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Heat and smoke pre-treatment of seeds to improve restoration of an endangered Mediterranean climate vegetation type

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Abstract Invasive alien plants impact ecosystems, which often necessitates their removal. Where indigenous species recovery fails following removal alone, an active intervention involving reintroduction of seed of native species may be needed. This study investigated the potential for a combination of the fire cues of smoke and heat as a pre-treatment of seeds in breaking dormancy and facilitating increased germination. Species were selected to represent different functional types within Cape Flats Sand Fynbos; a fire-prone, critically endangered vegetation type in South Africa. Seeds were exposed to either a heat pulse (temperatures between 60 and 300°C for durations of between 30 s and 20 min) or dry after-ripening (1 or 2 months at milder temperatures of 45°C or less). Thereafter, seeds were soaked in smoke solution for 18 h and subsequently placed on agar at 10/20°C for germination. Most species fell into one of two main groups: Seed germination in the first group was greatest following a lower temperature (60°C) heat pulse, an extended period of mild temperature (20/40°C or 45°C) exposure, or no pre-treatment with heat. Seed germination in the second group was promoted after brief exposure to higher (100°C) temperatures. No germination occurred in any species following heat treatments of 150°C or higher. Species which responded better to higher temperatures were mainly those possessing physical dormancy, but seed morphology did not correlate with germination success. This study showed that heat stimulation of seeds is more widespread in fynbos plant families than previously known and will enable the development of better seed pre-treatment protocols before large-scale sowing as an active restoration treatment after alien plant clearing.

Key words: active restoration, Cape Flats Sand Fynbos, dry after-ripening, germination facilitation, heat pulse, seed dormancy.

INTRODUCTION

Invasive alien plants impact ecosystems (Richardson *et al.* 2000) by decreasing diversity and altering ecosystem functionality (Levine *et al.* 2003). Management interventions include preventing introductions, eradicating or controlling invasive species and mitigating their impacts (Wilson *et al.* 2011). Maintenance control following removal will likely be necessary for decades to prevent reinvasion (Pretorius *et al.* 2008). Alien species removal alone often fails to achieve a functional native ecosystem due to the lack of active intervention (Reid *et al.* 2009). In areas which have a long history of invasion, this may be due to native seed bank depletion (Holmes 2002). In such cases, the vegetation may recover if missing species are re-introduced (Pretorius *et al.* 2008). However, propagation of seedlings is costly, and an active restoration intervention using reseeding after alien clearing may fail to achieve successful re-establishment if seeds possessing dormancy do not receive appropriate dormancy-breaking cues. This becomes more important, but also more complicated to achieve, in highly biodiverse ecosystems such as the Cape Floristic Region.

The fynbos vegetation of the Cape Floristic Region, South Africa, is mainly characterized by a Mediterranean climate with winter rainfall and summer drought. This vegetation is dependent on

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periodic fire for regeneration (Kruger 1984), as most species produce seeds that lie dormant in the soil or are stored in the canopy until a fire. Many species are killed by fire and rely entirely on seed to re-establish while other species survive fire by resprouting (Van Wilgen & Forsyth 1992). Determining pre-sowing treatments that improve percentage germination can increase the establishment of mature plants, thus improving restoration success.

While germination success of many fynbos species increases greatly following exposure to smoke (Brown 1993), heat pulse is another fire-related cue that is important in stimulating certain species to germinate (Jeffery *et al.* 1988; Van de Venter & Esterhuizen 1988; Cocks & Stock 1997; Keeley & Bond 1997). Heat pulse stimulated seed germination has been studied in the Mediterranean region (Reyes & Trabaud 2009) as well as Californian chaparral (Keeley *et al.* 1985) and Australian Kwongan (Tieu *et al.* 2001), but aside from the afore-mentioned studies, heat pulse stimulation has thus far received limited attention in the fynbos.

The majority of species in fire-prone ecosystems accumulate seeds in the soil, for which the most common mechanism for preventing germination is physical dormancy (Ooi *et al.* 2014). During the inter-fire period, vegetation insulates the seeds from temperature fluctuations, but after fire, exposed soil will experience higher fluctuations in temperature between day and night (Auld & Bradstock 1996). Some fynbos species respond better to widely alternating diurnal temperatures than a single heat pulse (Pierce & Moll 1994). Ooi *et al.* (2014) identified two groups of species with physical dormancy based on seeds stimulated by fire-induced high temperatures or by milder peak summer temperatures.

Increased biomass close to the soil surface following alien tree felling would result in an altered fire regime if a fire were to take place (Levine *et al.* 2003), resulting in increased fire temperature and duration. Fynbos seeds are potentially sensitive to hotter fires (Holmes *et al.* 2000; Blanchard & Holmes 2008), and in such cases increased seed mortality and an already depleted seed bank due to invasion (Holmes 2002) would negatively impact fynbos recovery. In the Cape, few studies have examined maximum temperature tolerance before seeds are killed (Jeffery *et al.* 1988; Cocks & Stock 1997). These studies focused on Fabaceae, where most species respond best to temperatures up to 100°C, or higher for a short duration. Fabaceae typically have a hard seed coat, and species with a soft coat may be less resilient to severe fires. Conversely, since many species are dependent on fire for regeneration it is important to understand their threshold temperatures for seed germination and mortality to determine if burning after alien tree felling is appropriate for existing soil-stored seed.

A restoration initiative at Blaauwberg Nature Reserve, Western Cape, South Africa aims to facilitate recovery of critically endangered Cape Flats Sand Fynbos following clearing of dense stands of invasive woody Australian acacias. The standard clearing practice involves felling of acacias followed by stacking and burning biomass, which does not appear to facilitate successful recovery in lowland fynbos. The restoration study investigated different clearing methods, one of which involved burning of felled acacia biomass to stimulate any fynbos seeds requiring fire to break dormancy and to deplete the acacia seedbank (Blanchard & Holmes 2008). However, the fuel load was denser and in closer proximity to the soil surface than in non-invaded fynbos and therefore would have burnt hotter and longer as a result (Holmes *et al.* 2000). This motivated the need for data on upper thermal tolerance limits for germination of fynbos species. Although slash and burn is specific to clearing of acacias, understanding seed dormancy is important for restoration in all habitats.

Owing to the *Acacia saligna* (Fabaceae) invasion, the fynbos seed bank appears to be depleted at the site (Holmes 2002). To mitigate this impact, seeds were sown *in situ* as an active restoration experiment immediately after a controlled burn. Many species showed poor germination success (Hall, unpubl. data, 2015), which could be due to a lack of dormancy-breaking cues being applied before sowing. Some species only germinated in the second year after sowing, giving invasive plant species a competitive advantage as they can establish quickly after fire. Increasing germination rates and success of native species can increase competitive ability of sown seed, facilitating better resilience to secondary invasions.

This study focused on heat treatment as well as dry after-ripening of seeds, in combination with smoke, as a means of facilitating restoration of critically endangered Cape Flats Sand Fynbos following clearing of invasive alien acacia stands. We determined whether seeds of species within this vegetation type germinate better in response to exposure to selected temperatures and time durations before sowing. We included the invasive species *A. saligna* to provide a comparison in terms of invasive species response to heat pulse. We also investigated the maximum thermal tolerance before exposure becomes lethal to seeds. It is likely that response of seeds to heat treatment is affected by permeability to water (Stone & Juhren 1951) or seed morphology, such as seed coat thickness (Wright 1931) or overall seed size (Bond *et al.* 1999). These variables were therefore analysed to determine whether seed morphology or imbibing ability can predict likely response of seeds to heat treatment. The outcomes of this research will improve restoration protocols in Fynbos and other fire-prone Mediterranean shrubland vegetation where

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active interventions are necessary following alien clearing.

MATERIALS AND METHODS

Study site, species selection, seed collection and seed processing

Seeds were collected from Blaauwberg Nature Reserve, close to Melkbosstrand in South Africa (33.75°S, 18.48°E). Fourteen species were selected to represent a range of vegetation structural components, growth-forms and seed morphologies (Table 1). This was a similar selection to that sown *in situ*. Seeds were collected within Cape Flats Sand Fynbos vegetation between late 2013 and early 2014 while each species was producing seed. All seeds were collected fresh from the field, except alien *A. saligna* for which the seed was collected by sieving leaf litter under trees. Seeds were then stored under conditions of 15% RH and 15°C until germination experiments were conducted in June 2014.

In this study, the term 'seed' refers both to true seeds and diaspores without easily detachable structures. The majority of species produce true seeds, whereas *Anthospermum aethiopicum* (Rubiaceae), *Pelargonium elongatum* (Geraniaceae), *Thamnochortus punctatus* (Restionaceae) and *Watsonia meriana* (Iridaceae) fall into the latter category.

Thamnochortus punctatus, *Serruria fasciflora* (Proteaceae), *A. aethiopicum* and *Passerina corymbosa* (Thymelaeaceae) seed collections were cleaned using a zig-zag seed aspirator (Zig-Zag type 1, Selecta Machinefabriek BV, Enkhuizen, The Netherlands) to eliminate lighter, partially filled or empty seeds. All other seed collections were visually inspected and cleaned manually.

To ascertain seed fill of cleaned seeds as an initial method of estimating potential viability, one subsample of 50 seeds from each species was X-rayed using a Faxitron digital X-ray machine (Qados, Sandhurst, UK). During commissioning the X-ray machine was internally validated and the settings of 22 kV and 0.3 mA for 20 s identified as optimal for the wide range of seed types that are encountered during routine processing at the Millennium Seed Bank. As *Erica mammosa* (Ericaceae) seeds were too small to accurately interpret seed

Table 1. Species used in the study testing the effect of different temperatures and durations of exposure on seed germination

Family	Genus	Species	Species code	Survival strategy	Growth form	Previous research on fire-cues	Date of seed collection	Seed quality (% filled)	No. seeds per replicate
Fabacaeae	*Acacia*	*saligna*	AS	Seeder/ resprouter	Tree	Heat (Jeffery *et al.* 1988)	March 2014	96	26
Rutaceae	*Agathosma*	*imbricata*	AI	Resprouter	Ericoid shrub		December 2013	82	24
Rubiaceae	*Anthospermum*	*aethiopicum*	AA	Seeder	Ericoid shrub		March 2014	42	50
Rutaceae	*Diosma*	*oppositifolia*	DO	Resprouter	Ericoid shrub		December 2013	74	27
Ericaceae	*Erica*	*mammosa*	EM	Resprouter	Ericoid shrub		March 2014	88	28
Aizoaceae	*Lampranthus*	*reptans*	LR	Seeder	Herbaceous perennial		January 2014	70	25
Asteraceae	*Metalasia*	*densa*	MD	Seeder	Ericoid shrub	Smoke (Brown 1993)	July 2013	90	28
Thymelaeaceae	*Passerina*	*corymbosa*	PCO	Seeder	Ericoid shrub	Multiple (Pierce & Moll 1994)	December 2013	60	42
Geraniaceae	*Pelargonium*	*elongatum*	PE	Seeder	Herbaceous annual	Scarification (Kakihara & Hondo 2013)	November 2013	96	26
Rhamnaceae	*Phylica*	*cephalantha*	PCE	Resprouter	Ericoid shrub	Acid scarification (Allsopp & Stock 1995)	December 2013	60	42
Proteaceae	*Serruria*	*fasciflora*	SF	Seeder	Ericoid shrub		December 2013	56	25
Restionaceae	*Thamnochortus*	*punctatus*	TP	Seeder	Graminoid shrub	Smoke (Brown 1993	June 2013	22	75
Rhamnaceae	*Trichocephalus*	*stipularis*	TS	Resprouter	Ericoid shrub		December 2013	84	30
Iridaceae	*Watsonia*	*meriana*	WM	Resprouter	Geophyte		December 2013	96	26

Estimated seed quality was determined by the percentage of filled seed from X-ray. Number of seeds sown was increased to compensate for empty seeds present in samples. Where too few seeds were available to sow an average of 25 viable seeds per replicate of each treatment, the number of seeds per tray was scaled down to the amount available.

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fill from X-ray, 50 seeds were dissected longitudinally to determine whether the embryo was visually healthy, apparent from a seed being plump and white on the inside, as opposed to a shrivelled or empty seed.

For each species, the percentage of full seeds from X-ray results was used to calculate the number of seeds to sow to obtain an estimated 25 full seeds per replicate, with the exception of some species for which this number was lower due to insufficient seed availability. Each treatment consisted of four replicates of between 24 and 75 seeds (Table 1).

Comparison of seed morphology and testing for seed coat dormancy

Six seeds from each species were bisected longitudinally under a Leica M125 microscope (Leica, Heerbrugg, Switzerland) and photographed using a DFC 320 Leica camera and Leica application suite 4.4.0 software to measure seed length and mean seed (or diaspore) coat thickness. As remaining seed quantities were limited, only six seeds were randomly selected, representative of the population.

The permeability of the seed or fruit coat (hereafter for simplicity referred to as 'seed coat') was checked by imbibing two subsamples of between 6 and 20 seeds per species in water and weighing seeds daily until water uptake levelled off (Baskin & Baskin 2003). Prior to imbibition, the seed coat of the first subsample of seeds was pierced with a scalpel while that of the second subsample was left intact (Cook *et al.* 2008). This allowed for seed coat imposed dormancy to be tested within the selected species.

Experimental design for heat treatment study

Treatments were selected to simulate conditions under which seeds would be exposed during a range of potential fire intensities that have been recorded in fynbos (Kruger 1984), and incorporating temperatures and durations that stimulate fynbos species of Fabaceae (Jeffery *et al.* 1988; Cocks & Stock 1997). Two control treatments without heat were included, one with and one without smoke treatment. Heat treatment temperatures included 60°C (10 and 20 min), 100°C (2.5, 5 and 10 min), 150°C (1 and 5 min), 200°C (0.5 and 1 min) and 300°C (0.5 min). Heat treatments were applied independently for each replicate, to avoid pseudoreplication (Morrison & Morris 2000). In addition to heat, seeds were also treated with smoke, as this more closely mimics natural conditions associated with a fire, and most species experience increased germination success, or at least no negative effect, from exposure to smoke (Brown *et al.* 2003). For ease of communication, the presence of a smoke pre-treatment is assumed to be part of all heat treatments hereafter.

Seed samples for each treatment were wrapped in aluminium foil. Heat pulse treatments involved preheating silver sand (Sporting Surface Supplies Ltd., Smallfield, UK) to the required temperature in an oven for at least 2 h and then checking sand temperature using a 250 mm Type K insulated stainless steel probe thermocouple attached to a Grant squirrel logger (Series 1200, Type 1203, Grant Instruments (Cambridge) Ltd, Shepreth, UK). Ovens were operated at a set temperature, with a maximum deviation of ±2°C. The exception was the 300°C treatment, for which sand was instead heated over a Bunsen burner and a temperature probe was used to determine when the sand had reached the correct temperature. In this case there was higher variability in the temperature, but it was kept within a maximum deviation of 20°C. Samples were placed into the sand along with a temperature probe to record temperature for the given duration (Cocks & Stock 1997). After heat-treatment exposure, seed samples were immediately removed from the sand and allowed to cool to ambient temperature, then removed from the foil package and placed into a plastic vial within a chamber of 100% humidity for 24 h to allow seeds to imbibe. Thereafter, seeds were soaked in smoke solution (1:10 dilution of aqueous smoke extract with distilled water) for a further 18 hours, a modification of the method used by Brown (1993). Aqueous smoke extract was obtained using the method of De Lange and Boucher (1990), in which smoke from burnt fynbos plant biomass was bubbled through water to dissolve the active chemicals. For control treatments, samples were wrapped in foil but not placed in an oven before being removed from foil and imbibed. The smoke-treated control samples were placed in smoke solution as was done for heat experiments while the control without smoke samples were placed in the same volume of distilled water.

After soaking, seeds were removed and placed onto Petri dishes containing 1% agar gel, and kept in an incubator (LMS Ltd., Sevenoaks, UK) with lateral illumination by 30 W cool white light. This was set at an alternating temperature of 10/20°C and a 12 h light and dark cycle, as alternating temperature is known to promote germination in fynbos species (Pierce & Moll 1994).

Petri dishes were monitored once every week after sowing. Seeds with a radicle of more than 2 mm in length were removed and recorded as germinated. When Petri dishes contained visible fungal contamination, seeds were removed, cleaned gently with tissue paper, and placed on fresh agar. Any seeds that had deteriorated when changing agar were discarded and recorded as dead.

Germination tests were terminated after 14 weeks if no germination occurred, once all seeds had germinated or if no further germination occurred for 4 weeks following a peak in germination. Remaining seeds were dissected with a scalpel to assess whether they were still fresh (and so assumed viable) or had deteriorated. This could indicate whether seeds did not germinate because of losing viability due to excessive heat exposure, or because dormancy had not been effectively broken. Viability of seeds following different experimental treatments was calculated by adding the number of seeds that germinated to those that were fresh on dissection for each replicate. Percent germination and viability were calculated in relation to total number of seeds sown per replicate.

Experimental design for the dry after-ripening experiment

A dry after-ripening (DAR) experiment exposed seeds to a temperature regime that would be expected in surface soil

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during summer and autumn conditions following removal of vegetation by fire (Auld & Bradstock 1996). A constant temperature of 45°C as well as an alternating temperature regime of 20/40°C was selected, both for four and eight weeks' duration in an incubator. Apart from the temperature and duration of exposure, all other conditions and treatments were the same as those described for the heat-treatment experiment. For ease of communication, the presence of a smoke pre-treatment is assumed to be part of all DAR treatments hereafter.

Data analysis

After plotting raw residuals and confirming that data were normally distributed, a one-way ANOVA was used to determine which treatment and duration worked best for stimulating germination in each species and also how heat treatments impacted seed viability. Independence of observations was satisfied as all replicates were separate heat treatments (Morrison & Morris 2000). Levene's test showed that most species did not have homogenous variances, with the exception of *Diosma oppositifolia* (Rutaceae), *E. mammosa* and *S. fasciflora*. A Fisher LSD *post-hoc* test was used for these three species, but for all other species a Games-Howell *post-hoc* test was more appropriate. Seed morphology data were analysed using an ANOVA to determine whether larger, heavier or thicker-coated seeds responded better to higher temperatures and longer durations of exposure than smaller, lighter and thinner-coated seeds. In all cases variance was not homogeneous, so a Games-Howell *post-hoc* test was used. The seed imbibition data were analysed between the two treatments using a *t*-test for independent variables.

Furthermore, a principal component analysis was performed to determine the relationship between temperature and duration of exposure in terms of the four replicates of germination success in each species.

RESULTS

Potential seed quality

Percentage of filled seeds ranged from 96% to only 22% (Table 1). Only three species had less than 60% of seeds filled.

Comparison of heat treatments

Species showed a range of responses to treatments which could be explained by two main groups (Fig. 1). One cluster of treatments included the four species *Agathosma imbricata* (Rutaceae), *A. aethiopicum*, *Metalasia densa* (Asteraceae) and *W. meriana*, which responded to the lower temperature (60°C), DAR, and control treatments. The second cluster of treatments included the six species

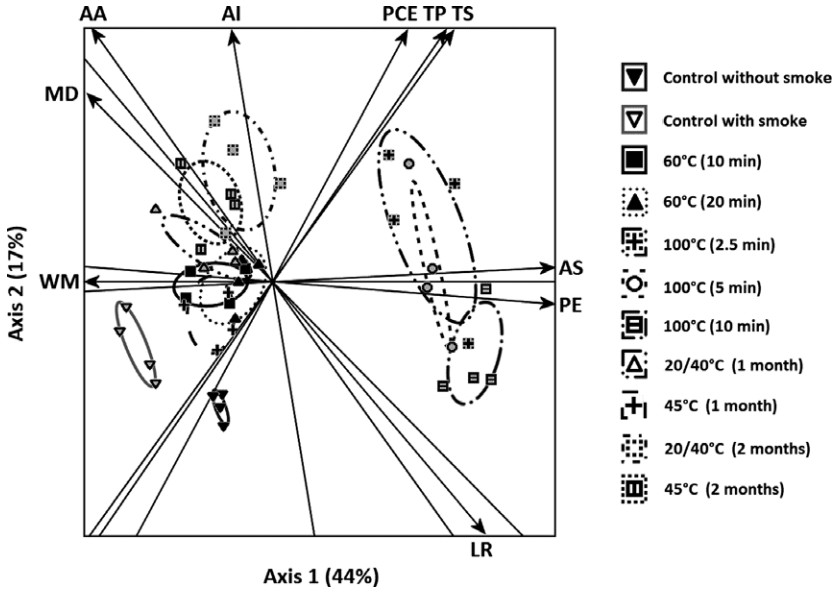

Fig. 1. Species responses to treatments testing the effect of different temperatures and durations of exposure on seed germination, in terms of both heat pulse and long-term dry after-ripening treatment of seeds, as determined by principal component analysis of temperature on axis 1 and duration of exposure on axis 2. Only species with $R^2 > 0.5$ were represented on the graph. *Passerina corymbosa, Diosma oppositifolia* and *Erica mammosa* ($R^2 < 0.5$) were not well explained by either of the principal components, and did not show a strong response to any treatment combinations. AA, *Anthospermum aethiopicum*; AI, *Agathosma imbricata*; AS, *Acacia saligna*; LR, *Lampranthus reptans*; MD, *Metalasia densa*; PCE, *Phylica cephalantha*; PE, *Pelargonium elongatum*; TP, *Thamnochortus punctatus*; TS, *Trichocephalus stipularis*; WM, *Watsonia meriana*.

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Table 2. Mean percentage germination and standard error values for each treatment testing the effect of different temperatures and durations of exposure on seed germination, using a one-way ANOVA

Treatment temperature	Treatment duration	AA	AI	AS	DO	EM	LR	MD
Control*	NA	21 ± 3.9^{bc}	21 ± 4.6^{abc}	1 ± 1.0^{c}	4 ± 1.5^{cb}	16 ± 2.5^{c}	1 ± 1.0	0 ± 0.0^{b}
Control	NA	55 ± 5.9^{ab}	27 ± 3.2^{b}	2 ± 1.1^{c}	11 ± 2.3^{a}	24 ± 4.3^{cb}	1 ± 1.0	15 ± 4.0^{ab}
60°C	10 min	50 ± 4.1^{a}	26 ± 5.3^{abc}	32 ± 2.9^{b}	5 ± 1.8^{cb}	33 ± 7.1^{ab}	0 ± 0.0	14 ± 1.8^{a}
60°C	20 min	50 ± 7.8^{abc}	27 ± 9.4^{abc}	36 ± 8.2^{bc}	2 ± 1.1^{c}	22 ± 8.2^{cb}	1 ± 1.0	19 ± 5.6^{ab}
100°C	2.5 min	34 ± 13.5^{abc}	38 ± 10.6^{abc}	100 ± 0.0^{a}	4 ± 1.5^{cb}	24 ± 5.2^{cb}	4 ± 4.0	0 ± 0.0^{b}
100°C	5 min	17 ± 9.7^{abc}	32 ± 17.5^{abc}	100 ± 0.0^{a}	1 ± 1.1^{c}	16 ± 5.5^{c}	4 ± 2.8	0 ± 0.0^{b}
100°C	10 min	1 ± 0.9^{c}	0 ± 0.0^{c}	100 ± 0.0^{a}	0 ± 0.0^{c}	21 ± 3.6^{cb}	18 ± 6.0	0 ± 0.0^{b}
20/40°C	1 month	47 ± 3.3^{a}	32 ± 3.1^{ab}	10 ± 3.3^{c}	11 ± 3.9^{a}	31 ± 5.4^{ab}	1 ± 1.0	11 ± 3.5^{ab}
45°C	1 month	38 ± 6.1^{abc}	20 ± 6.0^{abc}	3 ± 1.8^{c}	9 ± 1.7^{ab}	26 ± 1.4^{ac}	4 ± 1.7	13 ± 4.3^{ab}
20/40°C	2 months	47 ± 13.4^{abc}	45 ± 4.4^{ab}	13 ± 3.3^{bc}	4 ± 1.6^{cb}	39 ± 3.3^{a}	0 ± 0.0	24 ± 3.4^{a}
45°C	2 months	65 ± 8.7^{abc}	47 ± 2.9^{a}	1 ± 1.0^{c}	5 ± 1.8^{cb}	29 ± 3.3^{ac}	1 ± 1.0	23 ± 5.0^{ab}

Treatment temperature	Treatment duration	PCE	PCO	PE	SF	TP	TS	WM
Control*	NA	2 ± 1.1^{c}	4 ± 1.2	16 ± 4.9^{bc}	16 ± 2.8^{b}	0 ± 0.0^{b}	0 ± 0.0^{c}	97 ± 1.7^{a}
Control	NA	4 ± 1.6^{c}	19 ± 3.8	7 ± 2.0^{c}	28 ± 2.8^{a}	2 ± 1.3^{b}	4 ± 3.1^{bc}	100 ± 0.0^{a}
60°C	10 min	31 ± 2.9^{ab}	8 ± 2.1	23 ± 5.9^{bc}	27 ± 4.3^{a}	3 ± 1.3^{ab}	5 ± 2.7^{bc}	99 ± 0.9^{a}
60°C	20 min	33 ± 2.3^{a}	7 ± 1.7	27 ± 1.9^{b}	NA	3 ± 1.5^{ab}	10 ± 3.0^{bc}	96 ± 1.5^{a}
100°C	2.5 min	36 ± 8.4^{abc}	0 ± 0.0	82 ± 3.4^{a}	NA	15 ± 3.2^{ab}	37 ± 8.1^{abc}	0 ± 0.0^{b}
100°C	5 min	53 ± 6.6^{ab}	1 ± 0.6	62 ± 11.9^{abc}	4 ± 2.8^{c}	8 ± 2.0^{ab}	34 ± 2.1^{a}	0 ± 0.0^{b}
100°C	10 min	44 ± 7.0^{abc}	0 ± 0.0	72 ± 5.1^{a}	NA	12 ± 2.1^{ab}	26 ± 3.4^{a}	0 ± 0.0^{b}
20/40°C	1 month	33 ± 2.5^{a}	4 ± 1.1	3 ± 2.7^{c}	19 ± 3.0^{ab}	6 ± 2.0^{ab}	17 ± 1.5^{b}	97 ± 1.7^{a}
45°C	1 month	18 ± 1.5^{b}	4 ± 2.8	3 ± 2.0^{c}	27 ± 3.8^{a}	5 ± 1.3^{ab}	11 ± 2.1^{bc}	93 ± 2.5^{a}
20/40°C	2 months	46 ± 5.7^{ab}	6 ± 2.9	2 ± 1.1^{c}	NA	13 ± 2.5^{ab}	32 ± 6.8^{abc}	97 ± 1.7^{a}
45°C	2 months	33 ± 3.5^{abc}	9 ± 3.4	5 ± 2.2^{c}	NA	9 ± 0.8^{a}	20 ± 4.8^{abc}	99 ± 0.9^{a}

Statistical comparisons are between treatments and not between species. Values that are significantly different are indicated by different letters. All treatments included a smoke treatment except for the control treatment marked with an asterisk. Treatments not performed on *Serruria fasciflora* denoted by 'NA'. AA, *Anthospermum aethiopicum*; AI, *Agathosma imbricata*; AS, *Acacia saligna*; DO, *Diosma oppositifolia*; EM, *Erica mammosa*; LR, *Lampranthus reptans*; MD, *Metalasia densa*; PCE, *Phylica cephalantha*; PCO, *Passerina corymbosa*; PE, *Pelargonium elongatum*; SF, *Serruria fasciflora*; TP, *Thamnochortus punctatus*; TS, *Trichocephalus stipularis*; WM, *Watsonia meriana*.

that responded to higher temperature treatment (100°C). The species not represented in Figure 1 were not well explained by either of the principal components of temperature or duration of exposure.

Species not requiring germination cues

The control treatment (without smoke or heat) was statistically similar to all other treatments except 100°C for any duration for *W. meriana* and 100°C for 10 min for *A. imbricata*, while it resulted in intermediate germination success for *S. fasciflora*. All other species had lowest germination success in the control (Table 2).

Species responding to smoke without heat

Four species showed greatest germination response to smoke-treatment alone (Table 2). *Passerina corymbosa* appeared to respond best to the smoke without heat treatment, but this was not significantly different from other treatments based on the Games-Howell *post-hoc* test.

Species responding to smoke and heat pulse treatments

No germination was recorded for any species at temperatures of 150°C and above. Eight species showed greater germination success following one or more heat pulse treatments relative to the control (i.e. without heat or smoke); four of which also showed significantly greater germination compared with the smoke control (Table 2).

Phylica cephalantha (Rhamnaceae) had optimal germination under all heat pulse treatments up to 100°C, and also responded to three DAR treatments. *Acacia saligna*, *P. elongatum* and *Trichocephalus stipularis* (Rhamnaceae) performed better at 100°C than

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Table 3. Mean percentage of viable seed (including germinated seed) and standard error values for each treatment testing the effect of different temperatures and durations of exposure on seed viability, using a one-way ANOVA

Treatment temperature	Treatment duration	AA	AI	AS	DO	EM	LR	MD
Control*	NA	21 ± 3.9^{bc}	72 ± 2.6^{a}	88 ± 4.5	4 ± 1.5^{cd}	16 ± 2.5^{c}	1 ± 1.0	12 ± 2.1^{ab}
Control	NA	55 ± 5.9^{ab}	27 ± 3.4^{d}	92 ± 2.7	24 ± 2.5^{a}	24 ± 4.3^{ac}	1 ± 1.0	15 ± 4.0^{ab}
60°C	10 min	50 ± 4.1^{a}	88 ± 7.5^{ab}	94 ± 2.5	5 ± 1.8^{cd}	33 ± 7.1^{ab}	0 ± 0.0	14 ± 1.8^{a}
60°C	20 min	50 ± 7.8^{abc}	72 ± 5.5^{ab}	93 ± 3.3	2 ± 1.1^{d}	23 ± 9.0^{bc}	1 ± 1.0	19 ± 5.6^{ab}
100°C	2.5 min	34 ± 13.5^{abc}	38 ± 10.6^{abde}	100 ± 0.0	4 ± 1.5^{cd}	24 ± 5.2^{ac}	4 ± 4.0	0 ± 0.0^{b}
100°C	5 min	17 ± 9.7^{abc}	32 ± 17.5^{abde}	100 ± 0.0	1 ± 1.1^{d}	16 ± 5.5^{c}	4 ± 2.8	0 ± 0.0^{b}
100°C	10 min	1 ± 0.9^{c}	0 ± 0.0^{e}	100 ± 0.0	0 ± 0.0^{d}	21 ± 3.6^{bc}	18 ± 6.0	0 ± 0.0^{b}
20/40°C	1 month	47 ± 3.3^{a}	32 ± 3.1^{cd}	98 ± 1.1	11 ± 3.9^{b}	31 ± 5.4^{ab}	1 ± 1.0	11 ± 3.5^{ab}
45°C	1 month	38 ± 6.1^{abc}	20 ± 6.0^{cde}	96 ± 2.7	9 ± 1.7^{bc}	26 ± 1.4^{ac}	4 ± 1.7	13 ± 4.3^{ab}
20/40°C	2 months	47 ± 13.4^{abc}	45 ± 4.4^{bd}	97 ± 1.0	4 ± 1.6^{cd}	39 ± 3.3^{a}	17 ± 3.4	24 ± 3.4^{a}
45°C	2 months	65 ± 8.7^{ab}	47 ± 2.9^{bc}	100 ± 0.0	5 ± 1.8^{cd}	29 ± 3.3^{ac}	5 ± 1.0	23 ± 5.0^{ab}

Treatment temperature	Treatment duration	PCE	PCO	PE	SF	TP	TS	WM
Control*	NA	77 ± 3.7^{bc}	24 ± 1.7^{a}	88 ± 1.6^{b}	71 ± 3.5^{a}	0 ± 0.0^{b}	76 ± 3.5^{cd}	97 ± 1.7^{a}
Control	NA	80 ± 2.7^{ac}	56 ± 7.9^{ab}	92 ± 2.7^{ab}	49 ± 10.0^{b}	2 ± 1.3^{b}	91 ± 2.5^{ab}	100 ± 0.0^{a}
60°C	10 min	87 ± 2.1^{ab}	8 ± 2.1^{bc}	81 ± 8.5^{ab}	35 ± 6.8^{bc}	3 ± 1.3^{b}	83 ± 4.0^{bc}	99 ± 0.9^{a}
60°C	20 min	88 ± 1.1^{ab}	7 ± 1.7^{bc}	83 ± 10.9^{ab}	NA	3 ± 1.5^{b}	86 ± 3.0^{bc}	96 ± 1.5^{a}
100°C	2.5 min	36 ± 8.4^{e}	0 ± 0.0^{c}	82 ± 3.4^{ab}	NA	15 ± 3.2^{ab}	58 ± 4.4^{e}	0 ± 0.0^{b}
100°C	5 min	53 ± 6.6^{d}	1 ± 0.6^{c}	62 ± 11.9^{ab}	4 ± 2.8^{d}	8 ± 2.0^{ab}	46 ± 6.0^{f}	0 ± 0.0^{b}
100°C	10 min	44 ± 7.0^{de}	0 ± 0.0^{c}	72 ± 5.1^{ab}	NA	12 ± 2.1^{ab}	26 ± 3.4^{g}	0 ± 0.0^{b}
20/40°C	1 month	84 ± 3.8^{ac}	4 ± 1.1^{bc}	97 ± 2.7^{ab}	19 ± 3.0^{cd}	6 ± 2.0^{ab}	99 ± 0.8^{a}	97 ± 1.7^{a}
45°C	1 month	91 ± 2.5^{a}	4 ± 2.8^{c}	100 ± 0.0^{a}	27 ± 3.8^{c}	5 ± 1.3^{b}	99 ± 0.8^{a}	93 ± 2.5^{a}
20/40°C	2 months	71 ± 4.1^{c}	10 ± 4.3^{abc}	92 ± 4.3^{ab}	NA	22 ± 2.7^{a}	67 ± 4.5^{de}	97 ± 1.7^{a}
45°C	2 months	90 ± 3.8^{ab}	10 ± 4.1^{abc}	88 ± 5.9^{ab}	NA	15 ± 1.2^{a}	80 ± 5.3^{bc}	99 ± 0.9^{a}

Statistical comparisons are between treatments and not between species. Values that are significantly different are indicated by different letters. All treatments included a smoke treatment except for the control treatment marked with an asterisk. Treatments not performed on *Serruria fasciflora* denoted by 'NA'. AA, *Anthospermum aethiopicum*; AI, *Agathosma imbricata*; AS, *Acacia saligna*; DO, *Diosma oppositifolia*; EM, *Erica mammosa*; LR, *Lampranthus reptans*; MD, *Metalasia densa*; PCE, *Phylica cephalantha*; PCO, *Passerina corymbosa*; PE, *Pelargonium elongatum*; SF, *Serruria fasciflora*; TP, *Thamnochortus punctatus*; TS, *Trichocephalus stipularis*; WM, *Watsonia meriana*.

at 60°C, of which only *T. stipularis* germinated equally well at all 100°C and the 2 month DAR treatments. *Lampranthus reptans* (Aizoaceae) appeared to respond best to 100°C for 10 min, but this was not significantly different to the other treatments due to high variability of results. No species were primarily stimulated by exposure to 60°C.

Six species experienced a negative effect on germination at one or more of the 100°C treatments, including three species which responded optimally to smoke without heat treatment. In *D. oppositifolia*, germination was reduced by all heat pulse treatments.

Species responding to smoke and dry afterripening treatments

Erica mammosa experienced optimal germination under alternating temperature after 2 months exposure relative to control and higher temperature treatments, whereas *T. punctatus* experienced optimal germination under constant temperature after 2 months exposure relative to control treatments (Table 2). A further six species showed significantly greater germination for one or more of the DAR treatments compared with the control (without heat or smoke). Of these, *P. cephalantha* also showed significantly greater germination than the smoke control. Only two species showed a significant difference between treatments within the DAR experiment. *Diosma oppositifolia* had greatest germination in both 1 month DAR treatments (i.e. constant 45°C and alternating 20/40°C), whereas in *P. cephalantha* the only difference was within the 1 month treatment, with the alternating DAR temperature resulting in greater germination than constant DAR temperature. Only *D. oppositifolia* performed better in the DAR treatments than in any heat pulse treatments. However, only *A. saligna* and *P. elongatum* responded better to heat pulse treatments than the DAR experiment (both had highest germination at 100°C).

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Estimated viable seed at the end of germination

Species generally exhibited high variability in seed viability in response to different treatments (Table 3). Four species had low overall viability (<25%). Three species either germinated or died, as no fresh seeds remained in any treatment (i.e. viability (Table 3) was equal to germination (Table 2)), with the exception of 1% fresh *E. mammosa* seeds in the 20 min 60°C heat treatment. Of the remaining species, only two did not show a reduction in viability in any treatments compared with control (Table 3). No fresh seed remained in 100°C heat treatments in *P. cephalantha* (Table 2 cf. Table 3), and viability in these treatments was significantly reduced compared with all other treatments (Table 3). Similarly no fresh seeds remained in the 10 min 100°C treatment for *T. stipularis*, and fresh seeds were much reduced in the 2.5 and 5 min 100°C treatments compared with all other treatments in this species (Table 2 cf. Table 3). No fresh seed remained in DAR or 100°C treatments in both *A. imbricata* and *S. fasciflora* (Table 2 cf. Table 3), and almost all of these treatments showed significantly reduced viability (Table 3). *Passerina corymbosa* showed reduced viability in all treatments compared with the controls, except for the 2 month DAR treatments (Table 3), which exhibited greater, albeit not significant, germination than the 100°C treatments (Table 2).

Seed morphology and dormancy

Five species had large seeds, as determined by seed length (Table 4). *Diosma oppositifolia* and *T. stipularis* had very thick seed coats, the latter also having the heaviest seeds. While no species had highest values for all three seed morphology measurements, *A. saligna*, *D. oppositifolia*, *P. cephalantha* and *T. stipularis* were close to the highest values for each category. *Erica mammosa* and *L. reptans* had the smallest seeds, five species had equally thin seed coats, whereas *E. mammosa* and *M. densa* had the lightest seeds. Although no species fell into the lowest of all three measures,

Table 4. Three seed morphology measurements (seed length, seed coat thickness and seed weight) analyzed using a one-way ANOVA, and seed mass increase following imbibition to test for physical dormancy with standard error values for all study species, analyzed using a *t*-test for independent variables

Measurements	AA	AI	AS	DO	EM	LR	MD
Seed length (mm)	3.4 ± 0.07^{c}	2.7 ± 0.05^{d}	5.0 ± 0.08^{b}	5.8 ± 0.07^{a}	1.0 ± 0.06^{f}	1.2 ± 0.05^{f}	1.7 ± 0.08^{e}
Seed coat thickness (μm)	42.3 ± 3.68^{ghf}	68.7 ± 0.71^{e}	142.2 ± 3.64^{b}	191.2 ± 1.30^{a}	39.5 ± 2.32^{g}	27.0 ± 1.34^{h}	33.8 ± 0.60^{gh}
Seed weight (mg)	1.2 ± 0.09^{g}	2.9 ± 0.12^{f}	14.9 ± 0.40^{b}	14.1 ± 0.35^{b}	0.2 ± 0.04^{j}	0.4 ± 0.01^{i}	0.5 ± 0.07^{ijh}
Mass increase 1 (%)	78.3 ± 70.4^{b}	20.2 ± 2.3^{b}	0.4 ± 0.6^{a}	50.5 ± 16.4^{b}	40.9^{b}	11.1^{a}	26.9 ± 2.3^{b}
Mass increase 2 (%)	82.2 ± 21.8^{b}	26.3 ± 5.9^{b}	142.4 ± 7.0^{b}	32.0 ± 8.9^{a}	36.0^{b}	41.9^{b}	28.6 ± 2.6^{b}

Measurements	PCE	PCO	PE	SF	TP	TS	WM
Seed length (mm)	5.3 ± 0.08^{a}	2.8 ± 0.07^{d}	5.2 ± 0.13^{ab}	5.8 ± 0.25^{ab}	2.1 ± 0.05^{e}	4.2 ± 0.19^{bc}	11.0 ± 1.06^{abc}
Seed coat thickness (μm)	148.2 ± 4.09^{b}	103.8 ± 3.13^{cd}	73.3 ± 10.85^{cegh}	113.3 ± 4.15^{c}	44.5 ± 3.85^{ghf}	211.0 ± 7.58^{a}	75.2 ± 6.64^{edf}
Seed weight (mg)	11.7 ± 0.75^{bc}	3.4 ± 0.26^{ef}	4.2 ± 0.11^{e}	6.2 ± 0.29^{d}	0.8 ± 0.04^{gh}	19.6 ± 0.29^{a}	9.0 ± 0.13^{c}
Mass increase 1 (%)	43.2 ± 47.4^{a}	21.5 ± 10.9^{b}	25.7 ± 2.8^{a}	53.2 ± 13.5^{b}	40 ± 2.2^{b}	1.3 ± 1.1^{a}	97.8 ± 11.2^{b}
Mass increase 2 (%)	83.9 ± 57.8^{b}	18.3 ± 23.1^{b}	99.4 ± 0.1^{b}	48.9 ± 8.1^{b}	46 ± 4.6^{b}	109.8 ± 24.1^{b}	126.8 ± 22.3^{b}

Statistical comparisons are between species and not between measurement variables for morphology variables, whereas seed mass increase is compared between intact and pierced seed coat within species. Mass increase 1 refers to maximum percentage increase in seed mass after imbibition alone and mass increase 2 is that after imbibition with seed coat pierced. Species that are significantly different are indicated by different letters. AA, *Anthospermum aethiopicum*; AI, *Agathosma imbricata*; AS, *Acacia saligna*; DO, *Diosma oppositifolia*; EM, *Erica mammosa*; LR, *Lampranthus reptans*; MD, *Metalasia densa*; PCE, *Phylica cephalantha*; PCO, *Passerina corymbosa*; PE, *Pelargonium elongatum*; SF, *Serruria fasciflora*; TP, *Thamnochortus punctatus*; TS, *Trichocephalus stipularis*; WM, *Watsonia meriana*.

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E. mammosa, *L. reptans*, *M. densa* and *T. punctatus* were close to the lowest values in each category. The remaining six species were mostly intermediate in each category.

Five species were found to possess physical dormancy as determined by seeds imbibing significantly more water when pierced than when not (Table 4). These species also benefited from 100°C temperature treatments. Species with dormant seeds also had far greater viable seed than germinated seed in the control treatment, except for *L. reptans*. In *W. meriana* and *A. aethiopicum* germination occurred in pierced and unpierced seed while in *L. reptans*, as with *A. saligna* and *P. elongatum*, germination occurred only in pierced seed after imbibition.

DISCUSSION

The germination behaviour of the species in this study comprised two main categories, as determined by Ooi *et al.* (2014) – those which germinated best following 100°C temperature treatment and those which did not. Within the latter category, species could be further divided into those benefiting from smoke and those either having an intermediate response or else equally poor response to all treatments. However, while patterns were found within these categories, species exhibited a broad range of responses, with no two species having the same response across all treatments.

Species with heat and smoke-stimulated seed germination

Many species in fire-prone ecosystems accumulate seeds in the soil which have impermeable seed coats that are disrupted by the heat of a fire, thus triggering germination (Keeley & Fotheringham 2000). The four most strongly heat-stimulated species in this study all exhibited physical dormancy. *Acacia saligna* and *P. elongatum* seeds did not respond to DAR treatments, showing that they are dependent on a relatively high (100°C) temperature heat pulse for germination. *Acacia saligna* seeds are known to germinate *en masse* after fire (Richardson & Kluge 2008). *Pelargonium elongatum* seeds also germinated in large quantities after the Blaauwberg Nature Reserve burn (pers. obs.; Hall, 2013). The latter is a fire ephemeral species: it germinates, matures and flowers within 7 months after fire (Marais 2012).

Species within the family Rhamnaceae are known to produce seeds in which germination is stimulated by heat in both South Africa and California (Keeley & Bond 1997). Seeds of *P. cephalantha* and *T. stipularis* germinated after a heat pulse, and also responded well following longer duration DAR treatments. As has been found for *Phylica pubescens* (Witt & Giliomee 2004), these species possess an elaiosome (a fleshy structure attached to the seeds that often attracts ants) and so are presumably buried by ants at a depth at which seeds are stimulated to germinate (Cowling *et al.* 1994), rather than being killed by the heat of a passing fire. Since these species resprout and can set seed shortly after a fire, similar to *Phylica spicata* (Marais 2012), exposure of seed in bare soil to increased summer temperatures could also facilitate germination before substantial vegetation regrowth.

Germination in *T. punctatus* seeds was marginally greater following heat and DAR treatments. Seed germination shortly after fire would allow for rapid growth to maturity (Van Wilgen & Forsyth 1992) where seed is present in the soil. Alternatively, seed dispersed by wind (Brown *et al.* 1994) from adjacent unburnt vegetation into recently burnt areas, followed by extended periods of increased soil temperatures, could stimulate germination before the site is colonised by other plants.

Seeds of *E. mammosa* were not negatively affected by exposure to higher temperatures (100°C) relative to the control, but were stimulated by the DAR treatments. This is partly supported by observations of *Erica sessiliflora* seeds, which were not negatively affected by exposure to 96.5°C (Van de Venter & Esterhuizen 1988).

Species with smoke-stimulated seed germination

As expected, seeds of none of the species stimulated by smoke alone possessed physical dormancy. Since *S. fasciflora* and *D. oppositifolia* both possess an elaiosome (pers. obs.; Hall, 2014) and thus appear to exhibit myrmecochory (Cowling *et al.* 1994), seed of these species would likely be buried deep enough to escape a high heat pulse during a fire, yet would still be exposed to smoke residue in the soil as well as alternating temperatures of the exposed post-burn soil, resembling the DAR treatment (Auld & Bradstock 1996). *Anthospermum aethiopicum* and *M. densa* were primarily stimulated by smoke, and therefore would probably rely on patches of less intense fire or areas near the edge of a burn in order for populations to re-establish.

Species requiring neither heat nor smoke for seed germination

Seeds of some Fynbos species are opportunistic and germinate during a long inter-fire interval when some

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plants may become senescent, thereby opening up the shrub canopy (Van Wilgen & Forsyth 1992). Opportunistic species such as *P. corymbosa* can become dominant in such cases (Rebelo *et al.* 2011). Seeds of *W. meriana*, as with geophytes in general, did not require heat or smoke for germination. Instead, this species flowers and seeds during the first few years after fire (Le Maitre & Brown 1992). Smoke may stimulate flowering in this species, rather than seed germination, as has been found for *Watsonia borbonica* (Light *et al.* 2007), and is common in other Mediterranean-climate geophytes (Lamont & Downes 2011). *Agathosma imbricata* is a resprouting species, which can flower shortly after fire and is able to germinate and establish in the absence of fire (pers. obs.; Hall, 2015).

Although the succulent *L. reptans* showed low germination in all treatments, seeds were of reasonably high quality, as 70% were filled. However, this species does possess physical dormancy, as most of the pierced seeds were found to germinate within a few days while unpierced seeds did not. As high and alternating temperatures did not promote germination, but physical disruption of the seed coat did, it is possible that scarification by sand or a slow degradation of the fruit coat will break physical dormancy in this species, resulting in seed germination being spread out over time.

Dormancy and seed bank persistence

Seed morphology has been found to influence potential for seeds to exhibit dormancy (Keeley & Fotheringham 2000). However, physical dormancy was found not only for seeds with a thick seed coat or larger seeds (e.g. *L. reptans* has small seeds with thin coats, whereas *D. oppositifolia* does not possess physical dormancy in spite of a thick seed coat). Apart from these exceptions, the general trend followed that larger or thicker-coated seeds possessed physical dormancy and responded better to heat treatment.

Almost complete germination in the control (without smoke) occurred in *W. meriana*, suggesting that this species is unlikely to form a persistent soil seed bank (Le Maitre & Brown 1992). In addition, a further five species showed limited germination (i.e. >15%), suggesting that sporadic recruitment may occur in these species during favourable conditions for germination. Seeds of species such as *A. saligna* possess physical dormancy which requires a heat pulse to break the seed coat. Seeds of this species should persist in the soil seed bank until the next fire event (Richardson & Kluge 2008), with important implications for management of this invasive species.

Maximum temperature threshold

Cocks and Stock (1997) found optimal germination of fynbos legume species to be between 80 and 100°C, whereas highest mortality occurred at longer durations of 100°C exposure and 120°C. Similar trends were found by Auld and O'Connell (1991), whereas Reyes and Trabaud (2009) determined that exposure to 150°C is lethal to seeds.

In our study a number of species' seeds were negatively affected by exposure to 100°C. These species, however, possess adaptations to survive high temperatures. Resprouting species are not entirely dependent on seed surviving fire (Van Wilgen & Forsyth 1992), whereas non-sprouting species are more vulnerable in terms of regeneration ability. *Passerina* produces large amounts of seed (Pierce & Cowling 1991), which could counteract high seed mortality. *Metalasia* has a high dispersal ability (Pierce & Moll 1994), which combined with high seed production could facilitate recolonization after a hot fire. Seeds of the myrmecochorous species *Serruria*, *Phylica*, *Trichocephalus* and *Diosma* would be buried in the soil by ants (Slingsby & Bond 1983). This would prevent exposure to a more intense heat pulse during a fire, and being larger seeds they could still germinate from greater depths (Bond *et al.* 1999).

Germination success would be negatively affected by increased fuel load due to alien biomass, as this can lead to an increased severity of the heat pulse during a fire (Holmes 1989), resulting in increased temperature to a greater depth and for a longer duration.

The fact that even 150°C for 1 min killed all the seed in our study suggests that even the most heat tolerant seeds close to the soil surface would likely have been destroyed by burning acacia slash (Holmes 1989). *Acacia saligna*, one of the most heat-tolerant species in the study, mostly germinated from deeper in the soil following a prescribed burn at Blaauwberg Nature Reserve in April 2013, with a mean minimum depth of 10.5 mm (Karen Merrett, pers. comm., 2013). Seeds above this depth must have been exposed to temperatures greater than 100°C since this species was killed by higher temperatures tested in our study. This agrees with the finding by Auld (1986) that some seeds of the Australian species *Acacia sauveolens* were killed within 10 mm of the soil surface in a simulated hot fire. Less heat-tolerant species were likely destroyed to greater depths in the soil. Since larger seeds can emerge from greater depths than small seeds, the latter may have been too deep to survive following germination (Bond *et al.* 1999).

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Explanations for lower than expected germination

Even the best performing treatment still gave lower germination success than estimated viability for the majority of species tested. This could be due to seeds not receiving the right pre-treatment or combination of treatments to break dormancy, such as moisture combined with heat (Martin *et al.* 1975), desiccation (Brits *et al.* 1993) or acid scarification (Baker *et al.* 2005). Soil storage may also be important prior to heat and smoke treatment (Newton *et al.* 2006), or a longer period of drying before trying to germinate seeds (Bewley & Black 1994) since germination tests were set up within a year of seed collection and DAR experiments were a maximum of only 2 months in duration.

Alternatively, lower than expected germination success could indicate a bet-hedging strategy – not all seeds germinate in response to a single stimulus (Keeley 1991; Letnic *et al.* 2000). This would increase chances of population persistence, as a dry winter in the first season after fire decreases chances of seedling establishment (Mustart *et al.* 2012). If part of the seed bank is not stimulated by the heat pulse of the fire, but rather the extended period of alternating temperatures of the following summer before a more favourable winter, this would help to spread the risk involved in seedling establishment. For *W. meriana* and *A. saligna*, where all seeds germinated under certain tested treatments (Table 2), corms and vigorous resprouting ability, respectively, mean that these species are less reliant on seeds to persist (Van Wilgen & Forsyth 1992) if seedlings fail to establish in any given year.

Conclusions and implications for practice

Seeds which respond well to heat and smoke treatment or that maintain viability at higher temperatures and longer durations of exposure are not necessarily seeds with a thicker seed coat, or larger in size. Dormancy appears to be linked to seed coat thickness, but not in all cases.

The species for which heat treatment is detrimental (i.e. seeds are killed) would likely lose seeds from their seed bank when a fire goes through the area; non-sprouting species compensate for this loss by producing large numbers of seeds. Resprouting species are less reliant on seeds surviving fire as they can flower and set seed shortly after fire.

Previous fynbos heat studies involved species mostly within the Fabaceae (Jeffery *et al.* 1988; Cocks & Stock 1997). This study showed that species from genera in other families (*Trichocephalus* within Rhamnaceae and *Pelargonium* within Geraniaceae) also respond to heat and smoke treatment, which was not previously proven within the fynbos. This provides further evidence to support heat-stimulated seeds being specific to certain plant families, i.e. Fabaceae, Geraniaceae, Rhamnaceae, across different regions of the world (Keeley & Bond 1997). Other species, whose seeds contribute to the soil seed bank for which a heat pulse with smoke does not stimulate germination, respond to other dormancy-breaking cues associated directly (smoke) or indirectly (high alternating temperatures) with fire.

Vigorous germination of *A. saligna* after a higher heat pulse highlights the importance of considering the effect of fire and invasive species on native plant species. An altered fire regime, due to additional alien biomass, will also affect which species germinate post-fire; thus alien clearing and biomass removal prior to fire could be beneficial to restoration. Acacias are invasive in different regions of the world where they impact on fire regimes and therefore native vegetation (Le Maitre *et al.* 2011). The effect on indigenous seed banks and establishment success of alien species invading natural habitats is likely an underappreciated impact in many parts of the world.

Heat and smoke treatment can be a useful means of increasing germination success, thereby improving restoration effectiveness for relatively little extra effort involved. Heat and/or smoke treatments would need to be optimised for individual species before sowing trials in the field.

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