Arid land invasive weed *Salvia verbenaca* L. (wild sage): investigation into seedling emergence, soil seedbank, allelopathic effects, and germination

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Summary *Salvia verbenaca* L., wild sage (Lamiaceae) is native to western and southern Europe. In Australia *S. verbenaca* is considered an environmental weed with high invasiveness often growing on disturbed sites: tracks, roadsides and around earth tanks. Little is known about the ecology of this invasive species in Australia. Therefore, our objectives in this study were to determine: (i) seedling emergence in relation to sowing depth, (ii) density of soil seed bank, (iii) allelopathic effects, (iv) favourable conditions for seed germination of three month old and nine years old seeds, and (v) seed longevity.

Of four burial depths, only surface sown seeds germinated and survived. From the soil seedbank, *S. verbenaca* density was 343 ± 198 m^{-2}. For the allelopathic effects of *S. verbenaca*, neither the extract nor the leachate inhibited germination of *L. sativa*; however, the growth of the radicle of seedlings was shown to decrease. Germination was highest at 20°C under 12 h light/12 h dark. Burial of seeds in the field prior to germination reduced viability over time. Germination of three month old seed was significantly less than nine year old seed.

In conclusion, it is clear that *S. verbenaca* functions as a casual weed in arid and semi-arid environments of Australia. Understanding the plants' ecological characteristics in this study will help us take appropriate control measures for this species.

Keywords *Salvia verbenaca,* invasive weed, germination, allelopathy.

INTRODUCTION

Salvia verbenaca L. (wild sage) is a perennial herb, native to western and southern Europe, belonging to the family Lamiaceae (Claben-Bockoff and Speck 2000, Huang and van Staden 2002). In Australia *S. verbenaca* is considered an environmental weed with high invasiveness (DSE 2004). However, little is known about the ecology of this species in Australia. Therefore, the objectives of this study were to determine: (i) seedling emergence in relation to sowing depth, (ii) density of soil seed bank, (iii) allelopathic effects, (iv) favourable conditions for seed germination of three month and nine year old seed, and (v) seed longevity.

METHODS

Study site Nanya Research Station (E529680, N6335320) is located in south western New South Wales. The seasonal distribution of rainfall is even, but annual variation between seasons is high. Highest summer temperatures occur during February (mean daily maximum = 32°C; minimum = 16°C) and mild winter temperatures are experienced during July (mean daily maximum = 15°C; minimum = 5°C) (BOM 2013).

Seed collection Seed was obtained by collecting whole plants from dense infestations, in November 1996 and November 2004. Seeds were collected as seed capsules dried out, then stored in a labelled, air tight bottle and kept at room temperature until used.

Effects of seed burial The seeds were tested for germination at four different burial depths; 0, 2, 5 and 10 cm. Sterilised sand was used for the germination medium and four lots of twenty-five seed were placed in pots at each depth. The pots were placed on a bench in a glass house (20–25°C), watered twice a day and monitored for germination for 50 days.

Density and viability of soil seed bank Thirty two soil samples were collected from nine *S. verbenaca* infested sites in February 2005. At each site, four soil samples were collected along belt transects (Pavone and Reader 1982). Samples were taken from randomly selected points within plots and removed with a shovel to a depth of 15 cm (Florentine and Westbrooke 2005).

After being mixed thoroughly to ensure uniform distribution of seeds, each sample was spread evenly in labelled, paper towel-lined punnets, and placed in trays on benches in a glasshouse. All trays were watered twice a day and the number of emerged *S. verbenaca* seedlings was recorded. The germinable seed bank density was calculated as the number of *S. verbenaca* seeds that germinated under favourable water and temperature conditions within 90 days (Florentine and Westbrooke 2005).

Allelopathy: effect of exposure of seed to varying concentration of extract and leachate To determine the effect of metabolites produced by *S. verbenaca* on the germination and growth of other species, seeds of *Lactuca sativa* L. (Lettuce cv. Great Lakes; Yates seeds) were germinated in two different solutions; an aqueous extract and a leachate of *S. verbenaca*.

The aqueous extract was prepared from dried leaves and foliage of *S. verbenaca*, by placing 30 g of ground material in a 500 mL flask with 240 mL of deionised water and shaking the flask for two hours. The leachate was prepared by soaking 30 g of fresh leaves in 240 mL of deionised water for 24 hours. Both solutions were filtered and refrigerated until used.

Lactuca sativa seeds were germinated at a constant temperature of 20°C in four different concentrations of each extract; 0% (Control), 6%, 9% and 12.5%. To test for the effects of each solution on growth, the length of the radicle was measured three days after it had emerged from each seed.

Investigation of the temperature and light requirements of three month old and nine year old seeds Four lots of 25 seeds were surface sterilized with 6% sodium hypochlorite for one minute and placed in labelled Petri dishes that were lined with two moistened filter papers. Petri dishes were placed in incubators set at 20, 25, 30 and 35°C, under a 12 h light/12 h dark regime. For the 24 h dark treatment the dishes were wrapped in aluminium foil. All samples were kept moist until the experiment was terminated, after 50 days. Seeds were checked every 24 hours, with germinants counted and removed.

Germination of three month old and nine year old seeds after burial in the field To assess viability of seed following burial, twenty five small, fine-mesh nylon bags containing nine year-old and three month-old seed were buried in the field and retrieved over the duration of a year. Twenty five seeds were placed into each bag and the bags were buried in groups of five at a depth of 5 cm deep (Grigulis *et al*. 2001). Bags were removed periodically; at one week, three months, five months, and seven months after burial. After retrieval, seeds were sorted and assessed under a microscope for damage and germinated as per the optimum temperature and light requirements.

RESULTS

Seed burial Of the four burial depths, only surface sown seed germinated and survived. 52% of the seeds that germinated did so within eight days and the remainder over the next eight days (16 days in total). No seedlings emerged, from either the nine year old or the three month old seed, from the 2, 5 or 10 cm burial depths.

A significant difference (F 5.45, P = 0.04) was observed between the mean number of seedlings that emerged from nine year old seeds (3.18 ± 0.97) and three month old seeds (1.92 ± 1.42).

Soil seedbank A total of 2233 individuals belonging to ten species germinated from the soil seed bank. Of these, 64% were native species and 36% were exotic. The frequency of *S. verbenaca* was higher than any other species (24.74%), with a mean density across all collection sites of 343 ± 198 seedlings m^{-2}.

Between sites, the density of *S. verbenaca* differed significantly (F 4.01, P = 0.01) with the most significant (P <0.01) difference being attributed to seedling density at a single site, 1830 ± 61.85 seedlings m^{-2}. The seedling density for the other sites ranged from 0 to 655 seedlings m^{-2}.

Allelopathy Full germination was recorded for *L. sativa* seeds exposed to both the extract and leachate. The difference between the mean radicle length for each solution type was significant (F 37.67, P = 0.01). Growth of the radicle was inhibited by both the extract and the leachate, with the extract treatment having a stronger inhibitory effect (1.00 ± 0.50 mm) than the leachate (1.29 ± 0.27 mm).

Concentration of solution was the most significant influence on radicle growth overall, with the difference between the 0% and 12.5% concentrates (mean diff. 0.95 mm) and the lowest difference was between the 9% and 12.5% concentrates (mean diff. 0.18 mm).

Optimum temperature and light requirements There was no overall significant difference in the mean period of final germination (F 2.45, P >0.12) and the mean period for final germination was relatively short. Three month old seed germinated within 4.63 ± 1.07 days and nine year old seed germinated within 3.74 ± 1.27 days. Final germination varied according to age of seed (F = 12.00, P = 0.01) and was significantly higher for nine year old seeds (1.84 ± 0.22 seeds) compared to three month old seeds (1.62 ± 0.59 seeds).

The response to temperature was significant (F 6.12, P = 0.001) with the highest germination observed at 20°C under 12 h light/12 h dark (1.89 ± 0.26 days).

A significant difference (F 53.66, $P = 0.001$) was also observed between light treatments, due to lower germination under the 24 h dark treatment (1.49 ± 0.50 days).

Germination of seeds after burial in the field Viability of seed after burial declined for both ages of seed, with an overall total of 41% of three month old seed germinating and 7.4% of nine year old seed germinating under all temperature treatments. However, germination was observed to increase in seeds retrieved after periods of rainfall and lower temperatures, in July and September.

DISCUSSION

The soil surface appears to provide the best conditions for germination and survival of seedlings, suggesting that this species takes full advantage of available moisture and light. Seeds of this small size may require too much energy for seedlings to reach the surface from any depth of burial (Fenner and Thompson 2005). Alternately, it is likely that there is a light requirement, which may prevent a flush of germination occurring even though temperatures are favourable (Myers and Kitchen 1992). Seeds that are buried will become a part of the seed bank. As long as the seed remains viable, future disturbance events would bring the seed to the surface allowing for re-establishment of the species over time, thus protecting the population.

In conclusion, it is clear that *S. verbenaca* is functioning as a casual weed in arid and semi-arid environments. Understanding the plants' ecological characteristics in a study such as this at an early rather than late stage in the invasion will help us to take appropriate control measures of this species.

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