

The interaction of temperature, water availability and fire cues regulates seed germination in a fire-prone landscape

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Abstract Ambient temperature and water availability regulate seasonal timing of germination. In fire-prone landscapes, the role of fire-related cues in affecting the range of temperatures and water potentials (ψ s) across which germination can occur is poorly known, especially in non-Mediterranean landscapes. We examined interactive effects of temperature (15 or 25°C), ψ (0 to -0.9 MPa), and fire-related cues (heat and smoke) on germination for seeds of three shrub species from fire-prone southeastern Australia. Incubation temperature affected germination of untreated seeds of *Kunzea ambigua* and *Kunzea capitata* (Myrtaceae) (reduction at 25°C), but germination was uniformly low in *Epacris obtusifolia* (Ericaceae). Decreasing ψ reduced germination across both incubation temperatures. Fire cues increased germination at both incubation temperatures and across ψ s, although in *Kunzea*

the increase was smaller and occurred over a narrower range of ψ s at 25°C. Hydrotime analysis suggested that fire cues reduced the amount of water necessary for germination of *Kunzea* seeds. Post-fire germination of the three study species may occur during the warm season, although it is reduced and confined to wet periods for the two *Kunzea* species. Warm season germination of the study species is consistent with a trade-off between the increased risk of failure of a cohort of seedlings, and benefits of early establishment of a cohort that may survive in an environment with aseasonal rainfall.

Keywords Heat · Smoke · Hydrotime · Water potential · Soil seed bank

Introduction

Many species regenerate from persistent soil-stored seeds in landscapes with frequently recurring but unpredictable stand-replacing fires. Germination of soil-stored seeds may be restricted to a short time following a fire, allowing seedlings to capture the increased available resources such as light, space and nutrients (Bond and van Wilgen 1996). In addition, inter-fire germination is likely to produce poor recruitment due to competitive effects of established plants and predation (Specht et al. 1958; Tozer and Bradstock 1997). Mechanisms for limiting germination to the immediate post-fire environment include fire-related germination cues such as heat and smoke (Keeley et al. 1985; Thomas et al. 2003).

Post-fire germination may be restricted to seasons that are likely to result in adequate seedling survival (Vleeshouwers et al. 1995; Hilhorst 1998). Such germination in regions with Mediterranean climates is cued to provide maximum time

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for seedling establishment before summer drought, but also to avoid early rain that is light and sporadic, and freezing temperatures in regions where they occur (Bell et al. 1993; Pierce and Moll 1994; Skordilis and Thanos 1995). Thus, post-fire germination of gap-dependent species in Californian chaparral is delayed until the rainy seasons of late winter or early spring (Went et al. 1952; Keeley 1991). Post-fire germination is much greater following summer or autumn fire or smoke application than following winter or spring treatments in southern Australia (McMahon 1984; Roche et al. 1998), South Africa (Midgley 1989; de Lange and Boucher 1993) and in the Mediterranean (Trabaud 1974).

Germination may be restricted to seasons that favour seedling survival if fire-related germination cues are only effective in promoting germination when ambient temperature corresponds to a favourable season. Ambient temperature may restrict germination directly, through persistent soil-stored seeds that do not have dormancy (Thompson et al. 2003), or via a seasonal cycling of physiological dormancy (Baskin and Baskin 1998; Finch-Savage and Leubner-Metzger 2006; Ooi et al. 2004). In fire-prone habitats, there is evidence that cycling of physiological dormancy results in deeper dormancy during winter and no dormancy during summer. If fire cues are applied at the time when dormancy is not present, germination pulses can occur (Baker et al. 2005; Merritt et al. 2007). The restriction of post-fire germination and subsequent early plant establishment to cool wet seasons has adaptive value (sensu Antonovics 1976) in regions with a Mediterranean-type climate.

The frequency and strength of temperature-controlled germination in fire-prone regions with aseasonal rainfall is currently less well known. There is no drought season in Florida, but most germination occurs in spring, prior to the summer peak in rainfall. Because natural fires typically occur in spring and early summer, there is a post-fire delay of 9–10 months before germination (Carrington and Keeley 1999); whether this delay is controlled by temperature is currently unknown. In contrast, in the Sydney region where rainfall is also aseasonal many species germinate after fire during any season having adequate soil moisture (Auld and Tozer 1995; Whelan and York 1998; Ooi et al. 2004). Some persistent soil-stored seeds remain non-dormant, whilst other seeds have seasonal dormancy (Auld et al. 2000), and seasonally controlled post-fire germination (Ooi et al. 2004, 2006).

Seasonal temperature can affect the amount of water required for germination (Mayer and Poljakoff-Mayber 1989; Baskin and Baskin 1998), or water availability can directly affect post-fire germination. For most species that recruit after fire, the moisture availability required for germination, and its interaction with ambient temperature

or fire-related germination cues are currently unknown. Factors that promote germination also increase the range of water potentials (ψ s) at which germination can occur (Bradford 1995, 2002). Hydrotime (θ_H) modelling examines this effect. Gummerson (1986) showed that for seeds that germinate at reduced ψ , accumulated ψ units above a base or threshold ψ (ψ_b ; at which radicle emergence was inhibited), multiplied by duration of imbibition at that ψ was a constant (θ_H ; units MPa-days). Chronological time to germination shortened as ψ exceeded ψ_b of seeds; the total θ_H to radicle emergence was constant for all seeds in the population. However, individual seeds varied in ψ_b at which radicle emergence was prevented; thus the difference $\Delta\psi$ ($=\psi - \psi_b$) varied amongst seeds, giving a spread of germination times in the population; time to germination was inversely proportional to the amount by which ψ_b was exceeded (Bradford 2002).

Factors that increase germination either shift ψ_b to lower (more negative) values, or decrease the θ_H required for germination (Bradford 2002). A shift of ψ_b to more negative values means that seeds will germinate across a wider range of ψ , and will do so more rapidly (increase in $\Delta\psi$) (Bradford 2002).

We examined combined effects of fire-related germination cues, water availability and incubation temperature on germination for three common shrub species that regenerate from soil-stored seeds in heath and woodland within the fire-prone Sydney region. The two temperatures used (15 and 25°C) are reliable indicators of warm and cool seasons for this region (the average monthly maximum temperatures for Sydney are 26 and 16°C in summer and winter, respectively). The range of ψ s used simulated low to high water availability, and represent individual wetter or drier seasons, that are not predictable in this region (Bradstock and Bedward 1992). The experiments tested: (1) whether fire cues would alleviate adverse effects of reduced ψ on germination; (2) whether incubation temperature would affect germination, and how it would interact with fire cues and ψ ; (3) how fire-related germination cues and an environmental factor (post-fire temperature) affected the physiological status (θ_H parameters) of seeds.

Materials and methods

Seed collection, storage and characteristics

Seeds of *Kunzea ambigua* (Sm.) Druce (an obligate reseeder), *Kunzea capitata* (Sm.) Heynh. (Myrtaceae) (a facultative resprouter), and *Epacris obtusifolia* Smith (Ericaceae) (a reseeder with some resprouting capacity), were collected between March and July 2000. Seeds were

collected from Ku-ring-gai Chase National Park, north of Sydney (*K. ambigua* 33°40'S, 151°14'E; *E. obtusifolia* 33°39'S, 151°15'E), or from Blue Mountains National Park approximately 100 km away, to the west of Sydney (*K. capitata* 33°44'S, 150°23'E). Seeds or seed capsules were collected into cotton bags during seed release from single populations consisting of tens of plants for each species. Seeds were stored in these bags at approximately 20°C for a number of weeks until the cut-test was used to determine the external appearance of seeds with firm white endosperm; these seeds were selected. The seed lot was surface sterilized with 1% sodium hypochlorite for 10 min, rinsed with deionised water, and thence handled using sterile procedures. Air-dry seeds were then sealed in glass jars in darkness at approximately 20°C and 70% relative humidity for between 14 and 17 months until experiments were performed. No change in seed dormancy was observed under these storage conditions as indicated by germination trials (P. B. Thomas, unpublished data).

Seeds were small (air-dry mass: *K. ambigua* 39 µg, *K. capitata* 51 µg, *E. obtusifolia* 22 µg), and imbibed rapidly when in contact with water. The mean increase in air-dry weight 24 h after immersion was 29.7% for *K. ambigua*, 23.3% for *K. capitata*, and 20.3% for *E. obtusifolia*.

Experimental design and treatments

Fire cues (heat present or absent; smoke present or absent) were combined factorially with ψ (0, -0.3, -0.6 and -0.9 MPa) and temperature of incubation (15 or 25°C) to test how the combination of these factors would affect germination. Heat treatments were unheated controls, or 75°C heat for 5 min; smoke treatments were 0- or 10-min smoke duration. Temperatures of 75°C for 5 min duration have been recorded in the upper soil during the passage of medium-intensity fire (Bradstock and Auld 1995), and either smoke or fire can produce comparable levels of germination from soil-stored seeds (Roche et al. 1998).

To apply heat, seeds were heated in a fan-forced oven, on open glass petri dishes. Each replicate was treated separately with heat (for independence of applications of the treatment; Morrison and Morris 2000). Seeds were smoked on open glass petri dishes in a glass chamber, one replicate at a time. Smoke was produced in a bee keeper's burner by burning dry fine fuel litter from a eucalypt forest. The fuel was changed between each smoke application (Morrison and Morris 2000). An electric air-pump forced the smoke out of the bee keeper's burner, through a condensing tube, which both cooled and dried the smoke, and into the smoking chamber. The smoking chamber would rapidly fill with dense smoke, which circulated and exited through an outlet hole. For seeds that received both treatments, heat was applied before smoke.

After receiving fire-cue treatments, seeds were placed on boats of single layer Whatman no. 1 filter paper. The boats were placed on another single layer sheet of Whatman no. 1 filter paper, which was on a grid suspended above a well within an airtight container of 3.9 l capacity. The 350 ml of water or polyethylene glycol (PEG) solution within the well produced the appropriate degree of wetness on the paper surface on which the seeds were incubated. The required ψ s were produced with 6000 MW PEG and deionised water, using the formulae of Michel (1983). The large volume of PEG solution resulted in negligible change in ψ due to seed hydration, and the solution to paper ratio exceeded 30:1, so any change due to PEG exclusion by the paper was also negligible (Hardegree and Emmerich 1990). The air-tight seal of the containers prevented evaporative loss of water, whilst the large volume of air within the containers and the recurrent opening of the containers would have ensured that atmospheric composition did not appreciably change (Berkat and Briske 1982).

Experimental procedure

The maximum number of available seeds was used for each species to provide the most accurate estimate of the germination time course. Six replicates of 50 *K. ambigua* seeds, 20 *K. capitata* seeds, and 50 *E. obtusifolia* seeds were divided equally between two replicate containers for each of the four water potential treatments. Single containers were placed in replicate temperature-controlled cabinets at 15 or 25°C.

Containers were double wrapped in aluminium foil, and germination was monitored under safe light every 3 days whilst the rate of germination was high (the maximum rate of germination was almost 10% per day), then with decreasing frequency as the rate of germination declined. To minimize evaporative loss of water, the airtight containers were only opened within partially enclosed surrounds that were humidified by bubbling air through an open body of water (Berkat and Briske 1982). At the end of the experiment, to test whether ungerminated seeds were capable of germinating, *Kunzea* seeds were sequentially transferred into free water, then into the more favourable incubation temperature, then into light. *E. obtusifolia* seeds were transferred into free water and then treated with any fire cue(s) that they did not initially receive.

Data analysis

Effects of treatments on total germination

Data used for analysis were means of the proportion of seeds germinated (arcsine square root transformed) in water potential containers per treatment within the two

temperature-controlled cabinets. A split-plot ANOVA with temperature as the plot factor, and ψ and fire cues as sub-plot factors, was used to examine treatment effects; the analyses outlined below were used to partition the variance amongst treatments terms, rather than the usual main effects and interactions model.

A replicated regression analysis was applied to germination proportion versus ψ data in each germination treatment (Cottingham et al. 2005). Linear or quadratic models were fitted if either differences amongst means, or one of the models, was significant. The Akaike information criterion (AIC) was calculated for each model using the residual least squares for each fit, and Akaike weights derived (Anderson et al. 2000). The weights are a measure of the evidence that the given model is the best fit, and the model with the greatest Akaike weight was selected. In one case, the AIC weight for the quadratic fit was only 1.24 times that of the linear fit; the linear model was retained in this case. If the differences amongst ψ means, and fits of both models were not significant, the null model was accepted. These comparisons used 24 *df*, equal to the *df* for ψ and all its interactions in the full ANOVA model.

The remaining terms from the ANOVA were used to compare the effects of temperature (main effect and interactions with fire cues), and the fire cues (heat, smoke main effects, interaction), using 7 *df*. All tests combined equalled the sums of squares and *df* for comparisons amongst 31 means.

θ_H analysis

The θ_H model describes progress toward seed germination of different fractions of a population of seeds across a range of incubation ψ s. θ_H can be defined as

$$\theta_H = (\psi - \psi_b(g))t_g \quad (1)$$

where ψ is the ambient water potential, $\psi_b(g)$ is the ψ_b that will just prevent germination of percentage g , and t_g is the time required for the percentage g to germinate (the inverse of the rate of germination). The ψ_b of individual seeds varies as a normal distribution in the population of seeds (Gummerson 1986; Bradford 2002); as θ_H is a constant for the population, the difference $\Delta\psi$ ($=\psi - \psi_b$) between the ambient ψ and the $\psi_b(g)$ value of a particular percentage g is inversely proportional to the time required for that same percentage to germinate (t_g).

Application of the analysis requires adequate germination over a range of ψ s, with close to 100% germination at 0 MPa, and >50% in at least one other level of ψ . Analysis was only carried out if the germination requirement stated above was met in all four treatments. This occurred for *K. ambigua* at both incubation temperatures and *K. capitata* at 15°C only. Data on germination over time

for each species and treatment were analysed by the repeated probit technique of Ellis et al. (1986) to calculate values for θ_H , the median ψ_b [$\psi_b(50)$] and SD of ψ_b ($=\sigma_{\psi_b}$) (Bradford 1995, 2002). Cumulative germination (g) was probit-transformed and regressed as a function of $\psi - (\theta_H/t_g)$ using the model

$$\text{probit} = [\psi - (\theta_H/t_g) - \psi_b(50)]/\sigma_{\psi_b}. \quad (2)$$

Different values of θ_H were trialed iteratively until the best fit was obtained, using a program provided by K. J. Bradford. Once the θ_H which gave the best fit to the data was determined, $\psi_b(50)$ was taken as the $\psi_b(g)$ for $\text{probit}(g) = 0$, and σ_{ψ_b} as the inverse of the slope. Application of this method would ideally use data independently sampled in time; however, for practical purposes, sampling the time course of germination independently with separate seed lots or cumulatively with a single lot gives identical results (Campbell and Sorensen 1979), and the latter method was used.

Results

Effects on germination

K. ambigua

At 15°C incubation temperature, 91% of untreated *K. ambigua* seeds germinated in the zero ψ treatment (Fig. 1a; backtransformed data predicted from models are reported in the text; backtransformed means are given otherwise; means of raw data are given in full in Electronic Supplementary Material S1). Treatment with fire cues only slightly increased this (Fig. 1a). Decreasing ψ significantly reduced total germination of untreated seeds to 47% at -0.3 MPa, down to 3% at -0.9 MPa (Table 1; Fig. 1a).

The fire cues ameliorated this reduction in germination with decreasing ψ . Heat-treated seeds at 15°C maintained a high level of germination at -0.3 MPa, with a decline at lower ψ s (Table 1; Fig. 1a). Seeds treated with smoke alone maintained germination >90% down to -0.6 MPa, and still had total germination of 65% at -0.9 MPa (Table 1; Fig. 1a). Seeds treated with both heat and smoke showed the most striking amelioration of the adverse effects of declining ψ . Differences amongst ψ means were not significant for this treatment ($F_{3,24} = 0.58$, $P > 0.10$; full ANOVA in S2; Fig. 1a).

At an incubation temperature of 25°C, there was some reduction in germination in some treatments, but the overall temperature effect was not significant ($F_{1,2} = 10.04$, $P = 0.087$). Total germination of untreated seeds at 0 MPa declined to 76%. Fire cues increased germination of seeds at 0 MPa to >94% (Fig. 1b). Total

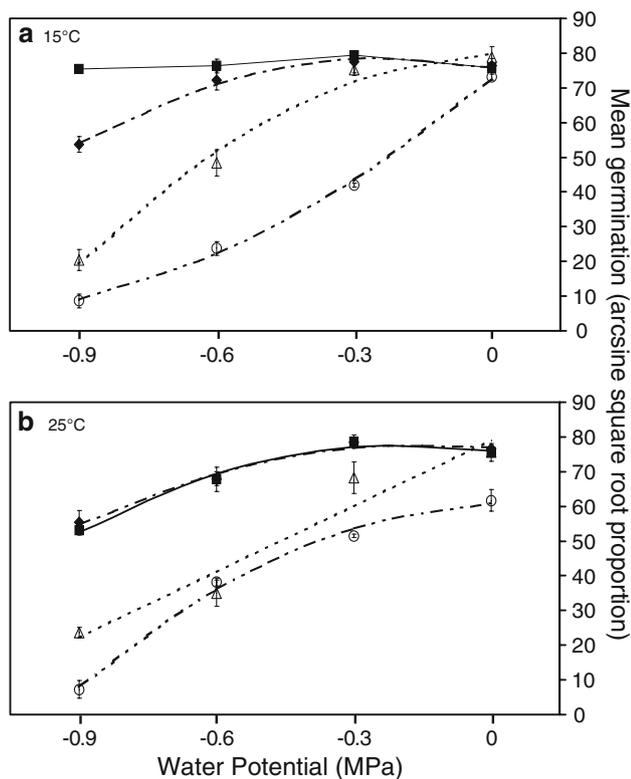


Fig. 1 Mean total germination (arcsine transformation \pm SE) of *Kunzea ambigua* for control (open circle), heat (open triangle), smoke (filled diamond), and heat plus smoke (filled square) treatments and incubated at **a** 15°C or **b** 25°C. Where no model was fitted (heat plus smoke at 15°C), a thin line joins the data points. Equations of fitted lines are given in Table 1

germination of untreated seeds declined with decreasing ψ , to only 2% by -0.9 MPa (Table 1; Fig. 1b).

At 25°C incubation, fire cues lessened the adverse effect of reduced ψ on total germination, but over narrower ranges of water availability than at 15°C. Heat-treated seeds at the 25°C incubation temperature maintained germination of 75% at -0.3 MPa, but germination declined below 50% at lower ψ s. Whilst the line for heated seeds lay above that for the control seeds (as at 15°C), the separation from controls was less at 25°C (Fig. 1b). The reduced effectiveness of heat in ameliorating the effects of ψ at 25°C was detected as a significant temperature \times heat interaction ($F_{1,2} = 525.4$, $P = 0.002$).

Seeds treated with smoke alone maintained a high level of germination at -0.3 MPa, but germination fell at lower ψ s (Table 1; Fig. 1b). The pattern of germination was very similar for seeds treated with heat and smoke (Table 1; Fig. 1b); the flat germination response to decreasing ψ that was present at 15°C was absent at 25°C.

The fire cues were significant in interaction ($F_{1,2} = 273.3$, $P = 0.004$); heat effects observed for unsmoked seeds were generally overridden if seeds were smoked (Fig. 1).

K. capitata

At 15°C incubation temperature, germination of untreated seeds kept at 0 MPa was 62%. Of the fire cues, smoke increased germination to 90% or more, whilst heat had little effect at this ψ (Fig. 2a). Total germination of untreated seeds declined significantly with a reduction in ψ , to 5% at -0.9 MPa (Table 1; Fig. 2a).

Fire cues lessened the adverse effects of reduced ψ , similar to results for *K. ambigua*. The heat treatment increased germination of seeds compared to controls at -0.3 MPa, but germination converged at lower ψ s for these treatments (Table 1; Fig. 2a). Smoked seeds, or heated and smoked seeds at 15°C, maintained germination over a wider range of ψ s (>81% over -0.3 to -0.6 MPa). Germination declined to 52–55% at -0.9 MPa (Table 1; Fig. 2a).

When incubation temperature was increased to 25°C, overall germination declined (temperature main effect; $F_{1,2} = 48.58$, $P = 0.019$; full ANOVA in S3). Total germination of untreated seeds at 0 MPa fell to 30% (Fig. 2b). Fire cues increased germination of seeds at 0 MPa, heat to 65%, and smoke or heat and smoke treatments to 90–97%. Total germination of untreated seeds declined with decreasing ψ , falling to 0% at -0.9 MPa (Table 1; Fig. 2b).

As with *K. ambigua*, fire cues lessened the adverse effect of decreasing ψ over a narrower range of water availability at 25°C than at 15°C. The ameliorating effect of heat was restricted to 0 MPa at 25°C (Table 1; Fig. 2b). At lower ψ s, germination of heat-treated seeds was close to that of controls (Fig. 2b). For seeds that were either smoked, or heated and smoked, a high level of germination was maintained only down to -0.3 MPa, and total germination declined to 16–19% at -0.9 MPa (Table 1; Fig. 2b).

Of the fire cues, smoke was significant as a main effect ($F_{1,2} = 845.75$, $P = 0.001$).

E. obtusifolia

A very low proportion of untreated *E. obtusifolia* seeds germinated in the 0 MPa treatment at 15°C (mean = 2%; Fig. 3a). Whilst heat alone did not increase germination, smoke alone increased germination to 25%, and smoke combined with heat to give greatest germination (69%). Germination of seeds at lower ψ s declined further from the low levels observed at 0 MPa in both the control, and heat alone treatments (Table 1; Fig. 3a).

Smoke alone, or in combination with heat stimulated germination at all ψ s (Fig. 3a). Germination of smoke-treated seeds was maintained above 22% down to -0.6 MPa, and then declined markedly. Greatest germination occurred in the heat and smoke treatment (Fig. 3a), with germination declining to 31% at -0.9 MPa (Table 1).

Table 1 Parameters of polynomial equations $y = a + bx$ (linear) or $y = a + bx + cx^2$ (quadratic) fitted to germination versus water potential (ψ ; arcsine square root) means, and r^2 of fitted model. SE shown in parentheses

Species	Incubation temperature (°C)	Treatment	a	b	c	r^2	
<i>Kunzea ambigua</i>	15	Control	72.6 (2.1)	110.5 (11.2)	44.4 (11.9)	0.998	
		Heat	80.0 (4.7)	6.8 (25.3)	-54.5 (8.2)	0.989	
		Smoke	76.0 (1.4)	-24.7 (7.8)	-54.5 (8.2)	0.994	
	25	Control	61.0 (3.2)	7.0 (17.0)	-57.7 (18.2)	0.994	
		Heat	78.9 (6.4)	63.0 (11.4)		0.938	
		Smoke	77.2 (2.3)	-10.8 (12.5)	-39.7 (13.3)	0.983	
		Heat + smoke	76.1 (2.1)	-18.7 (11.6)	-49.7 (11.8)	0.988	
	<i>Kunzea capitata</i>	15	Control	51.9 (6.4)	43.6 (11.4)		0.880
			Heat	54.3 (3.7)	14.9 (19.9)	-38.1 (21.2)	0.987
Smoke			72.5 (6.9)	-32.2 (37.0)	-68.1 (39.4)	0.914	
25		Heat + smoke	74.1 (2.5)	-8.5 (15.0)	-41.8 (16.0)	0.981	
		Control	33.5 (2.6)	-5.0 (14.1)	-47.7 (15.0)	0.990	
		Heat	53.5 (0.8)	87.2 (4.3)	41.5 (4.6)	0.999	
<i>Epacris obtusifolia</i>		15	Smoke	82.1 (4.5)	59.7 (8.1)		0.965
			Heat + smoke	72.0 (1.8)	-3.0 (9.8)	-62.7 (10.5)	0.997
			Heat	9.3 (0.4)	7.8 (0.6)		0.987
	25	Smoke	30.1 (1.6)	-33.8 (8.7)	-61.1 (9.3)	0.991	
		Heat + smoke	56.4 (1.1)	3.2 (6.0)	-24.4 (6.4)	0.996	
		Heat	18.9 (4.0)	56.9 (21.2)	50.0 (22.6)	0.898	
		Smoke	44.2 (2.6)	31.8 (4.6)		0.959	
		Heat + smoke	52.8 (1.9)	18.8 (3.4)		0.937	

The synergistic effect of heat and smoke on germination was detected as a significant interaction ($F_{1,2} = 27.12$, $P < 0.035$; full ANOVA in S4).

Temperature effects overall were not significant ($F_{1,2} = 4.69$, $P = 0.162$). Germination of untreated seeds increased slightly in the 0 MPa treatment compared to 15°C (mean at 25°C = 8%); germination of heated seeds increased to 10%. Smoke increased germination to 49%; seeds that were both heated and smoked showed the greatest germination. A decline in ψ reduced germination in the control and the heat alone treatments, and germination differed little between the two treatments (Fig. 3b).

As at 15°C, smoke stimulated germination over the lower ψ s at 25°C (Table 1; Fig. 3b). Greatest germination was again observed in the heat and smoke treatment, with mean germination ranging from 63% at 0 MPa down to 34% at -0.9 MPa (Table 1).

Germinability remained high throughout the experiment; for example, final germination of seeds originally within the lowest ψ , averaged across treatments was 91% for *K. ambigua*, 74% for *K. capitata* and 59% for *E. obtusifolia*.

θ_H parameters

Transformed time-course data gave a reasonable fit to the θ_H model (r^2 -values ranged from 0.78 to 0.90, Table 2).

Smoke reduced the $\psi_b(50)$ of *K. ambigua* and *K. capitata*. The estimated $\psi_b(50)$ for the controls was -0.39 to -0.57 MPa across both species, and more negative within the smoke alone (-1.06 to -1.16 MPa), or heat and smoke treatments (-1.13 to -1.65 MPa, Table 2). The heat treatment also slightly reduced the estimated $\psi_b(50)$ (-0.49 to -0.74 MPa), but smoke had the greater effect on $\psi_b(50)$.

The estimates of θ_H were consistently lowest for the heat alone treatment and greatest for treatments including smoke (Table 2). Effects of incubation temperature on θ_H parameters could only be compared for *K. ambigua*; in going from the 15 to 25°C incubation temperature, there were small increases in θ_H for the control, heat, and smoke treatments, and an increase in $\psi_b(50)$ for smoke and heat (Table 2).

Discussion

Temperature, ψ and fire cues

The negative effect of reduced ψ on final germination is well known (Bradford 1995); fire cues, especially when combined, clearly lessened this effect for all study species at both temperatures. The combination of cues provides the most reliable indicator that fire has passed directly over the

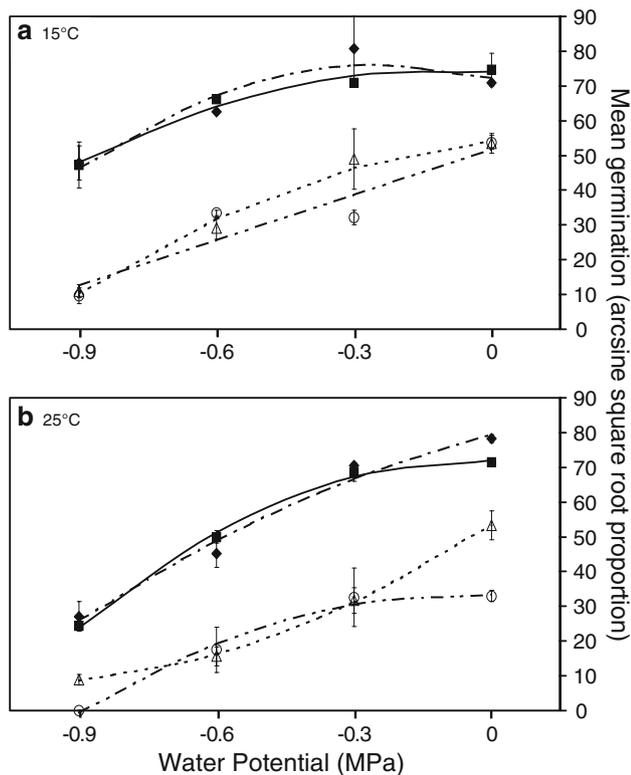


Fig. 2 Mean total germination (arcsine transformation \pm SE) of *Kunzea capitata* for control (open circle), heat (open triangle), smoke (filled diamond), and heat plus smoke (filled square) treatments and incubated at **a** 15°C or **b** 25°C. Equations of fitted lines are given in Table 1

seeds. Also, smoke alone consistently produced more germination than heat alone, and smoke is probably a more reliable indicator of the passage of fire than heat (Auld and Bradstock 1996). Increase in germination of *E. obtusifolia* in the current study due to a synergistic combination of smoke and heat has been previously recorded for *Epacris tasmanica* from southeastern Australia (Gilmour et al. 2000) and for *Kunzea ambigua* (Thomas et al. 2003).

Temperature, either directly or indirectly through its effect on relative dormancy (Bewley and Black 1994), was of primary importance in determining germination of *Kunzea* species. At the higher incubation temperature (25°C), germination of untreated seeds in free water was reduced, and there was a proportional decrease in the positive effect of fire cues on germination at lower ψ s.

The relationship between temperature and enhanced germination triggered by fire-related cues was different for *E. obtusifolia*. Relative to the *Kunzea* species, incubation temperature had little influence over the effect of the fire-related cues. Also, the trend towards increased germination at 25°C was the opposite of that found for *Kunzea*.

A limitation of the current study is that seeds had to be regularly exposed to safe light to assess cumulative

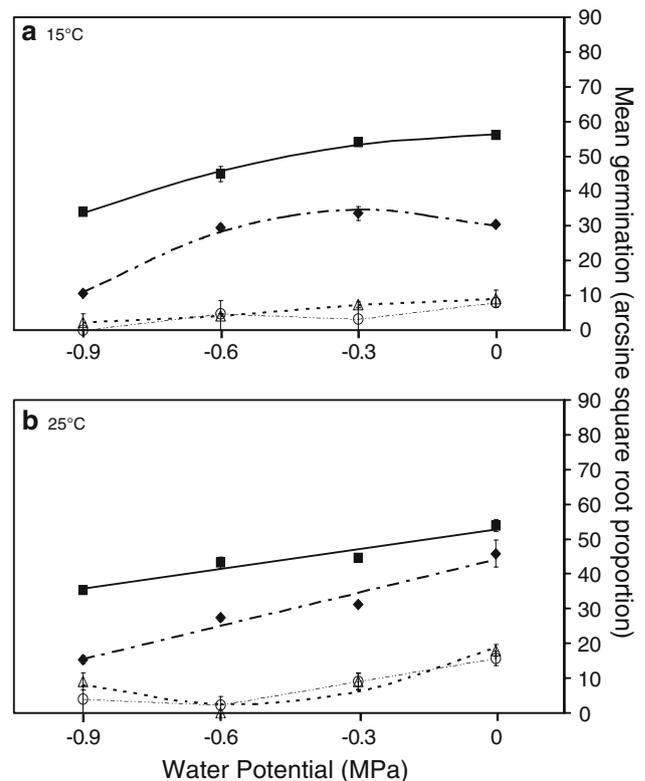


Fig. 3 Mean total germination (arcsine transformation \pm SE) of *Epacris obtusifolia* for control (open circle), heat (open triangle), smoke (filled diamond), and heat plus smoke (filled square) treatments and incubated at **a** 15°C or **b** 25°C. Where no model was fitted (control at 15 or 25°C), a thin line joins the data points. Equations of fitted lines are given in Table 1

germination over time. Long-term exposure to white light increased germination to uniformly high levels for *Kunzea* seeds that did not germinate during the current experiment. However, untreated seeds of the study species germinated before their first exposure to safe light, demonstrating that light is not required for germination. If brief exposures to safe light did increase germination of study species, then effects of incubation temperature and fire-related cues have probably been underestimated.

Fire cues and θ_H parameters

It is possible that changes observed in θ_H parameters of *Kunzea* seeds after treatment with fire-related cues were mediated through hormones such as gibberellic acid or abscisic acid. Smoke can alter levels (Schwachtje and Baldwin 2004) or sensitivity of seeds to these hormones (van Staden et al. 2000; Schwachtje and Baldwin 2004), and the hormones cause changes to θ_H parameters (Ni and Bradford 1993; Alvarado and Bradford 2005). However, large differences in germination across different incubation temperatures may not be accompanied by any change in θ_H

Table 2 Hydrotime (θ_H), median base water potentials (ψ_b ; MPa) (SD in parentheses), and r^2 -values for estimates of θ_H parameters

Species	Temperature (°C)		Treatment			
			Control	Heat	Smoke	Heat and smoke
<i>K. ambigua</i>	15	θ_H	12	9	16	22
		ψ_b	-0.46 (0.261)	-0.74 (0.248)	-1.13 (0.273)	-1.65 (0.426)
		r^2	0.90	0.89	0.90	0.78
	25	θ_H	16	11	20	19
		ψ_b	-0.57 (0.157)	-0.69 (0.183)	-1.16 (0.226)	-1.22 (0.305)
		r^2	0.88	0.80	0.82	0.80
<i>K. capitata</i>	15	θ_H	15	12	18	16.5
		ψ_b	-0.39 (0.439)	-0.49 (0.365)	-1.06 (0.385)	-1.13 (0.421)
		r^2	0.81	0.89	0.87	0.84

parameters (Huarte and Benech-Arnold 2005). Effects of temperature on germination of *K. ambigua* seeds were not mediated through a change in the level of water required for their germination.

Seeds of *Kunzea* species that were treated with heat and smoke required markedly lower water availability for germination (median $\psi_b = -1.22$ to -1.65 MPa) than seven species that regenerate from soil-stored seeds within a fire-free environment (mean median $\psi_b = -0.77$ MPa, range = -0.51 to -1.18 MPa) (Allen et al. 2000). *K. ambigua*, *K. capitata* and the seven species occur on sandy soils that are rapidly draining, hence a requirement for high water availability for germination is expected because risk of seedling desiccation is high (Allen et al. 2000). For species affected by stand-replacing fire, there may be an advantage for some seeds to take the first available opportunity for recruitment, given a buffer of residual seeds (Bradstock et al. 1997), and the possibility that germinants may survive the summer in the Sydney region (Purdie 1977; Auld 1987). The initial risk of seedling desiccation would be high for *Kunzea* seeds that germinate at low water availability, particularly at high ambient temperature (Auld and Bradstock 1996), but risk would diminish as seedlings accessed deeper soil moisture in an environment of low transpiration (Silva et al. 2006).

Predicted effect of season of fire

If germination responses to fire cues, ψ and incubation temperature observed in this study represent what happens in the field, some generalisations emerge. In doing so, it is assumed that responses to incubation temperature represent seasonal effects on germination, while responses to ψ represent effects of changes in soil moisture.

The effect of temperature on the *Kunzea* species, in interaction with water availability, would be predicted to lead to post-fire germination across a wider range of individual rainfall events in cool seasons. However, germination would be more restricted to wetter periods in the

warmer seasons. In contrast, post-fire germination of *E. obtusifolia* would be affected little by season per se, but would always depend on rainfall patterns within a particular season. Populations would have a wide range of seasonal responses in their soil-stored seeds if they produce seeds with different responses to temperature between years (Trewavas 1988; Meyer and Allen 1999).

Although only a limited number of species were examined in the current study, there is a major contrast in their germination characteristics when compared with species from highly seasonal Mediterranean climates. Species from the aseasonal region used in this study maintain successful germination at a temperature that is too high for germination of most Mediterranean species (Bell 1999). The key difference across regions is that there is potential for germinants to survive across all seasons when rainfall is aseasonal as shown in field sampling of seedling survivorship (Purdie 1977; Auld 1987). Although risk of seedling mortality may be greater for cohorts that are produced during summer, this must be balanced against the risk that another opportunity for germination may not occur for some time, or not at all, and that fitness is reduced in seeds that forgo what is an infrequent opportunity for recruitment (Harper 1967; Lewontin and Cohen 1969).

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