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Does smoke water enhance seedling fitness of serotinous species in fire-prone southwestern Western Australia?☆

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A B S T R A C T

Studies have begun to show the potential for smoke to improve seedling fitness of species from fire-prone environments. The seeds of serotinous species have rarely been known to exhibit any dormancy, or require any further cue for germination once seeds are released from the woody fruits. However, these seeds are often released into a post-fire environment that contains active smoke chemicals. Recent studies recognise chemicals from smoke may regulate diverse aspects of plant development; we hypothesised that smoke may have important effects on seedling fitness of serotinous species in fire-prone environments. To explore the role of fire on the post-fire recruitment processes of serotinous species we first conducted a germination experiment with smoke water treatments on eight serotinous species from southwestern Western Australia; with a replicated design, we subsequently tested the post-treatment seedling growth of the eight species in a glasshouse experiment. The results showed that while the seeds of the eight serotinous species readily germinated with or without smoke treatment, there were significant smoke responses with regards to enhanced seedling fitness in three species. *Petrophile filifolia*, *Isopogon divergens*, and *Banksia menziesii*, seedlings treated with Oaten Hay smoke-water demonstrated significantly greater mean shoot length (mm) ($F = 25.5_{1,4}$, $p = 0.007$), mean root length (mm) ($F = 31.4_{1,4}$, $p = 0.005$), and root dry-weight (mg) ($F = 12.8_{3,12}$, $p < 0.001$) respectively, than untreated seedlings. This study demonstrates the potential for some serotinous species to exhibit growth responses elicited by fundamental fire traits.

1. Introduction

Fire has played a major role in shaping ecosystems and the evolution of plant traits (Keeley et al., 2011; He and Lamont, 2017). Seed germination and seedling establishment are critical stages for plant population persistence. In regions characterised by drought, recurrent fires, and low soil fertility, such as southwestern Western Australia (SWA), successful seed germination and seedling establishment often requires adaptive strategies to overcome harsh conditions (Bell et al., 1993). The flora of SWA has developed a number of strategies to cope with, and even benefit from fire, as the post-fire environment can provide optimal conditions for seed germination and seedling establishment, with reduced competition, higher nutrients, and increased light levels (Keeley et al., 2011).

The utilisation of smoke as a germination cue is one fire adaptive strategy that has received significant attention since de Lange and Boucher (1990) reported on the smoke elicited germination response of the South African species *Audouinia capitata*. The smoke and burnt material produced from a fire contain a multitude of chemicals that have been recognised as playing an important role in overcoming dormancy and stimulating germination in many plant species (van Staden et al., 2000). Species which respond to smoke as a germination cue are often those that form soil seed banks with dormant seeds (Roche et al., 1997; Baker et al., 2005). In contrast, canopy stored, or serotinous species, are rarely recorded to have smoke-stimulated germination as serotinous species do not generally require any further trigger for germination once propagules are released from fruits after-fire [see Zhao and Ladd, 2014 for a rare exception] germinating readily in appropriate winter temperatures and moisture (Bellairs and Bell, 1990; Bell et al., 1993).

Many studies have investigated the potential for smoke to improve seedling fitness (albeit, mostly in agriculturally important species). For example, maize seedlings grown from seeds that had been exposed to aerosol smoke have been shown to produce longer roots and shoots (Sparg et al., 2006). However, to date,

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little work has been done with regards to the seedling fitness benefits of smoke water in native Australian species. Seedlings that grow faster and have a robust root system gain a competitive advantage in the postfire environment. Understanding the potential for post-germination seedling fitness benefits to species is important and highlights the potential for research into this often-neglected area of research.

The seeds of serotinous species are often released into a post-fire environment that contains active chemicals from smoke (Keeley and Fotheringham, 1997). Given this association of serotinous species with fire and exposure to smoke-related chemicals over extended periods of evolutionary time (Ne'eman et al., 2009; He et al., 2011) and recent studies recognising that chemicals from smoke (e.g. Karrikins) regulate diverse aspects of plant development (Nelson et al., 2012; Waters, 2017), we hypothesised that smoke may affect seedling fitness of serotinous species in fire-prone southwestern Western Australia.

To explore the role of fire on the post-fire recruitment processes of serotinous species rarely studied, we first conducted a germination experiment with smoke water treatments on eight serotinous species from southwestern Western Australia; with a replicated design, we subsequently tested the post-treatment seedling growth of the eight species in a glasshouse experiment.

2. Materials and methods

2.1. Study species

Eight serotinous species from the family Proteaceae were investigated in this study: *Banksia candolleana* Meisn., *Banksia menziesii* R.Br., *Isopogon divergens* R.Br., *Isopogon dubius* (R.Br.) Druce, *Isopogon* sp., *Petrophile anceps* R.Br., *Petrophile drummondii* Meisn., and *Petrophile filifolia* R.Br. Species selection was based on the availability of sufficient viable seed from serotinous species within the Proteaceae family inhabiting southwestern Western Australia, while *Isopogon* and *Petrophile* species were specifically included due to the relative lack of related research within the Proteoideae sub-family. Species selection was also contingent on having seed viability greater than 40% (estimated by a cut test; Ooi et al., 2004). Apart from *I. dubius* (which was ordered from the Nindethana seed company (Albany, WA), seeds were collected from the field across southwestern Western Australia, and seeds from different provenance were mixed. To avoid pre-treatment exposure to smoke, all seeds were extracted without the use of fire; *Petrophile* and *Isopogon* species seeds were removed from cones with forceps, while *Banksia* species follicles were sawed flush with the cone and seeds removed with forceps.

2.2. Smoke water treatments and seed germination

Treatments for *I. divergens*, *I. dubius*, *I.* sp., *P. anceps*, and *P. filifolia* species consisted of a control (sterile deionised water) and a 5% Oaten Hay smoke water treatment diluted using sterile deionised water (Downes et al., 2013). Thirty seeds of each species were placed in 90 mm Petri dishes ($n = 3$), with three squares of Wettex® and 10 mL of the treatment solution. Prior to treatment, all seeds underwent surface sterilisation to minimise fungal contamination as described in Downes et al. (2010).

Treatment solutions for species *B. candolleana* and *B. menziesii*, and *P. drummondii* consisted of a control (sterile deionised water), and three Oaten Hay smoke water concentrations (1%, 5%, and 10%), all diluted in sterile deionised water. Seeds of each species were then placed in Petri dishes containing 50 seeds, squares of Wettex® sponge (4 cm^2), treatment solution, and two sheets of Whatman® No. 1 filter paper, then sealed with Parafilm® ($n = 4$). Species with smaller seeds (*P. drummondii*) were placed in 120 mm Petri dishes, with four squares of Wettex® and 15 mL of treatment solution; larger seeds (*B. candolleana* and *B. menziesii*) were placed in 150 mm Petri dishes, with five squares of Wettex® and 20 mL of the treatment solution.

All Petri dishes were incubated for five weeks at 15 °C ± 2 °C (Bellairs and Bell, 1990; Bell et al., 1993) and set with a light/dark cycle of 12 h/12 h respectively. During the experiments, Petri dishes were randomised daily and checked for germination. Germination was counted when the radical length ≥ 2 mm. At the termination of the experiment any seeds that failed to germinate underwent a cut test to ascertain viability. The total number of seeds germinated from each species after the five weeks was then converted into a percentage of viable seeds germinated.

2.3. Glasshouse growth and seedling fitness

Seedling fitness studies were undertaken to examine treatment effects on the key growth parameters (determined to be effective key measurements in previous seedling fitness studies; Sparg et al., 2005, 2006; Zhou et al., 2011) of shoot length (mm), root length (mm), and shoot and root dry-weight (mg). Eleven germinants with a radicle length ≥ 5 mm were randomly chosen from the germination trials from their corresponding experimental unit within each treatment. Each seedling was planted in clean white sand in custom made pots – PVC tube 500 mm in height and 40 mm in diameter – to allow for root extension, then randomly allocated to a location within the study area (within replicate groups).

Seedlings were grown for five weeks before being harvested and root and shoot length recorded (mm). Seedlings were dried in an oven for 48 h at 80 °C (determined as constant dry mass using methods outlined by Campbell and Plank, 1998), upon which dry-weight was recorded. For species with large cotyledons (i.e. *B. candolleana* and *B. menziesii*) cotyledons were removed before shoot dry-weight was measured.

2.4. Data analysis

A one-way ANOVA was performed to assess the difference in treatments with regards to total germination, shoot length, root length, shoot weight, and root weight of each species. Percentage data were arcsine transformed. To correct for multiple variables within tests a Bonferroni correction was applied to an alpha level of 0.05 and a new alpha level set at 0.01. Each Petri dish was considered as an experimental unit/replicate. All data analysis was performed using SPSS Statistics® version 20 (IBM Corp. 2012).

3. Results

All eight serotinous species germinated readily without smoke treatment; Oaten Hay smoke-water treatments, regardless of the concentration, had no significant effects on the germination across all eight species (Fig. 1).

3.1. The effect of smoke water on seedling fitness

3.1.1. Shoot length and dry-weight

Shoot growth in all but one species was not significantly different between treatments (Table 1). For *P. flifolia*, seedlings treated with a 5% Oaten Hay smoke-water treatment significantly promoted seedling growth (mean shoot length; $F = 25.5_{1,4}$, $p = 0.007$). Mean shoot dry-weight was not significantly different between treatments in any of the species tested (Table 2).

3.1.2. Root length and dry-weight

Root growth in all but two species was not significantly different between treatments. *Isopogon divergens* seedlings treated with a 5%