²)	308		
Total quadrat area (m ²)	2.883		
Number of quadrats	30		
Number of quadrats with <i>S. ovata</i>	16	4	5
Total number of <i>S. ovata</i> in all quadrats	82	6	7
Mean number of <i>S. ovata</i> per quadrat (standard deviation)	2.73 (6.22)	0.20 (0.66)	0.23 (0.89)
Estimate of <i>S. ovata</i> (per m ²)	28	2	2
Estimate of total population of <i>S. ovata</i>	8757	641	748

TABLE 2. SEED NUMBERS COUNTED FROM *Sebaea ovata* INDIVIDUALS COLLECTED IN 1997/98.

PLANT	CAPSULES	EMPTY CAPSULES (ABORTED)	SEEDS PER FLOWER	LOOSE SEEDS
Packet 1	1 intact		F1=60	
Packet 2				
1	3		F1=45 F2*=24 F3=31	
2	6	5	F6=29	5
3	8	7	F4*=50	2
4	5	4	F5=50	6
5	4	4	N/A	
6	7	7	N/A	
7	5	5	N/A	
8	6	6	N/A	
9	9	9	N/A	
10	5	5	N/A	
11	14	14	N/A	
Extra seeds				898
Total	73	66 (4)	289	911

* Capsule was possibly open

Number of seeds per developed capsule (based on unopened capsules) = $215/7 = 31$

Number of seeds per developed capsule (based on total seed/capsule) = $(289+911)/73 = 16$

Thus an average output of around 30 seeds per fertilised capsule may be produced corresponding to 60 seeds per plant (based on observed counts of an average of two capsules with viable seed produced per plant) and an annual seed output of c. 50000 (1700 per square metre) assuming that there are two generations per year of *S. ovata* (represented by the December 1998 and March 1999 samples).

2.1.2 Determination of seed bank

Five cores (54 mm diameter × 200 mm) were taken from each of five sites (quadrats sampled in December 1998). Cores were taken back to the laboratory and sieved through a stack of 850, 500, 250, 125 and 76 µm sieves. Using seed collected the previous summer by Colin Ogle, it had been determined that the seeds were c. 250 µm in size.

A total of five cores were sieved, one from each of sites 4 and 5, and three from site 1. These quadrats had the highest populations of living *S. ovata* adjacent to the cores. All sieve fractions were checked under the microscope, and no seeds were found in any of the cores. The remaining cores were not sieved but kept intact for the germination study (see Section 3. Cultivation trials).

2.1.3 Sediment analysis

Sediment was collected from Whitiāu Scientific Reserve in February 2000, and later from the *S. ovata* site at Waitotara. Four samples were taken from sites where *S. ovata* had been previously recorded in Whitiāu. Samples were analysed by the Soil Fertility Service (AgResearch). The results are shown in Table 3.

TABLE 3. SOIL FERTILITY OF SAMPLES TAKEN AT WHITIAU AND WAITOTARA FROM AREAS SUPPORTING THE GROWTH OF *Sebaea ovata* .

TEST	pH	Ca	P	K	S(SO ₄)	Mg	Na	NH ₄	NO ₃
	1:2.1 v/v slurry	Ammonium acetate extr. 1g/40 × 10 ³ mL extr.	Olsen extr.	Ammonium acetate extr. 1g/250 × 10 ³ mL extr.	K phosphate extr.	Ammonium acetate extr. 1g/1 × 10 ⁶ mL extr.	Ammonium acetate extr. 1g/1 × 10 ⁶ mL extr.	KCl extr.	KCl extr.
SAMPLE		Calcium MAF QT	Phospho- or-us µg/ml	Potassium MAF QT	Sulphate/ Sulphur ppm	Magnes- ium MAF QT	Sodium MAF QT	Ammonium Nitrogen ppm	Nitrate Nitrogen ppm
Whitiau 1	8.4	4	3	1	2	2	4	<1	<1
Whitiau 2	7.3	12	4	2	4	10	8	<1	<1
Whitiau 3	8.1	3	1	1	3	2	3	<1	<1
Whitiau 4	7.8	9	1	1	5	4	5	<1	<1
Waitotara	7.7	5	1	1	1	4	5	8	<1

Macronutrient levels were comparable between the two sites apart from ammonium-nitrogen, which was much higher at the Waitotara site. Difference in available nitrogen between Waitotara and Whitiau may contribute to the lack of *S. ovata* plant height and vigour at Whitiau compared with *S. ovata* plants at Waitotara.

2.1.4 Vegetation supporting the growth of *Sebaea ovata*

Associate vegetation was assessed at Whitiau using the 30 randomly thrown quadrats (310 mm × 310 mm) within the dune hollow that *S. ovata* occurs. In December 1998, the presence of other plant species in each quadrat was recorded and vegetation cover was assessed in March 1999 and February 2000. The vegetation within the area supporting *S. ovata* may be described as *Schoenus nitens*-hawkbit-*Gunnera dentata* herbfield (Figs 1 and 2, Table 4)



Figure 1. *Sebaea ovata* at Whitiau Scientific Reserve. The *S. ovata* plants (centre right) are c. 10 cm high and bearing single flowers. Associate plants include *Schoenus nitens*, Yorkshire fog (*Holcus lanatus*) and oioi (*Apodasmia similis*).

Figure 2. Quadrat at
Whitiau Scientific Reserve.
Plants include *Gunnera
dentata*, *Sisyrinchium
'blue'* (in flower), and oioi
(*Apodasmia similis*). The
apparent bare patches are
covered in a dry mat of
algae.

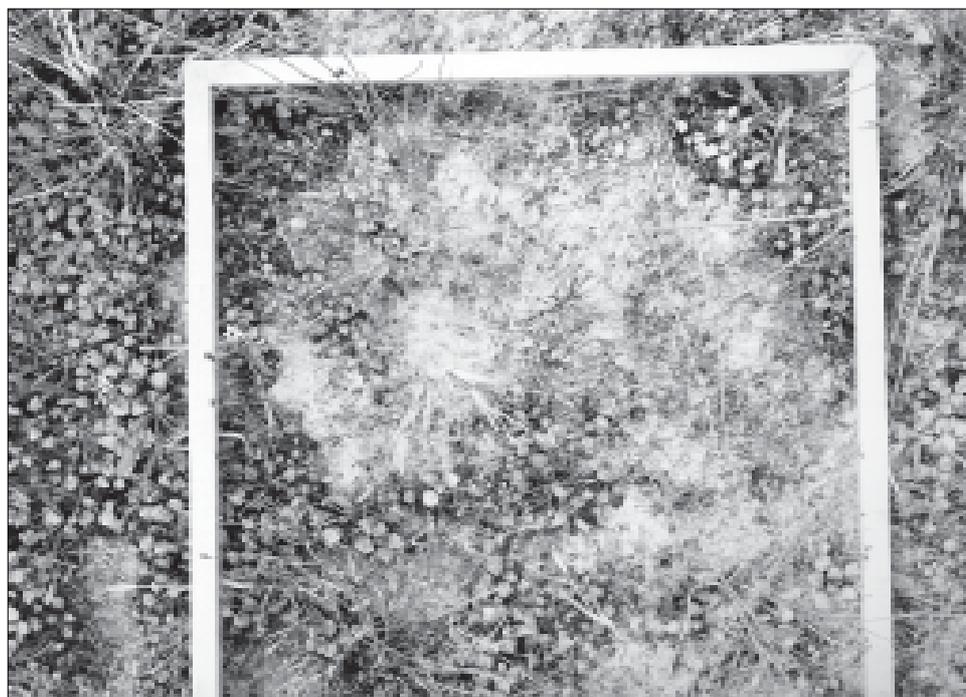


TABLE 4. ASSOCIATE SPECIES PRESENCE OR COVER IN 30 QUADRATS SAMPLING *Sebaea ovata* HABITAT AT WHITIAU.

SPECIES	% OF PLOTS WITH THE SPECIES	% OF PLOTS WITH THE SPP. AND <i>S. ovata</i>	AV. COVER	AV. COVER IN PLOTS WITH WITH <i>S. ovata</i>	AV. COVER	AV. COVER IN PLOTS WITH <i>S. ovata</i>
	DEC 1998	DEC 1998	MAR 1999	MAR 1999	FEB 2000	FEB 2000
* <i>Agrostis stolonifera</i>	3	6	1.5	0	0.1	0
<i>Apodasmia similis</i>	50	50	2.6	7	1.9	6.0
<i>Epilobium billardioreanum</i>	0	0	0.1	0	<0.1	<0.1
<i>Gunnera dentata</i>	83	75	12.1	4.5	1.9	<0.1
* <i>Holcus lanatus</i>	43	50	5.2	1.3	<0.1	1.0
* <i>Juncus articulatus</i>	40	56	2.1	1.8	1.6	2.0
<i>Juncus caespiticus</i>	0	0	0.6	0.3	0.3	2.0
<i>Lachnagrostis billardierei</i>	0	0	0	0	0.2	0
<i>Lachnagrostis filliformis</i>	0	0	0	0	0.1	0
* <i>Leontodon taraxacooides</i>	90	100	10.8	8.3	5.5	8.8
<i>Lobelia anceps</i>	10	13	0.7	0.8	0.1	0.4
* <i>Prunella vulgaris</i>	23	25	0.7	1	1.6	<0.1
* <i>Schedonorus phoenix</i>	17	13	0.7	0	0.8	1.0
<i>Schoenus nitens</i>	90	94	6.4	3.5	30.3	35
<i>Selliera rotundifolia</i>	57	63	2.1	1.5	0.9	0.2
* <i>Sisyrinchium blue</i>	63	69	0.8	0.8	<0.1	0
* <i>Sporobolus africanus</i>	56	38	3.3	0.5	0.4	0.4
* <i>Trifolium fragiferum</i>	3	0	0.9	0	<0.1	0
<i>Triglochin striata</i>	0	0	<0.1	0	0.1	0.2
Total cover			51.9	31	49	60.8

* Alien species are identified by an asterisk.

Figure 3. Typical habitat for *Sebaea ovata* in Torrens Gorge, South Australia.



with a mixture of low (< 100 mm) indigenous and alien herbs, grasses, rushes and sedges, with scattered areas of taller oioi (*Apodasmia similis*) and club rush (*Isolepis nodosus*). A total of 36 species (16 indigenous, 20 alien) were recorded in the quadrats, and these and additional species found within the vicinity are listed in Appendix 1. Vegetation cover was generally low in over half of these quadrats (Fig. 2 and Table 3). Table 3 presents a list of associate species with an average cover of at least 0.1% in one or more plots. These were compared with quadrats that contained plants of *S. ovata*. Presence of *S. ovata* does not appear to be correlated with other species in these areas, or with total vegetation cover.

2.1.5 Australian studies

Sebaea ovata was seen at a range of sites in southern Australia, where it occurred in very open escarpment woodland (Fig. 3) with occasional eucalypt trees (see Appendix 2) and where total vegetation cover was less than 50%. Associate species were predominantly annuals or geophytes, with a mixture of native and introduced species (Fig. 4). A list of associate species recorded at Warrandyte State Park, near Melbourne, Victoria is given in Appendix 2. Plants of *S. ovata* germinate during late winter/spring and are present until mid-summer. This seasonality is a response to periodic rainfall events in Victoria and South Australia (D. Van Boekel pers. comm.).

Plants of *S. ovata* were collected from two sites (Pound Bend and Jumping Creek) in Warrandyte (n = 6), near Zummsteins in the Grampian Range, Victoria (n = 5) and near Kangaroo Creek Reservoir in the Torrens Gorge, near Adelaide, South Australia (n = 6). No plants of *S. ovata* were found at three other Warrandyte sites that formerly were reported as supporting this plant, nor at Black Hill Conservation Park, near Adelaide.

Sebaea ovata was not common at any of these sites, occurring in scattered populations of c. 50–100 plants per square metre (estimated by five random

Figure 4. Plants of *Sebaea ovata* at Pound Bend, Warrandyte, Victoria (below and right of the marker peg). Associate plants include squirrel-tail fescue (*Vulpia bromoides*), wallaby grass (*Austrodanthonia* spp.), square cicendia (*Cicendia quadrangularis*), small poranthera (*Poranthera microphylla*), common sunray (*Triptilodiscus pygmaeus*) and tiny vetch (*Vicia hirsuta*) (see Appendix 2).



quadrats thrown in areas supporting plants of *S. ovata*). Plants were similar in stature to the Whitiau population, although one 100 mm-tall plant was noted amongst dense grasses.

Plants of *S. albidiflora* were collected from a salt marsh south of Salt Creek in the Coorong Region of South Australia ($n = 7$) (Fig. 5). This was the only site investigated that supported this plant, with searches of Murtnaghurt Lagoon near Lake Connewarre, near Geelong and inland salt marshes around several lakes (where the species was previously collected) failing to turn-up this species. Material of this species was collected to provide an outlier for genetic comparisons of *S. ovata* from Australia and New Zealand (see Section 4. Characterisation of *Sebaea*).



Figure 5. *Sebaea albidiflora* (centre) in salt marsh near Salt Creek, Lower Coorong, South Australia.

The habitat of *S. ovata* in Victoria and South Australia appeared to be quite different from that in New Zealand where seedlings germinate following the drying up of ephemeral ponds in its dune habitat, as opposed to a response to rainfall. Flowering plants in New Zealand may be present from October to May, (dependent on the disappearance of standing water), compared with August to December in Australia. Habitat in Tasmania is more similar to the New Zealand situation, with *S. ovata* occurring in dune flats and seasonally flooded areas of coastal forest along with dry open eucalypt forest (de Lange pers. comm.).

2.2 DISCUSSION

Field investigations were undertaken to estimate the *Sebaea ovata* population and seed bank, to describe the habitat of *S. ovata* (associate vegetation (Table 4, Appendix 1; and nutrient profile of sediments in Table 3), and the habitat of *S. ovata* in Australia, to allow a better prediction of the long term viability of *S. ovata* plants in New Zealand.

Since its rediscovery in 1989 the Whitiiau population of *S. ovata* has been declining (Ogle 1998b). In December 1994 the population was estimated to be some 5000–7000 plants, with 604 counted in a single metre square plot compared with December 1996 when there were an estimated 300 plants in total (Ogle 1998b). Similarly in the present study estimated numbers of *S. ovata* have declined from 28 plants per square metre in December 1998 to only 2 plants in February 2000. Although some variation in estimated plant numbers from year to year may be accounted for by seasonal climate variations, (e.g. surface water in the dune hollow until late November in 1996 may have resulted in later germination that year (Ogle 1998b), and hence plant numbers from the same months in different years (e.g. December 1994 to December 1996) may not be directly comparable), decline in *S. ovata* plant numbers has also been noted from later observations (author's pers. obs. in 2001 and 2002).

Further evidence of population decline is observed in the small stature of the Whitiiau plants, with few flowers and seed production compared with cultivated plants (see Section 3. Cultivation, Figs 10 and 11) and the lack of *S. ovata* seed in sediment cores taken adjacent to *S. ovata* rich areas. Plants at Whitiiau were typically small, less than 100 mm in height, compared with *S. ovata* in cultivation where 79% were taller than 100 mm and some plants were over 30 cm. Whitiiau *S. ovata* typically had only one or two flowers (Fig. 1) whereas 79% of cultivated plants had at least 3 flowers, which corresponds to 30–60 seeds per Whitiiau sourced plant, and over 150 seeds per cultivated plant.

Small plants with fewer seed may also account in part for the lack of *S. ovata* seed observed in sediment cores taken adjacent to *S. ovata* rich areas (see below under Cultivation trials: 3.1.2 Cores). The time of year for core sampling with respect to spring rainfall events, in which seed may float to new localities (Ogle 1998b) and the germination of seed prior to sediment sampling are also likely contributing factors.

The cause of the decline of the *S. ovata* population at Whitiiau is likely the result of several contributing factors. Threats that have been identified include habitat destruction, weed encroachment, browsing (plume moth caterpillar), and

cattle trampling (Dopson et al. 1999). Conversely cattle and rabbit browsing may assist in maintaining *S. ovata*, as it appears that this species is unpalatable (P. de Lange pers. com.) and these mammals could help provide an open habitat critical for *S. ovata*. La Cock & Ogle (1999) discuss the impact of decreased influence of rabbit browse and soil disturbance since their decline from rabbit calicivirus disease. Decrease rabbit activity was seen as likely to allow weeds to flourish with a loss of available *S. ovata* habitat.

Nutrient limitation may also be a significant factor contributing to the decline of *S. ovata* at Whitiau. Nutrient analysis of sediment samples from Whitiau and Waitotara has shown comparable macronutrient levels apart from ammonium-nitrogen, which was much higher at the Waitotara site. Difference in soil fertility between Waitotara and Whitiau has been suggested as contributing to the lack of *S. ovata* plant height and vigour at Whitiau compared with *S. ovata* plants at Waitotara (C. Ogle pers. com. 2000). Upon discovery of the Whitiau *S. ovata* site plants were recorded as being between 40 mm and 140 mm tall (Ogle 1989) which is similar to plant heights observed more recently during the present study, this is generally smaller than plants of *S. ovata* at Waitotara that have been described as up to 200 mm (C. Ogle pers. com. 2000). An initial study was conducted (see cultivation discussion) to assess if plant vigour (height and maturation) was related to nutrient levels using cultivated *S. ovata*. The results were not conclusive (i.e. no increase in plant vigour) using cultivated plants, which may be due to natural variation in the *S. ovata* seedlings. However cultivated plants using Whitiau sourced seed were still generally larger than *S. ovata* from Whitiau, which may indeed be an indication of nutrient limitation at that site.

The lack of open habitat for seedling establishment at Whitiau, due to weed encroachment, has been described as a major cause of *S. ovata* decline at that site (Ogle 1998b). Data from two summers (1998/99 and 2000) does not indicate a relationship between the presence of *S. ovata* in association with other species or with total vegetation cover in the quadrats sampled, however alien species were common, accounting for over half of the species present. Similarly, a mixture of both alien and native species was recorded amongst the associate flora of *S. ovata* at Warrandyte Australia. But at Warrandyte, unlike Whitiau, *S. ovata* occurred in very open escarpment woodland with occasional eucalypt trees and only a third of the species recorded were alien. The *S. ovata* sites at Warrandyte are potentially less impacted by adventive species than at Whitiau and *S. ovata*, though not a common species, is not in decline.

Like other indigenous annual herbs in New Zealand (Champion et al. 2000), *S. ovata* requires open vegetation (i.e. low-stature vegetation with bare substrate visible between the plants) in which to establish and maintain its population. At Whitiau (and the newly discovered site at Waitotara), these conditions appear to be provided by winter flooding of the dune habitat that might exclude more competitive perennial species. If the timing of flood events does not coincide with the life cycle of *S. ovata* (e.g. if flooding occurs before plants produce seed) they would be killed without contributing seed for future generations. Extinction of the population could occur following a series of atypical seasons.

3. Cultivation trials

Germination and cultivation trials were designed to assess the viability of fresh and sediment sourced *S. ovata* seed and to establish a technique for cultivating *S. ovata* that could provide information for the management of field sites as well as enable the establishment of a culture population.

3.1 METHODS AND RESULTS

3.1.1 Filter paper

Twenty-four seeds taken from material collected by Colin Ogle in 1997/98 were placed on moist filter paper (4 December 1998) and left by a windowsill (with high but not direct sunlight). After four months (12 April 1999), only three seeds had germinated. They were put in potting mix and left to grow on the windowsill. A fourth seed germinated several days later, however as with the first three transplanted seedlings it too failed to reach maturity (flower).

3.1.2 Cores

Sieved core fractions from five sediment cores (54 mm diameter.) from Whitiāu were placed in pots (65 mm diameter × 70 mm high) in 10 mm-deep water, and monitored for germination and seedling emergence. A further four sediment cores were split longitudinally, placed in a tray surrounded by potting mix, retained in 10 mm-deep water, and monitored for germination. The remaining 15 cores were divided amongst three water depth treatments: 10 mm (shallow), 35 mm (medium) and 65 mm (deep). At 65 mm, the water was level with the top of the sediment in the pots. At each water depth there were five pots, each containing one core surrounded by potting mix. Pots were regularly monitored and species presence recorded.

No plants of *S. ovata* were recorded following seven months of observations (from December to July), or indeed a year later on final examination. However a number of the associate species that are described with *S. ovata* in Whitiāu did germinate (Table 5).

3.1.3 Whitiāu sediment scrapes

Two patches of sediment and associated leaf litter, each 100 mm × 100 mm and c. 10 mm deep, were excavated from each of three sites, referred to as north end, mid-site (Jim Campbell's (DOC Wanganui) plot 6) and south end in the Whitiāu reserve. These sites had been rich in *S. ovata* within the past decade. Each block of sediment was sectioned into three and planted in pots (65 mm diameter × 70 mm high) partially filled with potting mix, then placed at one of three water depth intervals: 10 mm (shallow), 35 mm (medium) and 65 mm (deep). The pots were monitored regularly for emergence of *S. ovata*.

Although seedlings of *S. ovata* did emerge (Table 6) along with associate plants, few (i.e. only those that were moved to the glasshouse) grew to maturity. With

one exception, sediment from the south end site was the only sediment from which *S. ovata* emerged, regardless of the water depth.

3.1.4 Transplants

Six plants (less than 10 cm high) from Whitiāu and four from Waitotara were removed in sediment cores and transferred to pots in the glasshouse. The objective was to establish a population of *S. ovata* in cultivation from Waitotara, for future preservation (following seed set) and morphological and genetic comparison, and to determine if this was a feasible method for translocating *S. ovata* to establish alternative populations.

Four of the Whitiāu plants survived transplantation and grew to maturity. Although all of the Waitotara plants appeared to survive transplantation (i.e. continued growing for two months), only three plants went on to set seed.

TABLE 5. SPECIES EMERGENCE FROM SEDIMENT CORES TAKEN FROM WHITIAU (FROM DECEMBER 1998).

TREATMENT (CORE TYPE)	WATER DEPTH (mm)	SITE CORE NO.	19 JAN 1999	15 FEB 1999	1 MAR 1999	14 APR 1999
Sieved	10	1.1	Too small to identify	Sr, Sn	Sm	Sm
		1.2		Sn	Ja	Ja, Sn, I sp, Sr
		1.3		Sn		Ja, Sn
		4.1		Sn	Ja	Ja
		5.1		Sn	Sn	Sn, Sr
Split	10	1.4	Sn	Sm,	Ja	Ja, Sr, Sb
		2.1	Sn, Sr, Gn	Sm	Sn, Sr, Gn, Ja	Gd, I sp, Sr
		3.1	Sn, Sr	Sm	Sn, Sr, Ja	Sn, Sr, Ja, I sp, La
		4.2	Sn, Gd	Sm	Sn, Gd, Ja	Gd, Ja, moss
		5.2	Sr, J sp.	Sm, Sn	Sn, Sr, Ja	Ja, Sr, I sp., Ox
Intact	10	2.3	Sn	Sm	Ja	I sp, Ja, La
		2.5	Sn, Gd, <i>Juncus</i> sp.	Sm	Ja	I sp, Gd
		3.3	Sn, Sr	Sm	Sr, Ja	HI, Ja, Sr
		4.4	Sn, Sr	Sm	Sn	Sn, Ja, Sr, Lt
		5.3	<i>Epilobium</i> sp.	Sm	Sm	Eb
	35	1.5		<i>Juncus</i> sp.	Sn	Sn, Ja, moss
		2.4	<i>Gunnera</i> sp.	Sm, Sn	<i>Isolepis</i>	Gd, Ja, Ib
		3.4	Sn	Sm, <i>Gunnera</i> sp.	Sn	Ja, Pv, Lh
		4.3		Sm	Sn, Gd, Ja	Sn, Pv, moss
		5.5				Moss
	65	2.2	Gd, Sb	Sm, Sn	Gd	Sm
		3.2	Sn, Sr	Sm	Sn, Sr, Ja	Sn, Sr, Ja
		3.5	Gd	Sm	Sm	Sm
		4.5	Sr, Lt	Sm, Sn	Sn, Lt, Ja	Sn, Lt, Ja
		5.4	Sn, Sr	Sm	Sm	Sm

Abbreviations: Sm = same, sp. = species. Species list: Eb = *Epilobium billardioreanum*, Gd = *Gunnera dentata*, HI = *Holcus lanatus*, Ib = *Isolepis basilaris*, I sp. = *Isolepis marginata*, Ja = *Juncus articulatus*, Lt = *Leontodon taraxacoides*, La = *Lobelia anceps*, Lh = *Lythrum hyssopifolia*, Ox = *Oxalis*, Pv = *Prunella vulgaris*, Sn = *Schoenus nitens*, Sb = *Sisyrinchium 'blue'*, Sr = *Selliera rotundifolia*.

3.1.5 Glasshouse culture

Fresh seed collected from plants of *S. ovata* from New Zealand and Australia were sprinkled on top of trays of potting mix with a thin (< 0.5 mm) layer of fine sand. Trays were placed on mist beds in the glasshouse where they remained until seedlings were 5–20 mm in height (four weeks to four months). At this stage plants were individually pricked out put into pots and left on the mist beds for c. one week. Later they were transferred off the mist bed, and watered daily (Fig. 6). Plants were then monitored (plant height and flower development were recorded) through to flowering and seed set (Fig. 7). After pods had developed the seeds were collected off individual plants, counted before being sown or allowed to set in their own tray, which was then placed back under the mist or maintained in the glasshouse (Figs 8 and 9).

In relation to other techniques tested, successful plant cultivation was achieved. However, plant maturation rates were still highly variable given the amount of seed planted (Table 7) as were the seed production (Fig. 10), plant height and flower number (Fig. 11).

TABLE 6. EMERGENCE RESULTS FROM SEDIMENT SCRAPES TAKEN IN MARCH 1999.

WATER DEPTH	SITE	PLANT SPECIES PRESENT AT EACH MONITORING DATE			
		14 APRIL	6 MAY	12 MAY	21 JUNE
10 mm	North end -1	Sn, Unk dicot, leafy liverwort or moss	Sn, Ja, Unk dicot		G sp, moss Lt, Sn, Ja, Ib
	North end -2	Sb, Sn, HI, grass sp	Sb, Sn, grass sp, HI		Sb, Gd
	Mid (plot 6)–1	Sb, HI, Ib, Unk	Sb, HI		Sn
	Mid (plot 6)–2	Sb, Ja	Sb, Ja		HI, Sb, Sn
	South end–1a	Lt, Sn, Sr, moss	Lt, Sn, Sr, moss		Lt, Sr, Sn, Ib, moss
	South end–1b	Lt, Sn, HI, 2 × So?	Lt, La, HI, 2 × So	2 × So	Lt, Ja, HI, Sn, moss, So × 1
	South end–2a	Lt, Sn, Gd, Pv, Unk, 2 × So?	1 × So		
	South end–2b	Lt, Sn, Sr, 2 × So	3 × So		
35 mm	North end–1	Sn, Ja, <i>Isolepis</i> sp.	Ja		<i>Juncus</i> sp., Ja, Lt, Ib
	North end–2	Sn, Unk	Sn, Ja, hairy Unk		<i>Juncus</i> sp., Sn
	Mid (plot 6)–1	Lt, Sb, Ja	Lt, Sb, Ja		Sb, moss, Gd
	Mid (plot 6)–2	Ja, Sb, 2 × Unk dicots	Ja, Sb, 2 × Unk dicots	So	Sb, Ja, HI
	South end–1	Sb, Lt, Sn, 2 × composites	Sb, Ja, Sn, 2 × composites		Sb, Ja, La, Lt
	South end–1b	Ja, Sn, leafy liverwort, 3 × So	Ja, Sn, Sb, 3 × So		
	South end–2	Ja, Sn, Sr, HI, Tr	Ja, Sn, Sr, HI, Tr		Clover white, Ja, HI, Gd, Sn
	South end–2b	Ja, Lt, Sn, Sr, 6 × So	Ja, Lt, Sn, Sr, 4 × So	2 × So	7 × So, Lt, Ib, Sr, Ja
65 mm	North end–1	Ja, Sn, Unk	Ja, Sn, Unk		Ja, Sn
	North end–2	Lt, Sn, Unk dicot	Lt, Sn, Unk grass		Lt, HI, Ja, Sb, Sn
	Mid (plot 6)–1	Ja, Sb, Sn,	Ja, Sb,		Sb, Ja, Sn
	Mid (plot 6)–2	Ja, Sb, Unk	Ja, Sb,		Ib, Ja, Sb
	South end–1	Sb, Lt, Sn, HI.	Sb, Lt, Sn, Ja		Lt, Sb, HI, Ja, Sn
	South end–1b	Lt, Sn, Sr, 1 × So	Lt, Sn, 1 × So	So	Hp Lt, 1 × So
	South end–2	Lt, Sn, Sr, Pv	Lt, Sn, Ja, Pv		Pv, Lt, Ja, Sn
	South end–2b	Sn, Sr, Pv, 7 × So	Sn, Sr, Pv, Ja, 7 × So?	So	Pv, Sr, Sn, Ja, So × 2

Abbreviations: sp. = species, Unk = yet to be identified. Species list: Eb = *Epilobium billardioreanum*, Gd = *Gunnera dentata*, HI = *Holcus lanatus*, Ib = *Isolepis basilaris*, Ja = *Juncus articulatus*, Lt = *Leontodon taraxacoides*, La = *Lobelia anceps*, Lh = *Lythrum hyssopifolia*, Pv = *Prunella vulgaris*, Sn = *Schoenus nitens*, Sb = *Sisyrinchium* 'blue', Sr = *Selliera rotundifolia*, Tr = *Trifolium repens*, So = *Sebaea ovata*, So? = *Likely S. ovata*.

Figure 6. Young plants of *Sebaea ovata* potted up.



Figure 7. *Sebaea ovata* that has started to flower and grow over a larger tray.

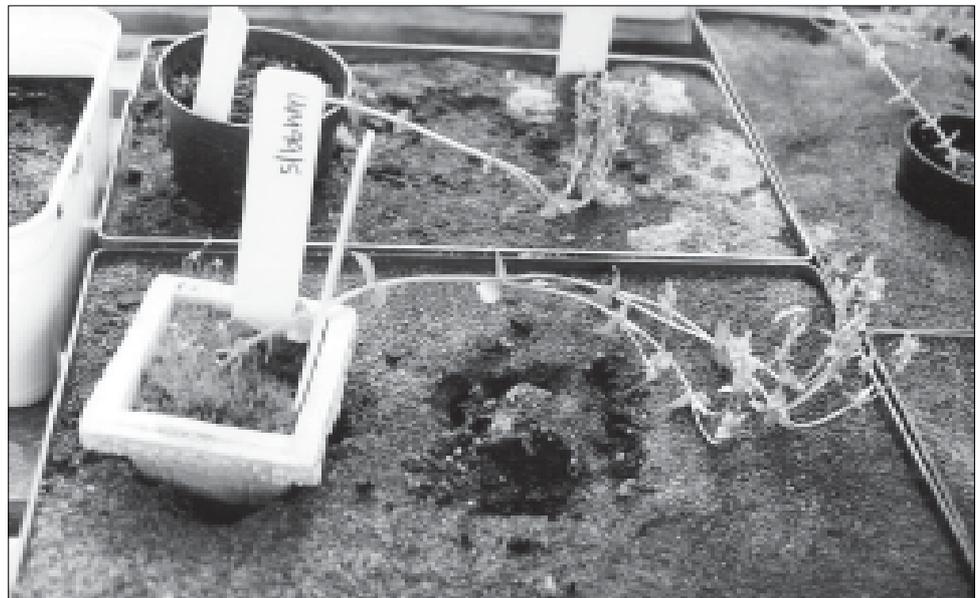


Figure 8. *Sebaea ovata* that was left to self-sow. The seed has not scattered, and upon germination has resulted in a cluster of seedlings.



Figure 9. Plants of *Sebaea ovata* that are ready for transfer to individual pots.



3.2 DISCUSSION

Germination and cultivation trials were designed to assess the viability of fresh and sediment sourced *S. ovata* seed and to establish a technique for cultivating *S. ovata* that could provide information for the management of field sites as well as enable the establishment of a culture population.

The most successful method, and the one currently used, for the cultivation of *S. ovata* involved fresh (light brown) seed sprinkled on potting mix, and placed on a heated mist bed for germination and seedling emergence, followed by glasshouse cultivation (either heated or ambient) for plant maturation. However, the highly variable percentage of seedling emergence from apparently healthy seed, and variability in plant maturation were still cause for concern.

TABLE 7. MATURATION (%) FROM SEED SOWN.

PLANT IDENTIFICATION	NUMBER OF SEEDS PLANTED	PERCENTAGE MATURED
U99/1	40	0
U99/2	17	0
U99/3	77	65
U99/4	105	27
U99/5	90	22
008/2	254	0
001/1	13	0
00345/2	210	0
005/2	20	0
W5	72	0
W6	5	0
W7/1	71	0
JCA21/1	134	23
JCA27/2	65	3
JCA27/3	39	8
JCA3/1	61	20
JCA3/2	43	60
PBA27	73	18
PBA12	155	7

In an effort to determine if this variability was sediment or nutrient based, amended substrate (with addition of Whitiua sediment, sand and/or nutrients) and hydroponics (Ruakura complete solution in a closed aerated system) were tested. In the sediment amended trials there was no change in the success rate of plant maturation. Using hydroponics the plants failed to grow although they remained healthy in appearance for a month. There was new leaf development

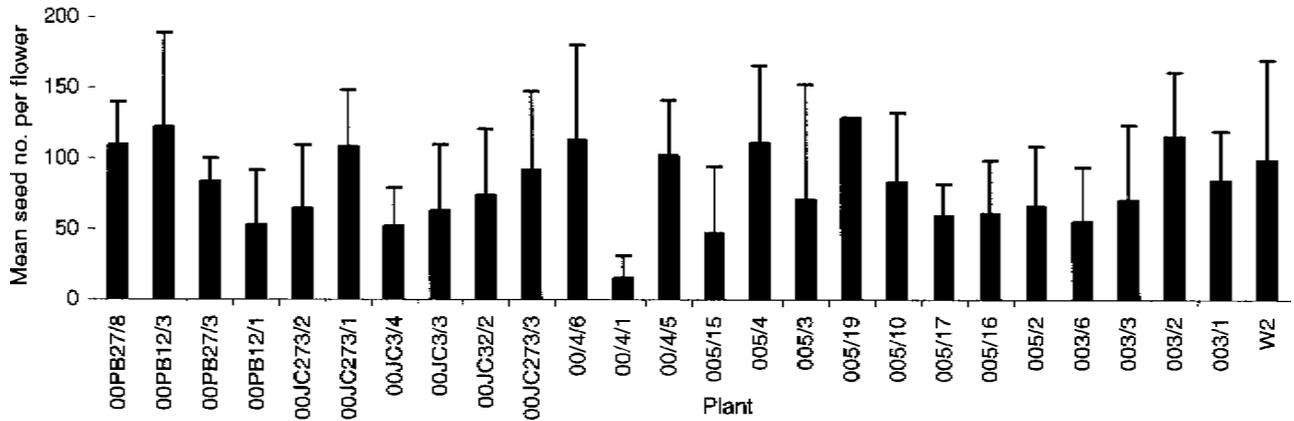


Figure 10. Mean seed number per flower on a plant.

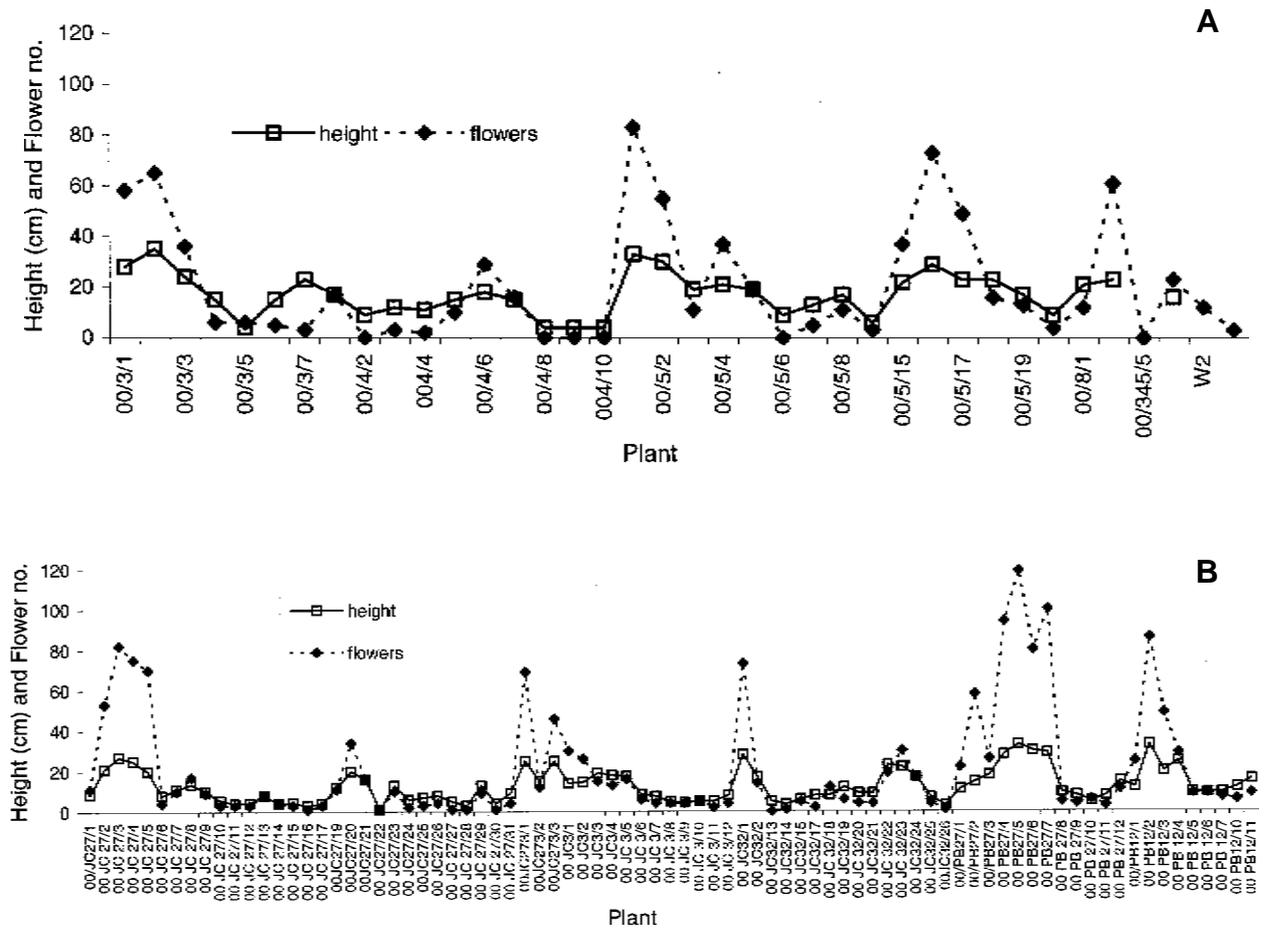


Figure 11. Plant height and flower number for (A) New Zealand plants, and (B) Australian plants.

or flowering, with plants eventually dying before maturation (before seed set). In addition, plants under the current cultivation system have variable health, with some plants developing bleached leaves, but this does not appear to be a nutrient response. Healthy plants that grew to set seed were also variable under the same growth conditions, ranging in height from 50 to 350 mm and in flower production from 1 to 119, which would correspond to a difference in seed numbers from c. 80 to 10 000. This does not appear to be related to seed origin. For example plants of *S. ovata* from New Zealand and Australia exhibit a similar range of plant height and flower number, and the trend for larger plants to have more flowers and seed prevails for both (Fig. 11).

Cultivation difficulties aside, there were approximately 60 plants in cultivation, with hundreds of seedlings emerging and thousands of seed at the conclusion of this project that were subsequently given to DOC for replanting or seeding.

4. Characterisation of *Sebaea ovata*

It has been suggested that New Zealand plants of *S. ovata* differed sufficiently morphologically from Australian plants to be considered a separate taxon (Ogle 1989). Australian plants of *S. ovata* have been described as being 500 mm tall, in grassland, forest country or dwarf scrub habitat, with no mention of coastal habitat. Such difference in form and habitat compared to New Zealand suggest they may be different taxa (Ogle 1989). Comparison of *S. ovata* from New Zealand and Australia genetically (using ITS (internal transcribed spacer) sequences) and in cultivation was carried out to determine the taxonomic status of *S. ovata* from these two countries.

4.1 METHODS

4.1.1 Plant material

Fresh plant material was collected from both Waitotara and Whitiua Scientific Reserve (Table 8). Plants were subsequently cultivated at the University of Waikato (see above), and morphological descriptions of the field and glasshouse cultivated plants were made. Dried and preserved specimens were obtained for *Sebaea ovata* and *S. albidiflora* from Australia (Table 8).

4.1.2 Genetic analyses

DNA was extracted from fresh plant tissue and herbarium samples using a modified CTAB method (Doyle & Doyle 1990). The tissue was ground in liquid nitrogen and scraped directly into a 1.5 mL Eppendorf tube containing 600 µL of preheated (65°C) CTAB isolation buffer (Doyle & Doyle 1990). The samples were incubated at 65°C for 1 hour with occasional agitation, after which 800 µL

TABLE 8. *Sebaea* SPECIMEN LIST.

<i>Sebaea</i> SPECIES	COUNTRY/SITE	TYPE	ISOLATE NO.
<i>S. ovata</i>	NZ, Waitotara	Culture at Waikato Uni	W3
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	SOUND5.1
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	SOUND5.2
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	003/1
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	003/2
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	003/3
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	00345/4
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	004/5
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	005/15
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	005/17
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	005/18
<i>S. ovata</i>	NZ, Whitiāu	Culture at Waikato Uni	005/19
<i>S. ovata</i>	AUS, Vic, Jumping Creek	Culture at Waikato Uni	SOJCA2
<i>S. ovata</i>	AUS, Vic, Jumping Creek	Culture at Waikato Uni	SOJCA3
<i>S. ovata</i>	AUS, Vic, Jumping Creek	Culture at Waikato Uni	00JC27/3
<i>S. ovata</i>	AUS, Vic, Pound Bend	Culture at Waikato Uni	00PB27/7
<i>S. ovata</i>	AUS, Vic, Pound Bend	Culture at Waikato Uni	00PB12/10
<i>S. ovata</i>	AUS, Vic, Grampians nr Zummsteins	Dried specimen	SOGZA5
<i>S. ovata</i>	AUS, SA, Torrens River Gorge	Dried specimen	SOTGA6
<i>S. ovata</i>	AUS, SA, Torrens River Gorge	Dried specimen	SOTGA2
<i>S. ovata</i>	AUS, SA, Torrens River Gorge	Dried specimen	SOTGA4
<i>S. albidiflora</i>	AUS, SA, Salt Creek, lower Coorong	Preserved specimen	SAF1.1
<i>S. albidiflora</i>	AUS, Vic, Lake Corangamite	Dried specimen	SACO
<i>S. albidiflora</i>	AUS, Tas, St Helens Saltmarsh	Dried specimen	SASH

Abbreviations: NZ = New Zealand, AUS = Australia, Vic = Victoria, Tas = Tasmania, SA = South Australia.

of chloroform/isoamyl alcohol (24:1, v/v) was added. The tubes were then centrifuged at 13 000 rpm in a micro centrifuge for 5 minutes. The aqueous phase was removed using a sterile pipette tip and transferred into a fresh tube. The extraction step was then repeated. The nucleic acids were precipitated by adding 400 µL of ice cold isopropanol and incubating at -20°C for approximately one hour, followed by centrifuging for 10 minutes at 13 000 rpm. The pellet was resuspended in 500 µL 1M NaCl at 37°C. The nucleic acids were again centrifuged (13 000 rpm) for 5 min, transferred to a fresh tube and incubated at 95°C for 30 minutes. The nucleic acids were then precipitated using 500 µL of ice-cold isopropanol, incubating at -20°C for 30 min and then centrifuging (13 000 rpm) for 15 minutes. The pellet was washed in 70% ethanol, followed by a second wash in 95% ethanol, and vacuum drying. The DNA was resuspended in 100 µL TE.

Amplification of the DNA via the polymerase chain reaction (PCR) was carried out in a 0.5 mL Eppendorf tube using a 100 µL reaction volume. Each reaction volume contained 4 µL of plant DNA, 100 µM of each dNTP, 0.2 µM ITS (internal transcribed spacer sequence) primers (or ETS (External transcribed spacer sequence) or LFY (leafy intron) primers), 1 U *Taq* Polymerase (Boehringer-Mannheim), PCR buffer (with extra Mg) and DMSO. The thermocycler (Eppendorf Mastercycler Gradient) programme had an initial denaturing for 5 min at 96°C, followed by 30 cycles of (30 seconds at 95°C, 30 seconds at

55°C, and 45 seconds at 72°C), and a final 10 minutes at 72°C. A portion (5 µL) of the amplification products were separated by electrophoresis in a 1% agarose gel, with 1xTBE and ethidium bromide. DNA bands were visualised under a transilluminator and the gel was photographed (Eagle Eye II, Stratagene). PCR products were then cleaned using Concert Rapid PCR Purification System (Life Technologies). Both strands (forward and reverse) were sequenced at the University of Waikato DNA sequencing facility. ITS sequences were edited and aligned in Sequencer 3.0 (Gene Codes Corporation).

4.2 RESULTS AND DISCUSSION

4.2.1 Morphology

Plants collected in the field and those grown in cultivation varied in size and flower number (Fig. 11), with smaller plants fitting Cunningham's description of *S. gracilis* (from Allan 1961). However, this range of plants occurred in both Australian and New Zealand material and all plants appear to fit within the same species (Figs 12–15).



Figure 12. (Right) *Sebaea ovata* from Whitiāu, New Zealand. Scale ×2.



Figure 13. (Above) *Sebaea ovata* flower (New Zealand), with lobes widely separated (before pollination). Scale ×2.

Plants grew up to 300 mm tall in culture, often with multiple branched peduncles bearing over 100 flowers per plant. Leaves were sessile and coriaceous, but varied from narrow ovate to suborbicular in shape. Flowers were uniform, with a lemon-yellow corolla tube (4 mm long) with five lobes (Fig. 13). Lobes are conspicuously spirally twisted after anthesis (Fig. 15). Calyx lobes are lanceolate, acute and erect at the apex. Capsules are ovoid, obovoid or ellipsoid containing seed of c. 0.3 mm diameter with a distinct reticulated pattern. The seed is pale brown when the capsules open, but darkens as it ages. Seed material from both New Zealand and Australia had a reticulated pattern as illustrated on page 208 of Webb & Simpson (2001).

Figure 14. (Right) *Sebaea ovata* from Pound Bend, Australia. Scale $\times 2$.

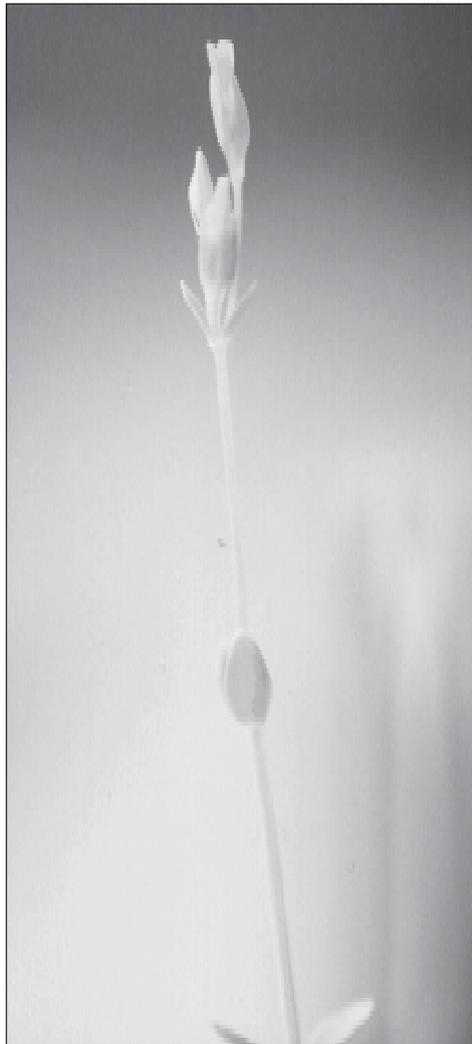


Figure 15. (Above) *Sebaea ovata* flower (c. 0.5 cm long) from Australia, showing twisted petals after pollination. Scale $\times 3$.

4.2.2 Genetic comparison

DNA was obtained from all isolates (Table 8). Single PCR products of approximately 900 base pairs and high-quality ITS sequences were obtained from all fresh plant material. However, some samples from dried or preserved specimens failed to amplify with the ITS primers.

Analysis of ITS sequence data revealed that the New Zealand and the Australian isolates of *Sebaea ovata* did not differ in ITS sequence. However *S. albidiflora* differed from *S. ovata* with 45 base pairs over 641. Analyses of the ITS region of nuclear ribosomal DNA (nrDNA) have proven particularly useful for uncovering the evolutionary and biogeographic history of a number of flowering plant lineages (Gemmill et al. 2002). In the present study ITS does show species level differences between *S. albidiflora* and *S. ovata* and supports the morphological descriptions that *S. ovata* from New Zealand and Australia are the same species.

An alternative to the use of the ITS region for the resolution of relationships among closely related taxa was also sought, and two other rapidly evolving molecular markers were trialled. The external transcribed spacer (ETS) region of nrDNA has been successfully employed to resolve relationships among closely related species (Markos & Baldwin 2002; Chan et al. 2001), and LFY second intron has been useful the species and population level in some species

(Hoot & Taylor 2001). No amplification was achieved using the ETS or LFY primers with *S. albidiflora* from Lake Corangamite, St Helens Saltmarsh and New Zealand *S. ovata*. Gradient experiments for PCR reaction conditions were conducted using both the ETS and LFY primers with *S. ovata* (00345/4 which had the best DNA profile) to determine if altering the reaction conditions would result in successful amplification. In the present study neither ETS nor LFY primers resulted in amplification products with *Sebaea* DNA.

Genetic analyses at two levels were anticipated at the onset of this study, the population level using RAPDs (Random Amplified Polymorphic DNA) and the species level to compare New Zealand and Australian plants and an outlier using ITS sequence data.

Although hundreds of plants have been in cultivation during this study, large scale RAPDs were not undertaken because the parentage of the New Zealand plants in culture is from only a few individuals from the south end of Whitiua Reserve. Limited genetic variation within this population was supported by preliminary RAPDs analyses as expected.

5. Summary and management recommendations

This project investigated the Whitiua field populations of *S. ovata* in New Zealand, field sites in Australia, cultivation of *S. ovata*, and morphological and genetic comparison of New Zealand and Australian plants.

Field investigations were undertaken to estimate the *S. ovata* population and seed bank, to describe the habitat of *S. ovata* (associate vegetation and nutrient profile of the sediments), and the habitat of *S. ovata* in Australia, to allow a better prediction of the long-term viability of *S. ovata* in New Zealand. Germination and cultivation trials were designed to assess the viability of fresh and sediment sourced *S. ovata* seed and to establish a technique for cultivating *S. ovata* that could provide information for the management of field sites as well as enable the establishment of a culture population. Morphological and genetic comparison of New Zealand and Australian *S. ovata* was undertaken because difference in form and habitat of *S. ovata* in the literature indicated that they may be different taxa (Ogle 1989).

Since its rediscovery in 1989 the Whitiua population of *S. ovata* has been declining (Ogle 1998b), from estimates in the thousands during the 1990's, to numbering only in the hundreds in February 2000. Further evidence of the population decline is the small stature of the plants at Whitiua, with few flowers and seed compared to cultivated plants from the same seed source, and the lack of *S. ovata* seed in the sediment cores.

The cause of the decline of the *S. ovata* population at Whitiua is likely the result of several contributing factors. Threats that have been identified include habitat destruction, weed encroachment, browsing from plume moth and cattle

trampling (Dopson et al. 1999). Sediment nutrient samples also indicate that nitrogen limitation may be a contributing factor in the decline of *S. ovata* at Whitiua compared with Waitotara.

Weed encroachment is a particularly serious threat to *S. ovata* habitat and grazing may be important to maintain habitat at Whitiua. DOC manipulated clearings at Whitiua have shown that *S. ovata* can readily establish successfully on bare ground when this is available. Trials to cultivate *S. ovata* in this present study have shown that *S. ovata* readily establishes on open trays of sediment, and that plant performance is better (size and maturation) when there were no alien species present (plants in individual pots) compared with those grown intact in sediment cores from Whitiua (where associate vegetation is present).

Cultivation of *S. ovata* was most successful using fresh seed, under glasshouse conditions with mist beds for seed germination, and weed free pots or potting mix for plant maturation (i.e. plants that were left in pots of with associate species from Whitiua did not perform as well).

Under culture conditions, and using dried plant specimens *S. ovata* from New Zealand and Australia appear to be the same species. This is supported by ITS sequence data. Although plants of *S. ovata* from Australia appear to be identical to those in New Zealand, this plant is still of major conservation concern here since it was once widespread but is now critically endangered (de Lange et al. 1999).

Recommendations for future management include maintaining a culture population of *S. ovata* for both reseeding in the wild and for insurance, and further habitat manipulation trials to remove or minimise encroaching weed species and provide suitable *S. ovata* sites.

Since *S. ovata* occurs in only two small field sites, both requiring winter flooding to maintain habitat, it is likely that one or both populations could be lost in the near future. It is therefore important to maintain plants in cultivation, which may be transplanted into new suitable field sites, and to continually provide a new (and viable) seed source. The present project has provided a method of cultivation for this species and plants (and seed) that remained in cultivation at the conclusion of this study have been given to DOC for replanting (or sowing).

The type of manipulation trials undertaken by DOC may be the key to allowing annual plants such as *S. ovata* to persist at sites where populations are known to have declined. However, the continued monitoring of such sites is required to evaluate the reproductive success of plants at these sites. Alternative manipulation of vegetation using a non-selective herbicide such as glyphosate, or the targeting of specific weeds or problem indigenous plants using selective herbicides (Champion 2000), could also be attempted in the future.

6. Acknowledgements

Jim Campbell and Colin Ogle provided field assistance and useful discussion on the New Zealand field sites and management aspects of this project. Peter de Lange provided useful discussion on various aspects of the project and helped in sourcing Tasmanian material. Dried plants were provided by Alex Buchanan and Andrew Rozefelds (Tasmanian Herbarium) and David Van Boekel (Warrandyte State Park, Victoria), who also assisted in the field and provided Australian seed of *S. ovata* for this project. Funding assistance was provided by the International Science and Technology Linkages Fund of the Royal Society of New Zealand (Bilateral Research Activities Programme) and Department of Conservation investigation no. 2483.

7. References

- Allan, H.H. 1961. Flora of New Zealand. Vol. 1. Government Printer, Wellington. 1085 p.
- Chan, R.; Baldwin, B.G.; Ornduff, R. 2001. Goldfields revisited: A molecular phylogenetic perspective on the evolution of *Lasthenia* (Compositae: Heliantheae *Sensu lato*). *International Journal of Plant Science*. 162(6): 1347–1360.
- Champion, P.D. 2000. Selective control of weeds in New Zealand wetlands using herbicides. NIWA Consultancy Report DOC00220. 31 p.
- Champion, P.D.; Beadel, S.M.; Dugdale, A.M. 2001. Turf communities of Lake Whangape and some potential management techniques. *Science for conservation* 186. 54 p.
- de Lange, P.J.; Heenan, P.B.; Given, D.R.; Norton, D.A.; Ogle, C.C.; Johnson, P.N.; Cameron, E.K. 1999. Threatened and uncommon plants of New Zealand. *New Zealand Journal of Botany* 37: 603–628.
- Dopson, S.R.; de Lange, P.J.; Ogle, C.C.; Rance, B.D.; Courtney, S.P.; Molloy, J. 1999. The conservation requirements of New Zealand's nationally threatened vascular plants. *Threatened Species Occasional Publication Number 13*. Biodiversity Recovery Unit, Department of Conservation, Wellington.
- Doyle, J.L.; Doyle, J. 1990. Isolation of plant DNA from fresh tissue. *Focus* 12(1): 13–15.
- La Cock, G.; Ogle, C. 1999. Possum woes, mistletoes and environmental impact assessments. *Ecological Management* 7: 39–40. Department of Conservation, Wellington.
- Gemmill, C.E.C.; Allan, G.J.; Wagner, W.L.; Zimmer, E.A. 2002. Evolution of insular Pacific *Pittosporum* (Pittosporaceae): Origin of the Hawaiian Radiation. *Molecular Phylogenetics and Evolution* 22(1): 31–42.
- Hoot, S.; Taylor, W.C. 2001. The utility of nuclear ITS, a LEAFY homolog intron, and chloroplast atpB-rbcL spacer region data in phylogenetic analyses and species delimitation in Isoetes. *American Fern Journal* 91(3): 166–177.
- Markos, S.; Baldwin, B.G. 2002. Structure, molecular evolution and phylogenetic utility of the 5' region of the external transcribed spacer of 18S-26S rDNA in *Lessingia* (Compositae, Astereae). *Molecular Phylogenetics and Evolution* 23: 214–228.
- Ogle, C. 1989. *Sebaea ovata* (Gentianaceae) and its habitat near Wanganui. *Wellington Botanical Society Bulletin* 45: 92–99.

- Ogle, C. 1998a. Whanganui plant list 2—Whitiau Scientific Reserve. Unpublished checklist, Department of Conservation, Wanganui, New Zealand.
- Ogle, C. 1998b. *Sebaea ovata* in the Whitiau Scientific Reserve. DOC Internal Correspondence (PLA 0002, 2 February 1998), Department of Conservation, Wanganui, New Zealand.
- Webb, C.J.; Simpson, M.J.A. 2001. Seeds of New Zealand gymnosperms and dicotyledons. Manuka Press, Christchurch.

Appendix 1

SPECIES FOUND AT WHITIAU

Plant species found within the dune flat sampled for *S. ovata* at Whitiau Scientific Reserve, Wanganui, New Zealand.

BOTANICAL NAME	COMMON NAME	ABUNDANCE RATING (OGLE 1998a)
* <i>Agrostis stolonifera</i>	creeping bent	l
<i>Apodasmia similes</i>	oioi	a
<i>Carex ?breviculmis</i>		u
* <i>Conyza albida</i>	fleabane	o
<i>Coprosma propinqua</i> × <i>robusta</i>		u
* <i>Cortaderia selloana</i>	pampas	o
<i>Epilobium billardioreanum</i>	sand willowherb	lc
<i>Gunnera dentata</i>	sand gunnera	lc
* <i>Holcus lanatus</i>	Yorkshire fog	l
* <i>Hypochoeris radicata</i>	catsear	o
<i>Isolepis basilaris</i>		l
* <i>Isolepis marginata</i>		lc
<i>Isolepis nodosa</i>	club sedge	a
* <i>Juncus articulatus</i>	jointed rush	lc
<i>Juncus caespiticus</i>		o
* <i>Juncus effuses</i>	soft rush	u
<i>Lachnagrostis billardierei</i>	sand bent	o
<i>Lachnagrostis filiformis</i>	wind grass	l
* <i>Leontodon taraxacoides</i>	hawkbit	c
<i>Leptospermum scoparium</i>	manuka	o
<i>Lobelia anceps</i>		o
* <i>Lotus pedunculatus</i>	lotus major	l
* <i>Lythrum hyssopifolia</i>	hyssop loosestrife	u
<i>Mazus novae-zelandiae</i> ssp. <i>impolitus</i>		la
* <i>Melilotus indica</i>	King Island melilot	o
<i>Microtis unifolia</i>	onion orchid	o
* <i>Oenothera stricta</i>	sand primrose	c
* <i>Parentucellia viscosa</i>	tarweed	u
* <i>Paspalum dilatatum</i>	paspalum	o
* <i>Poa annua</i>	annual poa	nr
* <i>Prunella vulgaris</i>	selfheal	lc
* <i>Schedonorus phoenix</i>	tall fescue	c
<i>Schoenus nitens</i>		a
<i>Sebaea ovata</i>		l
<i>Selliera rotundifolia</i>	half star	o

Continued next page>>

* Alien species, a = abundant, c = common, o = occasional, u = uncommon, l = local, lc = locally common, nr = not recorded by Ogle (1998a)

BOTANICAL NAME	COMMON NAME	ABUNDANCE RATING (OGLE 1998a)
* <i>Setaria gracilis</i>	knot-root bristle grass	lc
* <i>Sisyrinchium</i> 'blue'		u
* <i>Sporobolus africanus</i>	ratstail	o
* <i>Trifolium dubium</i>	suckling clover	o
* <i>Trifolium fragiferum</i>	strawberry clover	c
* <i>Trifolium repens</i>	white clover	lc
<i>Triglochin striata</i>	arrow grass	o
* <i>Ulex europaeus</i>	gorse	o
* <i>Verbascum thapsus</i>	woolly mullein	u

* Alien species, a = abundant, c = common, o = occasional, u = uncommon, l = local, lc = locally common, nr = not recorded by Ogle (1998a)

Appendix 2

SPECIES FOUND AT WARRANDYTE

Plant species found within the area sampled for *Sabaea ovata* at Warrandyte State Forest Park, Victoria, Australia.

BOTANICAL NAME	COMMON NAME
Canopy species	
<i>Eucalyptus macrorhyncha</i>	red stringybark
<i>Eucalyptus melliodora</i>	yellow box
<i>Eucalyptus polyanthemos</i> ssp. <i>vestita</i>	red box
<i>Eucalyptus radiata</i>	Peppermint
<i>Eucalyptus rubida</i>	Candlebark
Shrubs and subshrubs	
<i>Acrotriche serrulata</i>	trailing ground-berry
<i>Dillwynia cinerascens</i>	grey parrot-pea
<i>Exocarpos cupressiformis</i>	cherry ballart
<i>Kunzea ericoides</i>	Burgan
Perennials	
* <i>Arctotheca calendula</i>	Cape weed
<i>Gonocarpus tetragynus</i>	common raspwort
<i>Kennedia prostrata</i>	running postman
<i>Lomandra filiformis</i>	wattle mat-rush
Annuals and geophytes	
* <i>Aira caryophyllea</i>	silvery hair-grass
* <i>Anagallis arvensis</i>	Pimpernel
* <i>Anagallis minima</i>	Chaffweed
* <i>Anthoxanthum odoratum</i>	sweet vernal
<i>Arthropodium strictum</i>	chocolate lily
<i>Austrodanthonia</i> sp.	wallaby grass
* <i>Briza minor</i>	lesser quaking grass
* <i>Centaurium erythraea</i>	common centaury
<i>Cheilanthes austrotenuifolia</i>	green rock-fern
<i>Cheilanthes distans</i>	bristly cloak-fern
* <i>Cicendia filiformis</i>	slender cicendia
* <i>Cicendia quadrangularis</i>	square cicendia
<i>Daucus glochidiatus</i>	Austral carrot
<i>Drosera peltata</i>	tall sundew
* <i>Galium divaricatum</i>	slender bedstraw
<i>Geranium solanderi</i>	Austral cranesbill
<i>Hydrocotyle callicarpa</i>	small pennywort
<i>Hypericum gramineum</i>	small St. Johns wort

BOTANICAL NAME	COMMON NAME
<i>Hypochoeris glabra</i>	smooth catsear
* <i>Hypochoeris radicata</i>	Catsear
<i>Levenhookia dubia</i>	hairy stylewort
<i>Levenhookia sonderi</i>	slender stylewort
<i>Microlaena stipoides</i>	weeping grass
<i>Microtis unifolia</i>	onion orchid
<i>Ophioglossum lusitanicum</i>	Austral adder's-tongue
<i>Oxalis perennans</i>	grassland wood-sorrel
<i>Phyllangium divergens</i>	wiry miterwort
* <i>Polycarpon tetraphyllum</i>	four-leaved allseed
<i>Poranthera microphylla</i>	small poranthera
* <i>Romulea rosea</i>	common onion grass
<i>Sebaea ovata</i>	yellow sebaea
* <i>Soliva sessilis</i>	Jojo
<i>Thelymitra pauciflora</i>	slender sun orchid
<i>Themeda triandra</i>	kangaroo grass
* <i>Trifolium arvense</i>	hare's-foot clover
* <i>Trifolium campestre</i>	hop clover
* <i>Trifolium dubium</i>	suckling clover
<i>Triptilodiscus pygmaeus</i>	common sunray
<i>Veronica plebeia</i>	trailing speedwell
* <i>Vicia hirsuta</i>	tiny vetch
<i>Viola hederacea</i>	ivy-leaf violet
<i>Vulpia bromoides</i>	squirrel-tail fescue
<i>Wahlenbergia quadrifaria</i>	annual bluebell

* Alien species.