

Notes on the germination of the endangered species *Sclerolaena napiformis* (Chenopodiaceae)

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Abstract: *Sclerolaena napiformis* is found on fertile plains in northern Victoria and southern New South Wales and is endangered Australia-wide. Introductory work on its germination shows that seeds cannot germinate until the woody fruit has broken down. The seeds tolerate a wide range of temperatures for germination, suggesting that germination occurs regardless of season if sufficient rain falls. Seed ageing effects reduce seed viability, but some seed is still viable after two years storage. Flower buds first appear 21 weeks from germination and some fruits have matured by week 29. In the field, plants die back to their taproots in late autumn and resprout in spring. Ninety percent of tagged plants were still alive two years later. The physiological seed dormancy imposed by an intact fruit wall provides a mechanism for the development of persistent soil seed banks. Work on the ecological significance of such banks is needed. The literature on interactions between *Sclerolaena* fruit and seed biology and ants is briefly reviewed.

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Introduction

Sclerolaena (Chenopodiaceae) is a genus of perennial herbs or small shrubs containing 62 species, all endemic to Australia (Wilson 1984). While most species are from semi-arid to arid areas, a few are found in wetter areas where mean annual rainfall can be as high as 400 mm. One of these is *Sclerolaena napiformis*, the only *Sclerolaena* listed as endangered Australia-wide under the Environment Protection and Biodiversity Conservation Act 1999 as at December 2004 (Victoria: Department of Sustainability and Environment 2005). At present, it is confined to 20 small, remnant stands, mostly on roadsides, on fertile, clay loam plains in the Wimmera region of Victoria and the Riverina of northern Victoria and southern New South Wales (Alexander 2003, Mavromihalis 2004). Its endangerment is probably because suitable soil and climate have led to its virtual elimination by cropping and intensive stock grazing (Alexander 2003).

Detailed information on the ecology of the species is needed to work out how to maintain all existing populations and to increase those in biological reserves. Here, we begin this process by describing introductory work on germination behaviour. In addition, we summarize previous work on *Sclerolaena* fruit and seed ecology of likely relevance to *Sclerolaena napiformis* management, and give brief notes on seedling growth and phenology. For brevity, the *Sclerolaena* fruiting perianth will be called a fruit throughout.

Methods

Germination

Fruits were collected from eight Victorian *Sclerolaena napiformis* populations on various dates in 1997 and 1999 (Table 1). For exact locations of these, see Mavromihalis (2004).

Preliminary trials showed that germination would not occur from intact fruits. Partial removal of the woody fruiting perianth produced variable, often low germination, so complete removal was used.

For all germination trials, the number of empty fruits was counted. Abnormally small, shrunken or discoloured seeds were counted and discarded. The remaining seeds were surface sterilized for 3 min in 1% sodium hypochlorite, then washed in sterile water. There were five replicates per treatment and 20 seeds per replicate except where indicated in Table 1. Seeds were placed on a thoroughly moist Whatman seed test pad in a 9 cm Petri dish, covered in foil to exclude light. Germinated seeds were counted and removed daily. At that time, the dishes were exposed to light for approximately 30 sec. At 29 days from the start of germination, any ungerminated seeds were tested for viability by soaking in 1% tetrazolium chloride for 24 hr (International Seed Testing Association 1985).

For the germination/temperature trial, the seed was from the 1999 McKinley Road provenance (Table 1). All the growth cabinet treatments were 12 hr day/12 hr night, with temperatures as follows: 12°/8°, 15°/10°, 20°/12°, 25°/15°, and 30°/20°C. All other trials used 20°/12° C and the number of fruits opened per population was always 100 or more. All trials started in June 1999.

Seeding growth and phenology

Sclerolaena napiformis fruits were collected from Trevaskis Road, Wyuna on 16 February 1999. Seeds were removed, germinated and planted in potting mix in pots on 10 March 1999. The ten seedlings were watered every two days, fertilised regularly and grown in Melbourne until 6 October 1999 (week 30) in a glasshouse which was not temperature-controlled. Shoot phenology was observed in the field every month from February to May and again in August and October, 1999. At these times, observations were made on the microhabitats of existing plants at the sites listed in Table 1, including presence on ant nest-mounds.

Plant species nomenclature follows Ross & Walsh (2003).

Results and discussion

Germination and dormancy

In the temperature trial, germination percentages were always higher than 90% and did not differ greatly across the regimes tested. Germination speed was only significantly reduced in the 30/20° treatment (Table 2). The tolerance to a wide range of temperatures for germination is similar to that for *Sclerolaena bicornis* (Jurado & Westoby 1992) and *Sclerolaena birchii* (Auld 1976) and probably implies that germination can occur regardless of season if sufficient rain falls (Auld 1976, Plummer & Bell 1995).

The Creswicks Well data in Table 1 show conclusively that the intact fruit wall is capable of completely preventing germination as is the case with *Sclerolaena birchii*. In both species, there is a closely fitting, rigid, woody perianth with a small opening at the summit which in fruit is blocked by persistent remnants of styles, filaments and hairs. It is assumed that these can block the passage of water because germination can proceed once they are removed with a scalpel (see Auld (1976) for the case of *Sclerolaena birchii*). Thus, the presence of a persistent woody perianth can lead to seed dormancy as it can in some other chenopods such as *Halothamnus* ('*Aellenia*') *autrani* (Negbi & Tamari 1963) and *Atriplex confertifolia* (Warren & Kay 1984), although in the latter case the woody tissue is derived from the pericarp rather than the perianth. Such dormancy, found in these and some other Chenopodiaceae, is treated as a type of physiological dormancy by Baskin and Baskin (1998).

Seed set

The germination comparisons from three sampling dates in summer-autumn 1999 (Trevaskis Road, Table 1) show both low values of filled seeds and low percent seed viability for the (earliest) February sample, suggesting that seed maturation is incomplete at that time (Table 1).

Overall, of the eight locations from which seed was sampled between March and May 1999, seven samples had more than 80% of fruits with filled seeds and 98 to 100% viability of filled seeds (Table 1). The exception was O'Dea s Road (20

and 27% respectively). At this site, the plants and their fruits were sparse, dry and stunted, presumably due to locally dry conditions.

Seed ageing

The comparisons of current seed with seed collected about two years earlier (Table 1) showed that some seed from 1997 was still viable in all three cases, although in greatly reduced numbers. In two cases this reduction was due mainly to a large increase in numbers of fruits with shrunken seeds (or lacking seeds) but also to smaller decreases in percent viability of filled seeds (Table 1). These are likely to be effects of seed ageing. In *Sclerolaena birchii*, seed viability declines from 90% to 64% over 4 years 9 months (Auld 1981).

Seedling growth and phenology

In the glasshouse, the first flower buds appeared on the seedlings in week 21 and all plants were in flower by week 22. Flowering continued past week 29, by which time some fruits had matured and fallen. In contrast, *Sclerolaena birchii* flowers by six weeks (Auld 1976) and sets fertile seed by eight weeks (Parsons & Cuthbertson 1992). However, varying environmental conditions may contribute to these differences.

On mature plants in the field, live shoots with fruits were present from the first observations in 15 February 1999 until at least 1 May 1999. No live shoots were present on 14 August 1999, but new shoots had appeared by 9 October 1999. This supports the view that the species normally flowers and fruits in summer/autumn, dies back to its taproot in late autumn and resprouts in spring (Cook 1997).

The normal length of lifespan of *Sclerolaena napiformis* is unknown, but 90% of the 92 plants tagged by Cook (1997) in May of that year were still alive two years later (the present study). Normal lifespan is about one to three years for both *Sclerolaena birchii* (Auld 1981) and *Sclerolaena diacantha* (Leigh et al. 1979). During the field work (from February to October 1999) no seedlings of *Sclerolaena napiformis* were seen.

Interactions with ants

Sclerolaena napiformis is often found colonizing small bare areas, including the dome-shaped nest-mounds of the ant, *Rhytidoponera* species B (*sensu* Davidson & Morton 1981). Fruits of *Sclerolaena* species often have a hollow base filled with moist tissue when fresh (Wilson 1984). In at least some cases, this soft, moist mass is attractive to *Rhytidoponera* species B ants, which carry the fruits to their nest-mound, eat the food mass and discard the otherwise intact fruits (Davidson & Morton 1981). Of the species of *Sclerolaena* examined by Davidson and Morton (1981), two have fruits containing such a food mass and three do not. The fruits of *Sclerolaena napiformis* we examined lacked a food mass.

Table 1. Germination characteristics of *Sclerolaena napiformis* seeds from various Victorian locations and dates of collection, including one trial where seeds were left in intact fruits.

District	Location	Date of collection	% of fruits with filled seeds	No. of seeds tested †	% viability of filled seeds*	% of viable seeds germinating
Avon Plains	Creswicks Well (fruit intact)	1 May 99		No seeds germinated (100 tested)		
	Creswicks Well (fruit wall removed)	1 May 99	88	100 (5)	99	98
Avon Plains	Donald-Avon Plains Road	1 May 99	88	100 (5)	100	99
	Donald-Avon Plains Road	20 Apr 97	26	30 (2)	87	100
Avon Plains	McKinley Road	1 May 99	90	100 (5)	100	100
	McKinley Road	1 May 97	82	100 (5)	87	99
Mitiamo	Echuca-Serpentine Road	8 Apr 99	85	100 (5)	98	100
	Echuca-Serpentine Road	6 May 97	30	30 (2)	67	100
Shepparton	Trevaskis Road	16 Feb 99	36	40 (2)	43	40
	Trevaskis Road	16 Mar 99	60	100 (5)	98	100
	Trevaskis Road	8 Apr 99	82	100 (5)	98	100
Echuca	Andersons Road	15 Mar 99	93	100 (5)	100	100
Echuca	Echuca aerodrome	16 Mar 99	88	100 (5)	99	100
Shepparton	O'Deas Road	8 Apr 99	20	30 (2)	27	23

* Based on testing with tetrazolium chloride.

† Number of replicates in brackets.

Table 2. Germination characteristics of *Sclerolaena napiformis* seeds in five temperature regimes.

Temperature regime (°C)	No. of viable seeds*	Germination (%)	Days to reach final germination
12/8	99	98	12
15/10	100	100	13
20/12	99	99	15
25/15	98	96	15
30/20	97	93	29

* Based on testing with tetrazolium chloride.

Other ants, the seed-harvesting ants *Monomorium* ('*Chelaner*') *whitei*, carry large quantities of *Sclerolaena* fruits (both those with and without a food mass) back to their nests, where, just below the nest surface, they cut the fruits open and remove the seeds, which are then stored in granary chambers. Rings of *Sclerolaena* seedlings can be seen around the nests in wet years (Davison 1982, E.A. Davison pers. comm.). Work is needed to see if *Sclerolaena napiformis* is one of the species utilized.

Concluding discussion

Our work has shown that physiological seed dormancy in *Sclerolaena napiformis* can be imposed by the presence of an intact perianth wall and, for some seeds, that this can impose dormancy for at least two years. This is a mechanism for the development of persistent soil seed banks.

Ecologically, it is important to know how long such dormancy can last in the field. In the related *Sclerolaena birchii*, similar fruits can remain intact for at least 6 months on the soil surface but, on breakdown, allow abundant germination from 15 months to 2.5 years. However, buried fruits did not germinate until after two years and germination continued until at least 4.8 years (Auld 1981). This behaviour allows a soil seed bank to develop, producing a pool of germinable seeds increasing through time which can allow mass germination when conditions are favourable (Auld 1981, Navie et al. 1996). Similar strategies are known for a further five species of *Sclerolaena* (Kinloch & Friedel 2004). Preliminary study of soil cores showed the existence of *Sclerolaena napiformis* seed banks (the present work), but detailed field studies are needed to establish plant and seed lifespans, length of soil seed dormancy and how these interact with various sequences of wet and dry years.

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