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

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ABSTRACT

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. flfolia*, 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%

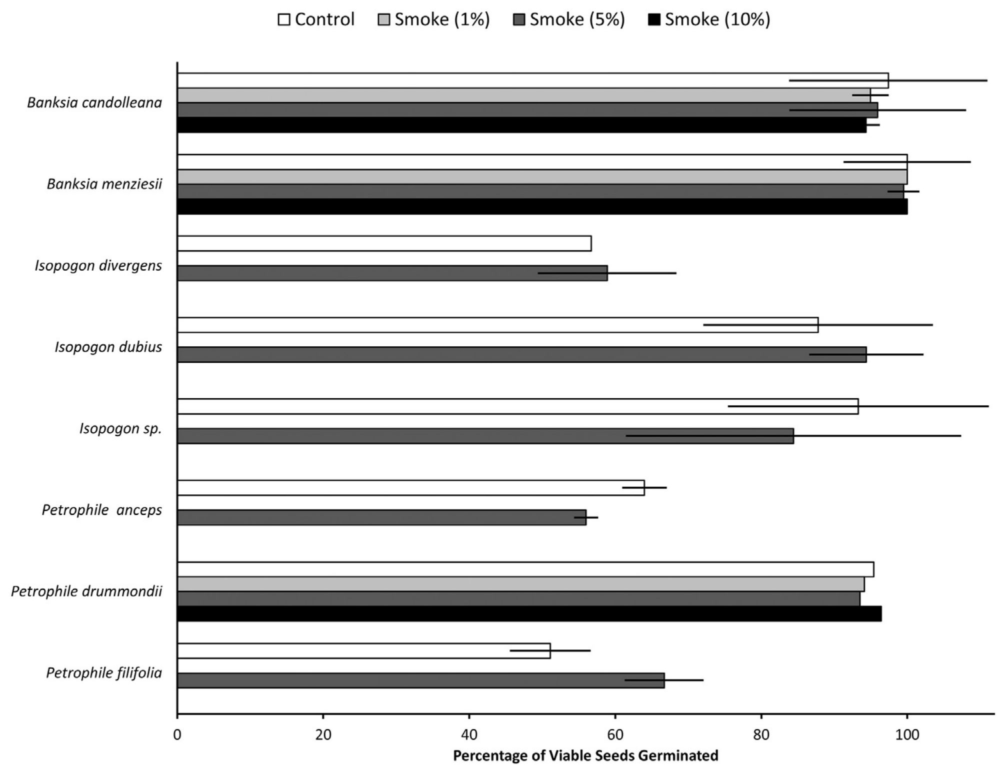

Fig. 1. Mean percentage (%) of viable seeds germinated in various smoke treatments in test species after five weeks with 95% CI.

Oaten Hay smoke-water treatment demonstrated significantly enhanced seedling growth in regard to mean root length ($F = 31.4_{1,4}$, $p = 0.005$; Table 3). *Banksia menziesii* seedlings treated with 10% Oaten Hay smoke water demonstrated significantly greater mean root dry-weight than untreated seedlings ($F = 12.8_{3,12}$, $p < 0.001$; Fig. 2; Table 4). However, *B. menziesii* seedlings treated with a lesser concentration of 5% Oaten Hay smoke water were not significantly different to untreated seedlings.

Table 1
Comparison of mean shoot length (mm) of species at various smoke-water treatments.

Species	Treatment	Mean (mm) ± SD	F	*p*
Banksia candolleana	Control	29.4 ± 6.7	$1.33_{(3,12)}$	0.312
	Smoke (1%)	30.9 ± 1.1		
	Smoke (5%)	34.5 ± 2,1		
	Smoke (10%)	29.8 ± 3.8		
Banksia menziesii	Control	27.2 ± 2.4	$4.44_{(3,12)}$	0.026
	Smoke (1%)	30.4 ± 3.6		
	Smoke (5%)	32.4 ± 3.7		
	Smoke (10%)	36.3 ± 4.3		
Isopogon divergens	Control	19.4 ± 0.7	$0.50_{(1,4)}$	0.520
	Smoke (5%)	20.0 ± 1.3		
Isopogon dubius	Control	25.3 ± 0.6	$5.92_{(1,4)}$	0.070
	Smoke (5%)	23.2 ± 1.4		
Isopogon sp.	Control	13.5 ± 0.9	$1.62_{(1,4)}$	0.272
	Smoke (5%)	12.0 ± 1.8		
Petrophile anceps	Control	19.9 ± 1.9	$5.61_{(1,4)}$	0.080
	Smoke (5%)	16.2 ± 2.0		
Petrophile drummondii	Control	11.6 ± 0.7	$0.56_{(3,12)}$	0.655
	Smoke (1%)	11.6 ± 1.2		
	Smoke (5%)	12.6 ± 5.3		
	Smoke (10%)	10.1 ± 1.4		
Petrophile filifolia	Control	28.7 ± 1.6	$25.5_{(1,4)}$	0.007*
	Smoke (5%)	39.0 ± 3.2		

Note: smoke-water derived from Oaten Hay.
* Significant results are denoted by an asterisk (α = 0.01).

Table 2
Comparison of mean shoot dry-weight (mg) of species at various smoke-water treatments.

Species*	Treatment	Mean (mg) ± SD	F	*p*
Banksia candolleana	Control	23.9 ± 6.9	$2.05_{(3,12)}$	0.160
	Smoke (1%)	29.9 ± 2.7		
	Smoke (5%)	26.4 ± 2.8		
	Smoke (10%)	23.4 ± 3.3		
Banksia menziesii	Control	17.1 ± 2.2	$3.55_{(3,12)}$	0.048
	Smoke (1%)	18.3 ± 3.9		
	Smoke (5%)	22.6 ± 4.5		
	Smoke (10%)	24.6 ± 4.0		
Isopogon divergens	Control	4.1 ± 0.5	$3.25_{(1,4)}$	0.146
	Smoke (5%)	4.9 ± 5.2		
Isopogon dubius	Control	11.3 ± 4.5	$4.07_{(1,4)}$	0.114
	Smoke (5%)	6.0 ± 0.8		
Isopogon sp.	Control	3.6 ± 0.5	$0.05_{(1,4)}$	0.830
	Smoke (5%)	3.5 ± 0.4		
Petrophile anceps	Control	6.7 ± 3.3	$2.57_{(1,4)}$	0.190
	Smoke (5%)	3.6 ± 0.3		
Petrophile drummondii	Control	5.4 ± 0.6	$4.18_{(3,12)}$	0.030
	Smoke (1%)	4.4 ± 0.4		
	Smoke (5%)	4.2 ± 0.3		
	Smoke (10%)	4.4 ± 0.7		
Petrophile filifolia	Control	5.5 ± 0.5	$11.90_{(1,4)}$	0.026
	Smoke (5%)	7.7 ± 1.0		

Note: smoke-water derived from Oaten Hay.
* Significant results are denoted by an asterisk (α = 0.01).

Table 3
Comparison of mean root length (mm) of species at various smoke-water treatments.

Species	Treatment	Mean (mm) ± SD	F	p
Banksia candolleana	Control	304 ± 32.8	$0.53_{(3,12)}$	0.670
	Smoke (1%)	330 ± 39.1		
	Smoke (5%)	323 ± 28.6		
	Smoke (10%)	331 ± 32.4		
Banksia menziesii	Control	318 ± 22.6	$0.76_{(3,12)}$	0.536
	Smoke (1%)	275 ± 50.4		
	Smoke (5%)	277 ± 57.0		
	Smoke (10%)	307 ± 60.4		
Isopogon divergens	Control	119 ± 9.2	$31.40_{(1,4)}$	0.005*
	Smoke (5%)	151 ± 3.8		
Isopogon dubius	Control	201 ± 1.6	$7.97_{(1,4)}$	0.048
	Smoke (5%)	177 ± 14.6		
Isopogon sp.	Control	148 ± 26.7	$0.05_{(1,4)}$	0.834
	Smoke (5%)	144 ± 17.0		
Petrophile anceps	Control	120 ± 9.2	$2.81_{(1,4)}$	0.170
	Smoke (5%)	103 ± 14.6		
Petrophile drummondii	Control	154 ± 18.7	$2.98_{(3,12)}$	0.074
	Smoke (1%)	121 ± 27.4		
	Smoke (5%)	107 ± 23.8		
	Smoke (10%)	136 ± 22.8		
Petrophile filifolia	Control	120 ± 10.3	$5.55_{(1,4)}$	0.080
	Smoke (5%)	159 ± 26.2		

Note: smoke-water derived from Oaten Hay.
* Significant results are denoted by an asterisk (α = 0.01).

Table 4
Comparison of mean root dry-weight (mg) of species at various smoke-water treatments.

Species	Treatment	Mean (mg) ± SD	F	p
Banksia candolleana	Control	33.9 ± 4.5	$3.67_{(3,12)}$	0.044
	Smoke (1%)	37.5 ± 2.7		
	Smoke (5%)	30.2 ± 3.1		
	Smoke (10%)	31.5 ± 2.7		
Banksia menziesii	Control	20.7 ± 2.3	$12.84_{(3,12)}$	<0.001*
	Smoke (1%)	21.5 ± 3.5		
	Smoke (5%)	17.7 ± 2.6		
	Smoke (10%)	33.6 ± 6.1		
Isopogon divergens	Control	2.9 ± 0.5	$1.33_{(1,4)}$	0.314
	Smoke (5%)	3.2 ± 0.3		
Isopogon dubius	Control	5.3 ± 0.4	$0.90_{(1,4)}$	0.400
	Smoke (5%)	4.8 ± 0.8		
Isopogon sp.	Control	1.9 ± 0.2	$2.04_{(1,4)}$	0.227
	Smoke (5%)	2.3 ± 0.4		
Petrophile anceps	Control	2.2 ± 0.2	$0.75_{(1,4)}$	0.440
	Smoke (5%)	2.7 ± 1.0		
Petrophile drummondii	Control	2.9 ± 0.4	$5.10_{(3,12)}$	0.017
	Smoke (1%)	1.8 ± 0.4		
	Smoke (5%)	2.1 ± 0.4		
	Smoke (10%)	2.7 ± 0.5		
Petrophile filifolia	Control	2.3 ± 0.1	$3.58_{(1,4)}$	0.130
	Smoke (5%)	3.1 ± 0.8		

Note: smoke-water derived from Oaten Hay.
* Significant results are denoted by an asterisk (α = 0.01).

4. Discussion

Serotinous species do not generally exhibit any dormancy or require any further trigger for germination once propagules are released from fruits, germinating readily in appropriate winter temperatures (Bell et al., 1993; Keeley and Bond, 1997). In our study, with eight serotinous species, no germination benefits of smoke water treatment were observed and seeds were able to reach maximum germination over the same timeframe without the addition of smoke water (see Appendix 2).

Our experiments did indicate that treatment with smoke water may have promoted an aspect of seedling growth in three out of eight serotinous species tested. Three different responses were observed across the three species: *P. filifolia* with greater shoot length, *I. divergens* with root length and *B. menziesii* with root dry-weight. As the difference in root dry-weight for *B. menziesii* was not reflected in root length, this suggests more branching and/or more robust roots rather than longer roots. The inverse can be seen in *I. divergens*, with the increased root length not being reflected in overall root dry-weight. This is also the case with the greater shoot

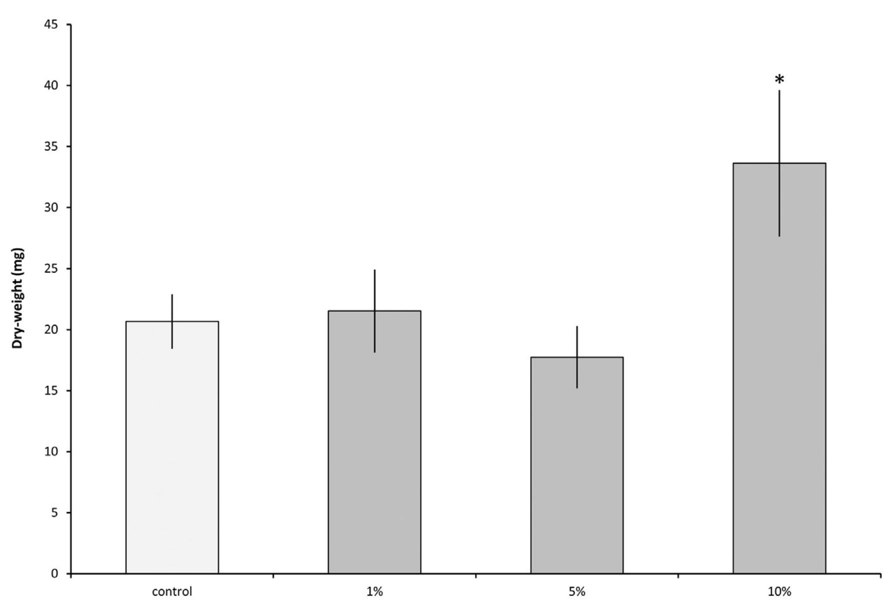

Fig. 2. Mean root dry-weight of *Banksia menziesii* at concentrations of Oaten Hay smoke-water. Note: * denote significant difference from control as determined by a Dunnett post-hoc ($p = 0.001$) ($\alpha = 0.01$).

length of *P. filifolia* not being reflected as greater shoot dry-weight. It is not unusual for species to respond differently to the effects of smoke, with variability due to differing life histories and functional traits (Lloyd et al., 2000). The seedling fitness-enhancing responses in this study were also not consistent across tested genera, suggesting possible species-specific mechanisms by which the smoke chemicals may affect seedling fitness.

Allocation of resources to roots is often associated with competition for nutrients and water, while allocation to biomass/shoot length is associated with competition for light. Tested species have differing traits (see Appendix 1), which may explain the different fitness benefits that were elicited with smoke treatment. *Petrophile filifolia* grows in naturally wetter parts of the landscape compared to *B. menziesii* and *I. divergens*, and therefore may not necessarily need longer roots as a competitive advantage following fire. Therefore, more resources may be allocated into other areas of development (e.g. growth) than to root extension (Craine, 2009). *Banksia menziesii* is usually weakly serotinous, and while inter-fire recruitment success is considered rare it is possible that the post-fire regeneration of a population may rely on only one or two years of seed cohort. Enhanced fitness through responding to smoke chemicals may ensure successful population regeneration with limited seed source in this case. While variation in species serotiny level was considered when selecting study species, there is no evidence that response of seedling growth in this study was related to serotiny level in the test species; *Isopogon* species generally exhibit low serotiny, while *B. candolleana* is among the most serotinous species, both exhibited no response to smoke water treatment.

While this study has revealed that some serotinous species may show seedling fitness responses to smoke treatment, not all species tested did. The specific mechanisms behind why some species responded and others did not is unknown. Smoke from burnt vegetation contains many chemicals, and much work has gone into trying to isolate the active compounds within smoke. In 2004, research revealed a new family of butenolides (3-methyl-2H-furo[2,3-c]pyran-2-one), referred to as 'karrikins' (Flematti et al., 2004; van Staden et al., 2004). Karrikins have been found to not only be responsible for stimulating germination, but also shown to play a role in seedling vigour (Nelson et al., 2012; Waters, 2017). However, karrikins alone cannot explain all plant responses to smoke (Downes et al., 2010) and there is still a lot yet unknown about the mechanisms of smoke stimulation both in germination and seedling fitness. Chumpookam et al. (2012) discussed the enhanced growth of papaya seedlings when treated with smoke water by the promotion of mineral and nutrient uptake, where the authors observed greater levels of nitrogen in roots and shoots, and greater levels of magnesium in shoots. Smoke chemicals may also trigger the expression of certain proteins (e.g. KAI2) that play a role in light sensing and signalling pathways regulating plant growth and development (Nelson et al., 2012; Waters, 2017).

5. Conclusions

Smoke related plant responses have received significant attention, particularly in the fire-prone Mediterranean-climate regions such as California (Keeley and Fotheringham, 1998), South Africa (de Lange and Boucher, 1990; Brown, 1993), and Western Australia (Bell et al., 1993; Dixon et al., 1995). The continuation of research into the smoke responses of a range of species in SWA is necessary when building a knowledge base of plant–fire relationships, both when considering the past as to how these adaptions may have evolved, and when looking into the future for how fire management strategies may impact the various species within a system. Furthering research into the post-germination seedling response of Australian native species is of particular importance when considering the need to better understand our native ecosystems in the face of a changing climate and fire regimes.

It should be highlighted that this study used Oaten Hay smoke water; results may vary with different smoke exposure types and concentrations, such as Regen® or aerosol smoke. Aerosol rather than liquid smoke has been documented to stimulate different effects to smoke water (Roche et al., 1997; van Staden et al., 2000). Serotiny level of species used in this study have comparatively longer timeframes of canopy seed storage, and further study could examine a variety of serotiny levels to establish if the level of serotiny has an impact on smoke response. In addition, further study should be conducted to explore whether these initial seedling fitness benefits that this study observed (over a limited timeframe), persist longer-term into the summer, where the real-life advantage would be tested.

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Appendix 1

Table A.1

Species	Code	Seed source	Collection location	Serotiny level	Viability %	Habitat	Life form	Fire response
Banksia candolleana	n/a	Field collection	Eneabba	Strong	95	Heathland	Shrub	Sprouter
Banksia menziesii	n/a	Field collection	Kwinana	Medium	100	Woodland	Tree	Sprouter
Isopogon dubius	n/a	Nindethana	Unknown	Weak	90	Heathland	Shrub	Non-sprouter
Isopogon divergens	ARCPP52	Field collection	Hill River	Weak	85	Woodland	Shrub	Non-sprouter
Isopogon sp.	ARCPP89	Field collection	Stirling range	Weak	95	Woodland	Shrub	Non-sprouter
Petrophile anceps	ARCPP14	Field collection	Stirling range	Medium	50	Woodland	Shrub	Non-sprouter
Petrophile drummondii	n/a	Field collection	Eneabba	Medium	40	Heath	Shrub	Non-sprouter
Petrophile filifolia	ARCPP112	Field collection	Stirling range	Medium	75	Woodland	Shrub	Non-sprouter

Appendix 2

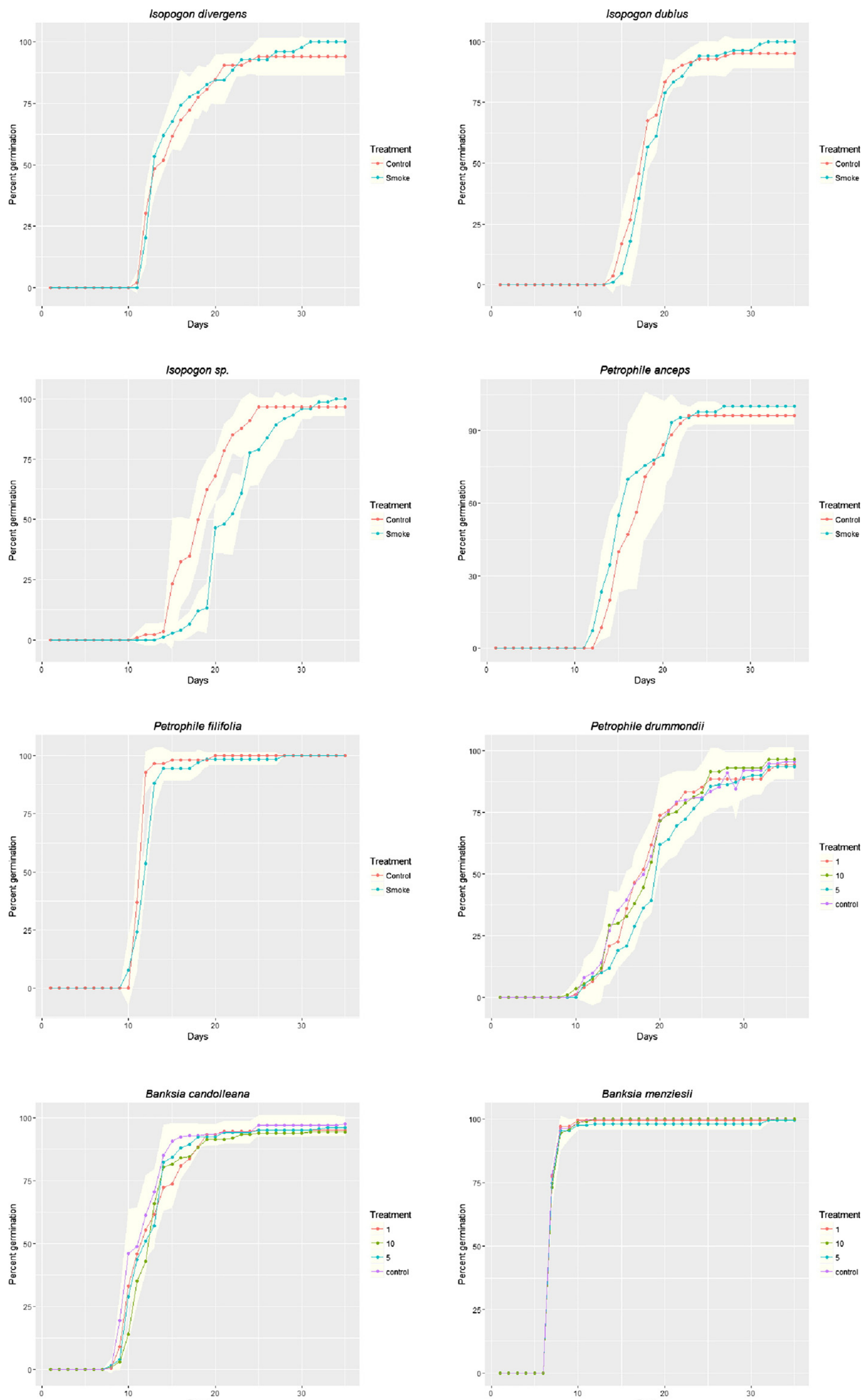

Fig. A.1. Percent of viable seeds germinated over time for each species used in this study, shaded area indicates 95% CI.

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