Germination of *Veronica parnkalliana* seeds in response to seasonal and fire cues

Jenny Guerin[1], Michael Thorpe[1], Dan Duval[1], Manfred Jusaitis[1,2] and Phil Ainsley[1,3]

[1.] Botanic Gardens of Adelaide, Adelaide, South Australia, [2.] School of Earth and Environmental Sciences, University of Adelaide, South Australia, [3.] Zoos SA, Adelaide, South Australia

Key Words
Burial, fire, seed germination, *Veronica parnkalliana*

Abstract

Veronica parnkalliana (Scrophulariaceae) is an endangered herb endemic to South Australia, with only six herbarium specimens recorded since its first collection in 1909. A targeted search of a fire scar in the Flinders Ranges during the spring of 2008 found prolific regeneration of the plant after a prescribed burn during the previous autumn. This post-fire discovery prompted an investigation into the seed germination requirements for this species. *In vitro* laboratory studies showed that germination was stimulated by gibberellic acid (GA_3), heat or smoked water. A seed burial experiment suggested that an annual cycle of dormancy was present, as germination of seeds that were exhumed after spring and summer was stimulated by a combination of heat and smoked water, but this did not occur after autumn or winter. Seeds buried at different depths before a fire were later monitored for seedling emergence *in situ*, and germination of exhumed seeds was also assessed. The depth of seed burial was a critical factor affecting germination. Results indicated that *V. parnkalliana* is a fire-ephemeral species with morphophysiological dormancy. These findings will provide valuable direction for future conservation planning for this species.

Introduction

Veronica parnkalliana was first recorded from the Eyre Peninsula in South Australia in 1909. It is a perennial herb with erect to ascending branchlets reaching a height of up to 40 cm. Leaves are opposite with serrated, tooth-like margins and during spring plants produce attractive white flowers with purple striations in the center of the petals. There are no further records of the plant until it was collected in the southern Flinders Ranges from 1984 to 1986. A more recent record was made after a targeted search of a fire scar in the Mount Remarkable Conservation Park during spring of 2008. A total of 12,000 viable seeds were collected during the following summer and are currently in long-term storage (at -20 °C) in the seed bank at the South Australian Seed Conservation Centre. *Veronica parnkalliana* is endemic to South Australia and is listed as Endangered under the National Parks and Wildlife Act, 1972. Assessment under IUCN (2001) criteria also leads to a rating of Endangered (EN B1&2ac(ii)(iii)(iv)).

To safeguard this species from extinction, it is important to understand its seed biology, and develop effective germination techniques to facilitate potential future translocations. The post-fire sighting of this endangered plant indicated that *V. parnkalliana* may belong to the group of plants known as fire-ephemerals. These plants are generally short-lived and mostly germinate after fire, producing seeds that persist in the soil seed bank between fire events (Baker *et al.* 2005). Seeds remain dormant in the soil and germination is stimulated by components of fire rather than by soil moisture or seasonal changes. Triggers for germination in fire-ephemerals often include heat and smoke, acting separately or concurrently.

The seed biology of *V. parnkalliana* was investigated using a combination of *in vitro* germination experiments and *in situ* seed burial. Seeds were exhumed and tested for germination at the end of each season to determine whether burial time affected seed dormancy. We were able to test the natural response to wildfire by burying seeds prior to a prescribed burn in the autumn of 2010. Seedling emergence was monitored at two sites and seeds were also exhumed for laboratory germination experiments. This provided a unique opportunity, as very few studies have combined burial and bushfire in this way. The aims of this study were to develop an effective protocol for

germinating seeds of *V. parnkalliana* and to determine how germination is affected by burial and fire.

Methods

Seeds were collected from Mount Remarkable in December 2008 and maintained in storage at 15 °C and 15% relative humidity until use.

In vitro *germination experiments*

Germination experiments were set up in replicates of four. Each replicate contained 25 seeds plated onto agar plates (1% (w/v)) and placed in a thermogradient plate with temperatures ranging from 5 ˚C to 40 ˚C. Germination was scored when the radicle had grown to at least half the length of the seed coat. GA_3 (250 mg/L) was added as a supplement to the agar plates.

In situ *burial*

V. parnkalliana seeds were buried on 21 December 2009 at four sites in the Mount Remarkable Conservation Park. Seeds were enclosed in wire mesh in packets of 100 and four were buried at each site, within the region where the natural population was found in 2008. A packet of seeds was exhumed from each site at approximately 3-monthly intervals, timed to coincide with the end of each season. After retrieval, seeds were tested for viability using a vital stain (tetrazolium chloride), and for germination using the following treatments: control (no treatment), GA_3 (250 mg/L), smoked water (soaked for 24 h in 10% (v/v)), dry heat (15 min at 90 °C) and a combination of smoked water and dry heat. Seeds were incubated at 10 °C 12 h dark, 22 C °12 h light. Burial control seeds were not buried and were tested at the start of the experiment.

Prescribed burn burial experiment

To examine the effects of wildfire on seedling regeneration, two sites were selected in the area to be burnt, and a control site was selected in an adjacent unburnt area. At each site, 100 *V. parnkalliana* seeds were sown (in 20 x 20 cm quadrats) in each of three treatment plots; soil surface, buried at 1 cm, or buried at 2.5 cm. These plots were individually covered with wire cages to protect against disturbance. The prescribed burn took place on 24 April, 2010. Early seedling emergence was monitored monthly, with the highest number of seedlings being recorded approximately 4 months after the fire.

Prior to the prescribed burn 100 seeds were encased in wire-mesh bags and buried at the same depths as in the previous experiment (0, 1, 2.5 cm). Two bags were buried at each depth at burn each site. Seeds buried in the unburnt control site were buried at 1 cm depth. The seeds were exhumed on the morning after the burn and were subsequently tested for germination *in vitro.* Four replicates of 25 seeds were tested on agar plates (1% (w/v)) and four replicate positive controls of 25 seeds each were placed on agar (1% (w/v)) supplemented with GA3 (250 mg/L). Plates were incubated as above.

Results and Discussion

In vitro *germination experiments*

Initial *in vitro* germination tests showed that seeds had a high level of dormancy, with no germination recorded over a wide temperature range (5 - 40 ˚C). However, high levels of germination (80 to 90%) were observed after treatment with GA_3. Germination in the presence of GA_3 was assessed at temperatures between 5 ˚C and 40 ˚C using a thermogradient plate. Optimal temperatures for germination were between 10 °C and 18 °C. Germination declined at temperatures over 20 °C and no germination was recorded at 30 °C or above.

Seed embryos were linear and underdeveloped (Figure 1), which indicates that embryos need to grow within the seed before germination can occur. Seeds within this class often respond to the application of GA_3 and have been shown to have seasonal cyclic dormancy that may be affected

by periods of cold, or warm followed by cold stratification (Ooi *et al.* 2007). Several reports have suggested that seed burial may also affect dormancy and germination (Baker *et al.* 2005; Ooi, 2010).

Germination after in situ burial

This experiment showed that seed retained viability during the burial period, although a slight decline was observed throughout the year (Figure 2). Mean germination levels for GA_3-treated seeds were above 75% for all burial times, also confirming maintenance of seed viability throughout burial. Germination of control seeds was negligible throughout the burial experiment, indicating a high level of dormancy. Seeds responded positively to both the heat and smoked water treatments, and a synergistic effect was observed when both these treatments were combined, yielding increased germination.

Fire components stimulated germination after burial during summer. However, there was no response from seeds buried throughout autumn and winter. In the following, spring seeds germinated in response to heat and smoke treatment. Such seasonal responses have been reported for seeds with cyclic dormancy, where seeds are responsive to certain environmental cues and cycle through seasonal periods of dormancy (Baskin & Baskin, 2004).

In situ burial of seeds prior to prescribed burning.

The germination of seeds that were buried prior to the prescribed burn and exhumed the following morning is shown in Figure 3. The highest level of seed germination was recorded at a burial depth of 1 cm. Seeds recovered from the surface were mostly charred and nonviable, and seeds that were buried at 2.5 cm remained dormant.

These results supported those from the seedling emergence counts at site 1, where 51 seedlings were observed from a depth of 1 cm, while only a single seedling was observed at each of the 0 and 2.5 cm placements after 4 months. Thus it appears that burial depth had a major influence on seed germination, although this pattern was not observed at site 2 where only a few (5) seedlings were counted from the surface-sown seeds. These results suggest that several factors could have an important role in stimulating germination. Factors that may have varied between the two sites could be fire temperature, the density of smoke, or the level of nitrates in the soil post-fire. No seedlings emerged at the unburnt control site.

Conclusions

Germination may be induced following stratification at high or low temperatures, and/or after a period of after-ripening to alleviate dormancy. The present results showed that germination of *V. parnkalliana* did not occur after burial alone and that treatment with GA_3, smoke or heat was necessary for germination to be initiated. The observed cyclic dormancy pattern changed through the warm and cool seasons. Seeds responded to fire cues in December and March, at a time when bushfires are likely to occur in the natural habitat. Factors influencing the emergence of *V. parnkalliana* can be deduced using the data obtained from both the field and the laboratory. We propose that fire during the warmer months delivers the fire cue to the seeds at a time when dormancy is at its lowest. Germination would then occur when moisture conditions were maintained by sufficient rainfall and temperatures were conducive to germination. These conditions would occur naturally during winter, resulting in plants growing and flowering during spring with seeds ripening during early summer.

Broadening the understanding of the seed biology of our threatened plant species increases our ability to monitor biodiversity and restore habitats. Identifying *V. parnkalliana* as a fire ephemeral species greatly increases the chances of locating this plant in its vegetative stage. These results provide new insight into how the interplay of environmental conditions affects the life cycle of this endangered and endemic species.

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Figure 1. A) Flowers, buds and leaves B) seeds and C) longitudinal cut showing the underdeveloped embryo.

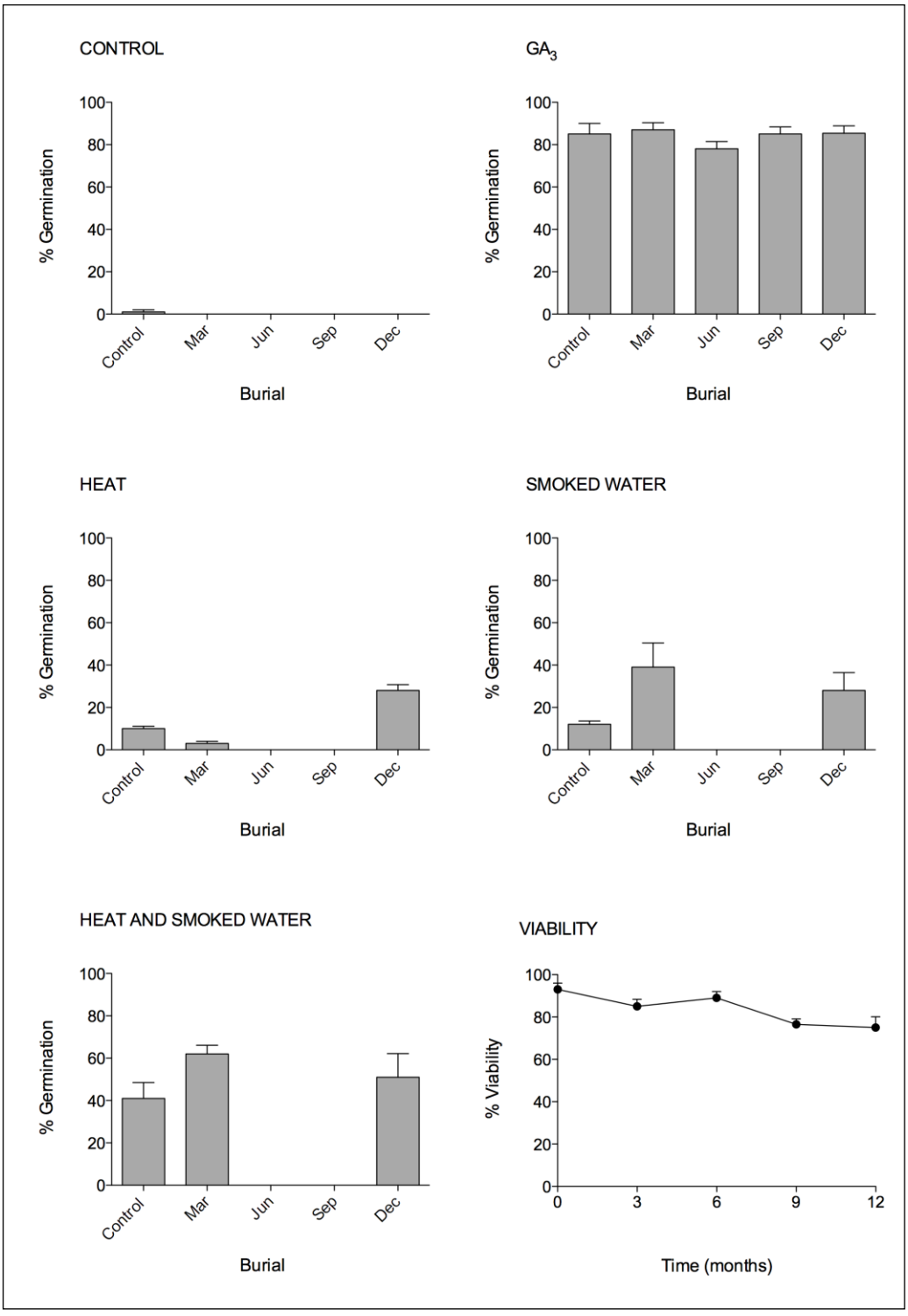

Figure 2. Germination data from the *in situ* burial experiment. Seeds were buried in December 2009 and exhumed at the end of each season in 2010, then tested for germination. Seed treatments are shown on top of each graph and the month that seeds were exhumed after burial is shown on the x axis. Burial control seeds were not buried. Viability was determined using a tetrazolium chloride stain. Bars represent standard error.

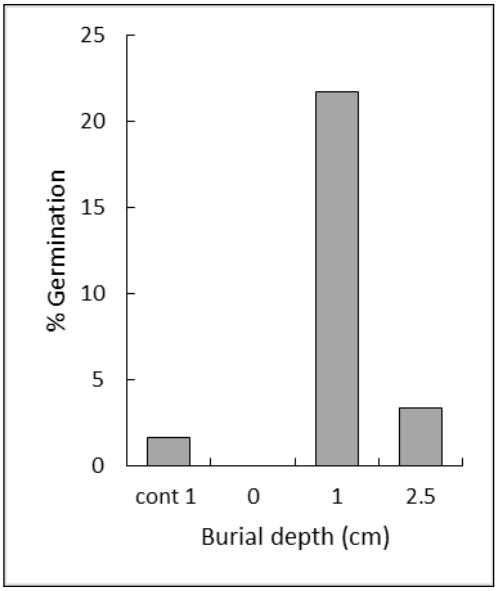

Figure 3. Germination of buried seeds exhumed after a prescribed burn. Seeds were buried at different depths prior to the prescribed burn. The control site (cont 1) was unburnt and seeds were buried at 1 cm depth. The % germination shown is the number of seeds that germinated with no treatment relative to the number of seeds that germinated in the positive control (supplemented with GA_3)