

Research Paper

Cite this article: Lamont BB, Gómez Barreiro P, Newton RJ (2022). Seed-coat thickness explains contrasting germination responses to smoke and heat in *Leucadendron*. *Seed Science Research* **32**, 70–77. <https://doi.org/10.1017/S0960258522000113>

Received: 12 January 2022
Accepted: 24 May 2022
First published online: 5 July 2022


Key words:

heat; *Leucadendron*; Mediterranean-type ecosystems; seed-coat thickness and permeability; serotiny; smoke; soil-stored seeds

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Seed-coat thickness explains contrasting germination responses to smoke and heat in *Leucadendron*

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Abstract

Fire stimulates the germination of most seeds in fire-prone vegetation. Fruits of *Leucadendron* (Proteaceae) are winged achenes or nutlets that correlate with their requirements for smoke and/or heat in promoting germination. We describe five possible smoke–heat dormancy–release/germination syndromes among plants, of which *Leucadendron* displays three (no response, smoke only, smoke and heat). As seed-coat thickness varies with seed-storage location (plant or soil) and morphology (winged or wingless), we tested its possible role in water uptake and germination. Species with winged seeds achieved 100% germination in the absence of smoke/heat, seed coats were an order of magnitude thinner, and their permeability greatly exceeded that of nutlets. As seed-coat thickness increased (1) imbibitional water uptake declined at a decreasing rate, and (2) the response to smoke, and to a lesser extent heat, increased linearly to reach levels of germination approaching those of winged seeds. For species responsive to smoke and heat, there was no additive effect when applied together, suggesting that they may have promoted the same physiological process. We conclude that seed-coat thickness holds the key to germination requirements in this genus, independent of seed-storage location or morphology. By what mechanisms (1) the smoke response is greater the thicker the seed coat and (2) smoke chemicals might increase water permeability to explain the non-additive effect of smoke and heat, warrant further investigation.

Introduction

Seeds in temperate, fire-prone ecosystems are usually stored in the soil but, in a number of regions, on the plant as well (Rundel et al., 2018). Three germination syndromes can be recognized in response to a summer–autumn fire event. (1) Soil-stored seeds that are impermeable to water rely on fire-heat to render them permeable before they can germinate (Tangney et al., 2020). (2) Soil-stored seeds that are permeable to water rely on smoke-associated chemicals to promote germination (Moreira et al., 2010). (3) Seeds stored on the plant (serotiny) are released from their fruits/cones in response to fire (branch death). These are then exposed to the soil environment and germinate as soon as substantial rain falls and daily temperatures drop below a threshold (Lamont et al., 2020). These three syndromes are usually considered discrete but there are increasing reports of their overlap. For example, despite insulation by their supporting structures, serotinous seeds are exposed to heat during a fire and a heat pre-treatment may sometimes promote germination (Midgley and Viviers, 1990; Hanley and Lamont, 2000). Some serotinous seeds may even respond to smoke chemicals that are released from soil particles following rain (Preston and Baldwin, 1999; Brown and Botha, 2004). Of particular interest are the (unexpected) reports of responses by species with soil-stored seeds to both smoke and heat (Kenny, 2000; Morris, 2000; Mackenzie et al., 2016).

The genus *Leucadendron*, which originated in the Cenozoic when climates became more seasonal and fire-adapted traits diversified (Sauquet et al., 2009; Lamont et al., 2019), is a major woody shrub component of fire-prone, sclerophyll shrublands (fynbos) mainly in the Western Cape of South Africa. It comprises 85 species with many subspecies (Rebello, 2001). Most species are killed by fire but regenerate from seeds stored on the plant (serotiny) following fire-stimulated seed release from the parent cone (pyriscence). Other species store their seeds in the soil with fire-stimulated dormancy release via smoke chemicals in particular and/or heat to a lesser extent (Newton et al., 2021). Fruits in this genus are single-seeded and thus can be treated as seeds; thus, their pericarps are called seed coats. Newton et al. (2021) reported that untreated seeds of all but 2 of 31 serotinous species showed high levels of germination without any pre-treatment (mean of 96% at 20/10°C diurnal treatment in water agar, Table 1). The two serotinous species with low germination had nutlets rather than the winged achenes of the other serotinous species and either responded positively to aqueous smoke (*L. linifolium*) or germinated poorly (<50%) irrespective of treatment (*L. album*).

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Table 1. 40 *Leucadendron* species whose seeds were subjected to simulated fire [namely, smoke (water) and/or heat (80°C for 20 min)] plus a dry, 40/20°C diurnal pre-treatment to simulate a warm postfire summer before incubation at optimal winter temperatures (moist, 20/10°C)

Leucadendron	Plant- or soil-stored	Nutlet or winged	Seed coat (μm)	Imbibition (%)	Germination of controls (%)
<i>album</i>	P	N	200	37.0	36.2
<i>argenteum</i>	P	N	568	28.5	91.0
<i>brunioides</i>	S	N	316	38.5	3.6
<i>chamalaea</i>	S	N	228	–	81.1
<i>comosum</i>	P	W	24	57.3	99.8
<i>coniferum</i>	P	W	33	–	99.7
<i>corymbosum</i>	S	N	160	–	24.7
<i>discolor</i>	P	W	63	–	99.0
<i>dregei</i>	P	N	250	–	95.5
<i>elimense</i>	S	N	237	28.8	10.0
<i>eucalyptifolium</i>	P	W	39	–	100.0
<i>flexuosum</i>	P	W	48	–	100.0
<i>foedum</i>	P	W	44	–	100.0
<i>galpinii</i>	P	N	320	32.7	59.5
<i>gandogeri</i>	P	W	44	–	28.8
<i>lanigerum</i>	P	W	53	–	18.0
<i>laureolum</i>	P	W	40	–	100.0
<i>laxum</i>	S	N	234	34.1	28.8
<i>linifolium</i>	P	N	575	31.6	18.0
<i>loranthifolium</i>	S	N	154	–	24.9
<i>meridianum</i>	P	W	31	–	100.0
<i>microcephalum</i>	P	W	94	–	99.7
<i>modestum</i> ^a	P	N	236	–	63.5
<i>muirii</i>	P	W	24	–	99.4
<i>nervosum</i>	P	N	57	67.8	98.2
<i>nobilis</i>	P	W	27	55.5	99.8
<i>procerum</i>	P	W	70	–	100.0
<i>rourkei</i>	P	W	26	49.4	100.0
<i>rubrum</i>	P	N	51	–	99.8
<i>salicifolium</i>	P	W	58	–	99.8
<i>salignum</i>	P	W	47	–	100.0
<i>sericeum</i>	S	N	193	–	4.1
<i>spissifolium</i>	P	W	57	–	99.0
<i>stelligerum</i>	P	W	54	–	99.8
<i>strobilinum</i>	P	W	50	–	100.0
<i>teretifolium</i>	P	W	33	–	100.0
<i>thymifolium</i>	S	N	505	38.0	24.9
<i>tinctum</i>	S	N	955	–	48.1
<i>uliginosum</i>	P	W	140	40.9	88.1
<i>xanthoconus</i>	P	W	103	–	100.0

Seeds plant-stored (P) or soil-stored (S). Fruit a nutlet (N) or flattened and winged (W). *L. nervosum* has small spindle-shaped achenes with long hairs that are unlike other nutlets listed here but is treated as W as it is wind-dispersed. Seed coat = arithmetic mean seed-coat thickness. Water content after imbibition for 72 h. SDs were 10–20% of the means (Newton et al., 2021, Supplementary Table S5, provides error terms for germination %) and have not been included here preserve legibility and because error terms were not used in any analyses. Germination data are posterior means.

^aSince this study was undertaken this species was found to be *L. galpinii*, although it was from a different collection, it has slightly different properties and is retained here.

Smoke promoted germination of all seven species that possessed nutlets (increasing from a mean of 24% among the controls to 92% germination when smoke-treated) in the study by Newton *et al.* (2021). Of these, five were unaffected by 20 min of heat at 80°C and heat promoted germination in two. In earlier studies, germination of *L. daphnoides* and *L. tinctum* was quadrupled by scarification or removing the embryo from the seed (Brown and van Staden, 1973; Brown and Dix, 1985), consistent with physical barriers to germination. For the closely related *Leucospermum*, Brits and Manning (2019) showed that high temperatures resulted in desiccation and tearing of the endotesta that removed its impermeability to oxygen and enabled germination to occur.

Soil-stored nutlets can be expected to have thick seed coats to ensure resistance against decay agents, granivores and the digestive tract of animal dispersers (Calviño-Cancela *et al.*, 2008; Hudaib, 2019; Dalling *et al.*, 2020) and eventually fire heat, whereas serotinous seeds are protected and insulated by their supporting woody fruits or cones (Lamont *et al.*, 2020). The latter can be expected to have thin seed coats since they germinate as soon as the soil is cool and moist. Thus, we wondered if seed-coat thickness might play a role in explaining the differences in germination requirements between these seed types. For example, water permeability decreases with increasing seed-coat thickness, independent of its hardness, in various legumes and grasses (Noodén *et al.*, 1985; Frączek *et al.*, 2005; Richard *et al.*, 2018). Seeds that respond to fire-type heat usually have thick, dense, cutinized coats that are impermeable to fluids until heat opens up the dedicated ‘water gap’ in the seed coat (Moreira *et al.*, 2010; Gama-Arachchige *et al.*, 2013; Burrows *et al.*, 2018). Among other species whose germination is promoted by heat (Hanley and Lamont, 2000; Kenny, 2000), no water gap is evident and general tearing of seed coat tissues seems to be involved (Brits and Manning, 2019).

Smoke-stimulated germination involves the promotion of seed-dormancy release and thus germination by several products of plant-matter combustion among many species (Downes *et al.*, 2014; Keeley and Pausas, 2018; Cao *et al.*, 2021). Seeds that respond to smoke are weakly to moderately permeable (Moreira *et al.*, 2010). This indicates a compromise in seed-coat thickness between protecting the embryo from deleterious agents in its environment and allowing smoke chemicals to reach the embryo. Thus, seed-coat thickness might provide a clue as to whether, and what sort of, a fire-related property is required to overcome dormancy in *Leucadendron*. If the seed coat is thin, consistent with lack of soil storage and readiness to germinate as soon as dispersed, then the seeds will be highly permeable and there should be no fire response; if the coat is sufficiently thick to render it tardily permeable, then the seeds will respond to heat; if the coat is moderately permeable, then it will allow smoke chemicals to enter the seed and act catalytically (Flematti *et al.*, 2004; Lamont *et al.*, 2019) or increase its water permeability (Ghebrehiwot *et al.*, 2008; Jain *et al.*, 2008; Footitt *et al.*, 2019).

We therefore tested the following hypotheses:

1. The need for smoke and/or heat to promote germination of *Leucadendron* species is a function of seed-coat thickness.
2. Imbibitional water uptake is a negative function of seed-coat thickness and is inversely correlated with the promotory effect of smoke and/or heat on germination.
3. Serotinous seeds in winged fruits germinate readily in the absence of fire-related properties and will have thin seed

coats that are highly permeable to water, whereas nutlets whose seeds require smoke and/or heat to stimulate germination have relatively thick seed coats that are only weakly permeable.

Materials and methods

Seed germination

The 40 species of *Leucadendron* examined are listed in Table 1. An orthogonal design was used to test the individual and combined effects of smoke and heat on germination of seeds in the postfire environment. Seeds were exposed to a temperature of 80°C for 20 min to mimic a heat pulse expected among seeds buried to a depth of 40 mm (Newton *et al.*, 2006). A postfire treatment was applied to all treatments, including the control, that consisted of 8 weeks of dry storage at 40/20°C, which simulated temperatures experienced by soil-stored seeds after fire (Bond and Slingsby, 1983; Lamont *et al.*, 1993; Auld and Bradstock, 1996). Seeds receiving a smoke treatment were soaked in a 1:10 solution of Regen2000® Smokemaster liquid solution (diluted with distilled water) for 24 h at 20°C, simulating the release of smoke chemicals adsorbed to soil particles at the beginning of the rainy season (Preston and Baldwin, 1999). Non-smoke-treated seeds were similarly soaked in distilled water. Following these pre-treatments, seeds were sown on distilled water agar and incubated at 20/10°C (12 h light, 12 h dark) to simulate near-surface autumn–winter temperatures in postfire fynbos (Brits, 1987; Brown and Botha, 2004). Further details of the experimental design are given in Newton *et al.* (2021). *L. tinctum* showed low germination levels under all treatments and was not included in the analyses (but is considered in the Discussion).

Seed-coat thickness

To determine seed-coat thickness, five representative intact seeds were selected from the collections used for the germination experiments. Pericarps were bisected manually with a microtome blade. They were then positioned with the cut surface held horizontally under a Stemi dissecting stereoscope, model SV11, with a camera (AxioCam, Carl Zeiss, UK) attachment. Distance between outer and inner surfaces of the pericarp was measured in mm to 4 decimal places (AxioVision 4.8.1, Carl Zeiss). To examine the extent to which water permeability was a function of seed-coat thickness, 13 species were chosen to cover the three seed types and the full range of seed-coat thicknesses. Ten representative seeds per species were selected and their air-dry weights were taken. Seeds were individually immersed in distilled water using a compartmentalized Petri dish, patted dry and weighed to 0.1 mg with a microbalance (UMT2, Mettler, Toledo) at 1, 3, 7, 24, 48 and 72-h intervals, returning the seeds to the Petri dishes each time. Water content, expressed as a percentage, was determined as (wet weight – dry weight)/dry weight and only the final result is reported here as only then had imbibition stabilized.

Statistical analysis

Bayesian inference was used to analyse the germination data and model parameters were estimated using Bayesian Markov Chain Monte Carlo methods. An absolute difference of 10% in germination probability between treatments was chosen as the minimum

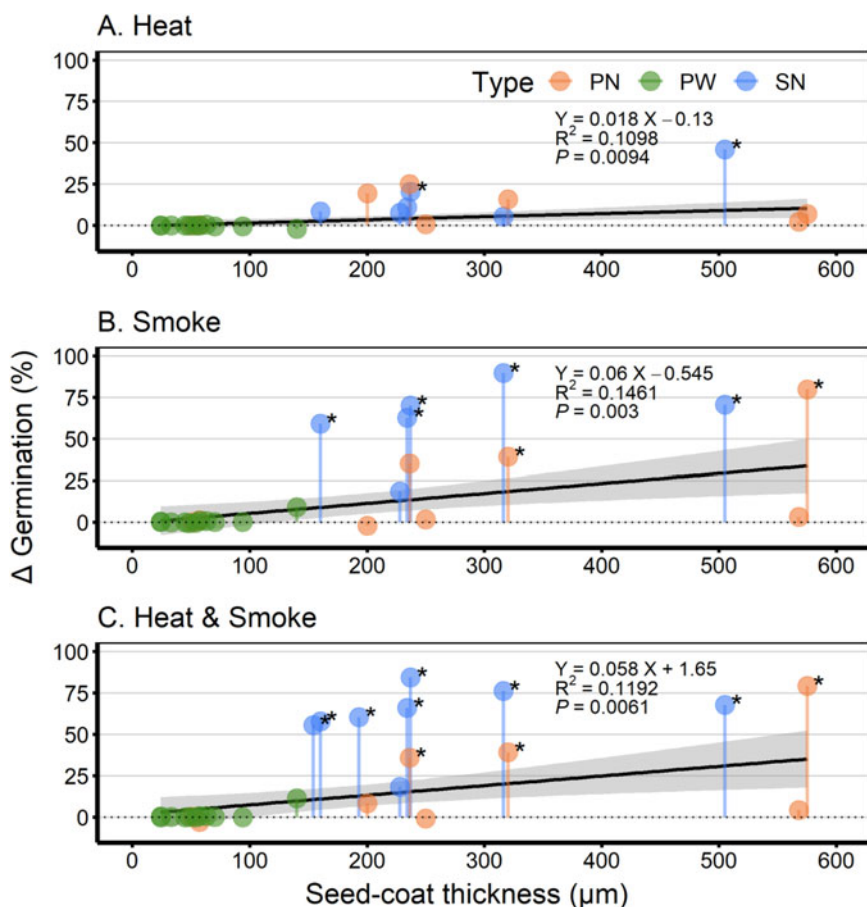


Fig. 1. Relationship between seed-coat thickness and germination increase (Δ = difference between average treatment and average control germination) for all species whose controls were <100% germination. The 13 species with 100% germination among the controls are represented here by one value to minimize bias in the data. (A) Following heat pre-treatment (80°C for 20 min), (B) Following smoke water pre-treatment and (C) Following both heat and smoke. Seed types are plant-stored nutlets (PN, orange), plant-stored winged achenes (PW, green) and soil-stored nutlets (SN, blue). Biologically significant results [according to Newton et al. (2021)] have an asterisk next to them. Linear regression lines are also given with their equations and probabilities, together with 95% confidence interval in grey. Germination averages were calculated from posterior mean germination values extracted from Supplementary Table S5 of Newton et al. (2021).

effect size of biological interest. Control germination of 50% or more was interpreted as evidence against an obligate requirement for direct fire stimuli. Treatment effects with 95% highest-density intervals falling outside the Region of Practical Equivalence (ROPE) interval $[-10$ to $+10\%$] were classified as 'biologically non-trivial'; treatment effects falling within the ROPE were classified as 'biologically trivial' and treatment effects overlapping the ROPE were classified as 'uncertain'. Further details on the statistical methods and allocation to smoke and/or heat responsiveness are given in Newton et al. (2021). Germination data for the controls and treatment giving the highest mean result were extracted from Supplementary Table S5 there, their classification noted as above, and plotted against the seed-coat thicknesses obtained here. The difference between germination levels of the controls and the three fire-related pre-treatments, and % water absorbed after 72 h, were plotted against mean seed-coat thicknesses. The lines with highest coefficients of determination (R^2) among four curvilinear options [Microsoft® Word for Mac 2011 or R (<https://www.stats.bris.ac.uk/R>) or] were fitted to the data. Germination and seed-coat thickness data for species with winged, and non-winged plant or soil-stored seeds, or that did or not respond to fire-type properties were also grouped and compared by conventional ANOVA/Tukey's statistics (<http://vassarstats.net>, R. Lowry©2021).

Results

Thirteen species, all plant-stored with winged seeds and seed-coat thickness of $45 \pm 19 \mu\text{m}$ (mean \pm SD), germinated at $\sim 100\%$

among the controls. For the remaining 26 species (omitting *L. tinctorum*), data for the controls fitted the linear equation: $Y = -0.13X + 91.01$, where $Y = \%$ germination and $X =$ seed-coat thickness in μm ($R = 0.543$, $P = 0.0041$). Thus, estimated $Y = 90.4\%$ when $X = 50 \mu\text{m}$ and $Y = 13.0\%$ when $X = 600 \mu\text{m}$ (the limit of seed-coat thicknesses recorded here). For the treatment yielding greatest response to smoke and/or heat, $Y = 95.8\%$ ($R = 0.123$, $P = 0.5541$), independent of seed-coat thickness, i.e. the treatment brought mean germination almost to the level of the 13 species not requiring any treatment to yield 100% germination.

Overall, the difference between germination levels of the controls and those treated with heat ($\Delta\%$) increased slightly with increase in seed-coat thickness, with zero difference at $23 \mu\text{m}$ and 10.7% at $600 \mu\text{m}$ (Fig. 1A). The addition of smoke increased the mean difference to 35.5% at $600 \mu\text{m}$ (Fig. 1B). The addition of smoke plus heat gave no further increase with a mean difference of 36.5% at $600 \mu\text{m}$ (Fig. 1C).

The 13 species selected for the study of imbibition showed similar trends to the total species in the study: these best obeyed a negative logarithmic function, with the controls ranging from $\sim 100\%$ germination at $\sim 25 \mu\text{m}$ to $\sim 10\%$ at $\sim 600 \mu\text{m}$. As an index of permeability, seed water content after soaking for 72 h best declined in a power-function manner with increasing seed-coat thickness (Fig. 2). Estimated mean water content fell from $\sim 60\%$ at $25 \mu\text{m}$ seed-coat thickness to $\sim 42\%$ at $140 \mu\text{m}$ (the range of thickness values for winged achenes), and $\sim 50\%$ at $\sim 50 \mu\text{m}$ to $\sim 30\%$ at $600 \mu\text{m}$ (the range for nutlets).

Placing the data into the three location-morphology categories summarizes the previous results (Fig. 3). Germination responses

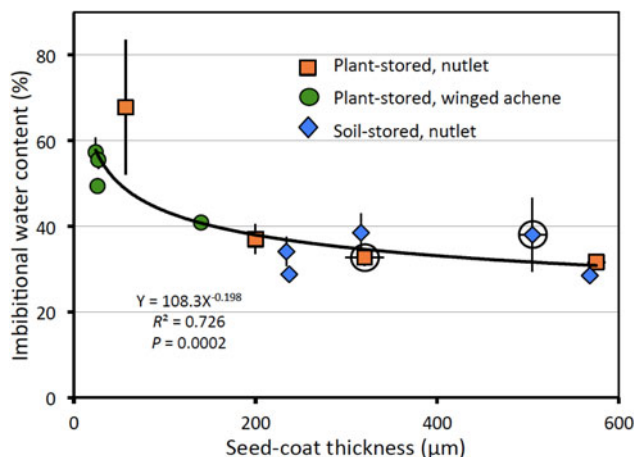


Fig. 2. Mean \pm SE imbibitional water contents after 72 h of soaking for the 13 species selected to represent the range of mean \pm SE seed-coat thicknesses. The two species that responded to both smoke and heat are ringed. The best-fit curve to the data is given as well as its formula and probability.

to smoke (and heat) increased from winged to non-winged plant-stored seeds to soil-stored seeds. However, seed-coat thicknesses of the nutlets, whether plant- or soil-stored, were on average about six times thicker than the winged achenes (Fig. 3B). The only plant-stored seeds not to require a fire-related property for high germination levels were winged (74%); the only plant-stored seeds benefitting from smoke and/or heat were nutlets (10%) and their seed coats were >7 times thicker on average, although with a large error term (Fig. 3C). All but 12% (one species uncertain) of the soil-stored nutlets responded to fire-related properties, particularly smoke.

Discussion

Among fire-prone seed plants generally, five fire-response dormancy-release/germination syndromes can be recognized (Table 2). *Leucadendron* has representatives in three of them. Syndrome 1 with almost all species having plant-stored, winged, single-seeded fruits lacking any need for a fire property to stimulate germination, and these possessed thin seed coats. Syndrome 2 with plant/soil-stored, nutlet-bearing species responding to smoke but not heat, with relatively thick seed coats. Syndrome 5 with a few soil-stored nutlet-bearing species responding non-additively to both smoke and heat, also with relatively thick seed coats. Overall, the thicker the seed coat, (1) the lower the level of germination in the absence of fire-related properties and (2) the greater the germination response to fire-related properties, such that the difference between the controls and fire-treated seeds increased linearly with increase in seed-coat thickness (Results, Fig. 1). Smoke was far more effective at increasing germination levels among the species with thicker seed coats, such that the co-presence of heat made a negligible difference to the outcomes (Fig. 1B, C). Even so, fire-type heat (80°C for 20 min) alone increased the germination response to a minor extent (Fig. 1A).

Thus, seed-coat thickness is a reasonable predictor of the extent to which fire-related properties, especially smoke, will bring germination levels up to those of species that do not require heat or smoke ($\sim 100\%$). Water permeability is an inverse function of seed-coat thickness [as also shown by Noodén et al. (1985) and Frączek et al. (2005)], with winged achenes (seed-coat thickness 25–140 μm) much more permeable than nutlets (150–600 μm),

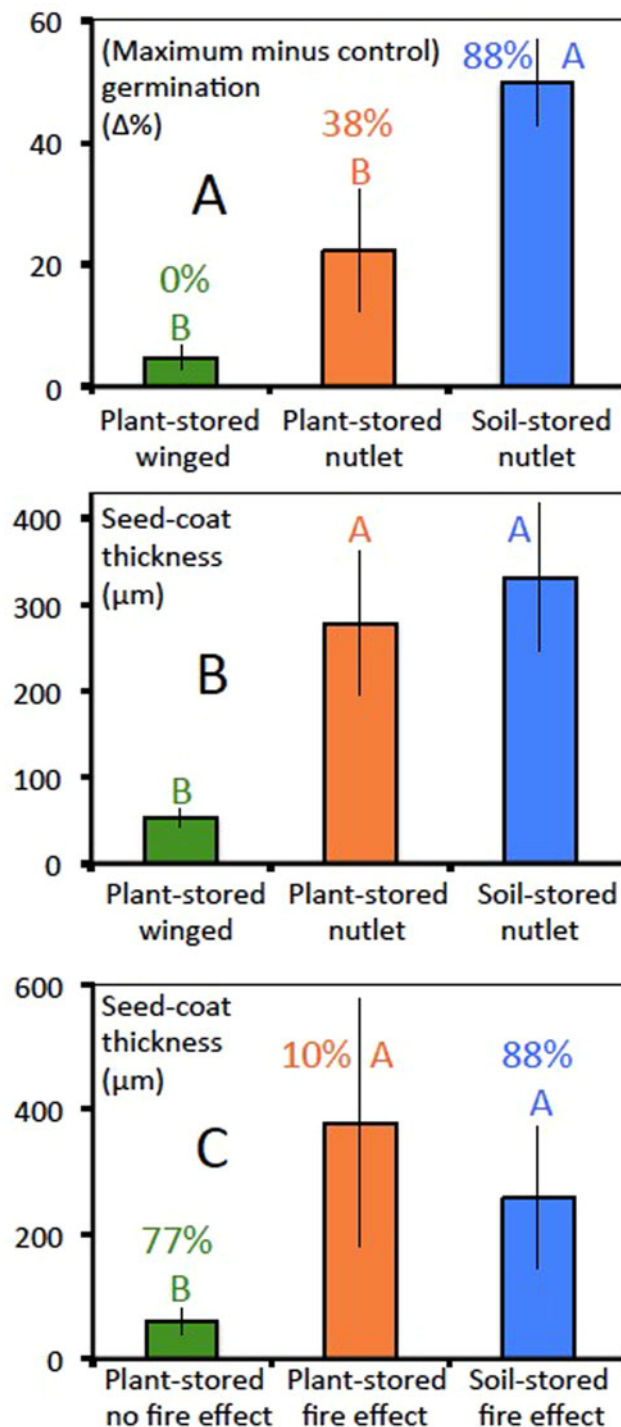


Fig. 3. Means \pm 95% CIs for all species in each of three seed categories based on storage location and fruit morphology, plus results for ANOVA of weighted means followed by Tukey's HSD test – letters significantly different at $P < 0.01$. (A) (Maximum value among the three fire-type treatments minus control) germination levels plus % of species showing biologically significant differences within each category; (B) Seed-coat thicknesses for the three categories and (C) Seed-coat thicknesses plotted against plant- or soil-stored seeds that do or do not have biologically significant germination levels in response to a fire (smoke) property plus % of species showing biologically significant differences within each category.

such that seed permeability declined in a power-function manner (best-fit curve). Since the amount of smoke chemicals absorbed is a function of water uptake (Baxter et al., 1994), and thicker seed

Table 2. Five possible dormancy-release/germination syndromes based on individual effects of heat (H) and smoke (S) and additive effects of both (H + S) on germination that should apply to all fire-prone species, with examples from this study of 36 *Leucadendron* species (excluding *L. tinctum*) and elsewhere if not observed here

1. $C = H = S = (H + S)$
Conclusion: neither H nor S required for promoting dormancy release (thin seed coat – already highly permeable)
Example: most serotinous *Leucadendron* species, especially if fruits winged (25/27)
2. $C = H < S = (H + S)$
Conclusion: S, but not H, promotes dormancy release (moderately thick, permeable seed coat – smoke chemical adsorption enables enzyme synthesis, possibly increases permeability)
Examples: most *Leucadendron* species with soil-stored seeds (5/6)
3. $C = S < H = (H + S)$
Conclusion: H, but not S, promotes dormancy release (thick, impermeable seed coat – now rendered permeable)
Examples: none among 36 *Leucadendron* species, possibly *L. tinctum* (Brown and Dix, 1985), most hard-seeded legumes
4. $C < \max(H, S) < (H + S)$
Conclusion: H and S promote different dormancy-release processes, or contribute to different parts of the same process (moderately thick seed coat – increased permeability, smoke chemical absorption enables enzyme synthesis)
Examples: none among 36 *Leucadendron* species, *Mimosa leiocephala*, *M. somnians* (Zirondi et al., 2019)
5. $C < \max(H, S) = (H + S)$
Conclusion: H and S probably affect the same dormancy-release process (moderately thick seed coat – increased permeability in both?)
Examples: a few *Leucadendron* species with soil-stored seeds (2/8): *L. elimense*, *L. thymifolium*

= same effect, < former has a lesser effect than the latter. (...) refers to likely mechanism of dormancy release as supported by our results and relevant literature cited in the text. $\max(H, S)$ = maximum effect of H or S.

coats are less permeable (shown here), it raises the possibility that (at least some of) the promotive chemicals in smoke served to increase permeability to water and/or oxygen (Brown and van Staden, 1973; Ghebrehiwot et al., 2008; Jain et al., 2008; Brits and Manning, 2019).

Of the ten species with biologically significant smoke responses, two (*L. elimense*, *L. thymifolium*) responded significantly to both smoke and heat. Heat and smoke can be distributed patchily during a fire (Auld and Bradstock, 1996) and the ability to respond to more than one germination property has been suggested to maximize the capability of seeds to sense the passage of a fire (Kenny, 2000; Morris, 2000). Once heated, the hard seeds of many legumes become permeable (Burrows et al., 2018) and will now germinate as soon as the soil is moist and cool, so that smoke sensitivity would be redundant. Smoke-sensitive seeds are stored in a permeable state that allows smoke chemicals after fire to enter them, so that now heat-sensitivity is redundant. However, it is possible for smoke chemicals to drift into unburnt or scorched patches through diffusion and leaching (Ghebrehiwot et al., 2013), where the seeds are unlikely to have received a heat treatment. However, patches already occupied by plants are unlikely to lead to recruitment because they are outcompeted by the plants already present (Lamont et al., 2019). Thus, a dual response is most likely to be adaptive when (1) seeds are in patches that will receive both heat and smoke and (2) the response is additive, especially if it is synergistic (syndrome 4 in Table 2).

In a worldwide survey of 589 species subjected experimentally to both heat and smoke, 14.5% responded positively to both heat and smoke (Pausas and Lamont, 2022) so that this phenomenon is not common, but it is also not rare. In some cases, hard seeds become smoke-sensitive after they are heated and there is an additive or synergistic effect (Zirondi et al., 2019; syndrome 5A/B). The logical interpretation is that these environmental properties affect different processes that are well-known: heat renders the seeds permeable and smoke chemicals have a catalytic effect on the seed's physiology. However, for *Leucadendron*, the two heat-responsive species were not impermeable to water, and

their seed-coat thickness and permeability were not greater than some other smoke-responsive-only species (Fig. 2). Furthermore, germination was no greater than with smoke plus heat than with smoke alone. But germination was greater than with heat alone that in turn was greater than for the controls.

These responses imply that smoke and heat may affect the same process (syndrome 5). Since heat probably increases permeability (no other function for a fire-type heat pulse on dormant seeds is known), and the thicker the seed coat the proportionately greater the smoke response, it seems in this case that heat supplements the permeability-enhancing role of smoke chemicals (Ghebrehiwot et al., 2008; Jain et al., 2008; Footitt et al., 2019). Although difficult to envisage a relevant scenario, it appears that a non-additive response to smoke and heat is only likely to be adaptive when seeds receive fire-type heat in the absence of smoke. The improbability of such a situation might explain why this dual response is not better represented among fire-prone floras. This topic clearly needs further investigation, especially the relative role of heat and smoke in raising the permeability of non-hard seeds.

Our results also raise the interesting issue of *L. tinctum* that has 1.5 times seed-coat thickness of the next thickest but germinated poorly despite high viability and did not respond to any treatment. In another study with this species, germination was raised from 12 to 45% with smoke and to 70% with smoke plus scarification (Brown and Botha, 2004), equivalent to syndrome 4 (Table 2). Brown and Dix (1985) showed that the seed coat was essentially a mechanical barrier preventing germination, as germination increased from 20 to 80% after scarifying the seeds then covering them in lanolin. If our batch had exceptionally thick seed coats, they could act like conventional 'hard' seeds and only respond to heat. It is possible then that our heat treatment (80°C for 20 min) was not 'severe' enough to scarify most seeds in this species, although the complete lack of a smoke response is not easily explained.

That it is not just an issue of winged achenes versus nutlets is demonstrated when the eight species with plant-stored nutlets are considered. These are as thick as the soil-stored nutlets on average

but their response to smoke is much less (Fig. 3A, B). Two were as thin as the winged seeds (controls 99% germination) and six were on average 50 µm thicker than the mean of the soil-stored nutlets (controls 61% germination). The three plant-stored species showing a biologically significant response to smoke had seed coats five times thicker than the plant-stored species that did not benefit from smoke (Fig. 3C). So, the key to their germination requirements is seed-coat thickness rather than storage location or morphology. Thus, some plant-stored nutlets may be just as permeable as the winged achenes as they have similar seed-coat thicknesses (Fig. 2). This may have functional significance – the confines of the cone are much less hazardous for survival than in the soil, and germination can proceed readily following postfire release as with the winged seeds. However, the six species with thick-walled, plant-stored nutlets double their options: if they arrive in a suitable microsite on release, they can germinate that winter (Lamont et al., 2021) or, if unsuitable, they can remain viable in the soil until the next fire.

Thick, weakly permeable seed coats serve to increase longevity and heat tolerance in the soil but then the seed must rely on a special fire-related property, smoke, as distinct from just cool wet winters, to signal (and respond to) the onset of ideal recruitment conditions. Thus, this genus possesses ideal taxa to pursue the mechanisms by which stimulatory smoke chemicals serve to break dormancy, as closely related species that do not require smoke for germination also exist. This includes the possibility of acting to improve permeability (Ghebrehiwot et al., 2008; Jain et al., 2008; Footitt et al., 2019) that needs further investigation. The role of fire-type heat is usually considered to break the impermeability of hard seeds. Yet here we have instances of seeds with moderate permeability that can also benefit from heat that suggests it may also serve to further increase the permeability of seeds that are already (weakly) permeable. Evidence available so far indicates that this dual response to heat and smoke may exist among many hundreds of fire-prone species. This genus is ideal for pursuing the mechanisms by which seeds may respond to both smoke and heat and their functional significance.

Acknowledgements. We thank a colleague who reviewed a draft of Newton et al. (2021) and suggested the possible significance of seed-coat thickness in explaining our initial results, Berin Mackenzie for introducing the concept of biological significance to this work, Richard Cowling and Tianhua He for early support, Juli Pausas for advice, and the three reviewers for their useful comments.

Author contributions. B.B.L. and R.J.N. conceived the project and designed the experiments; P.G.B. performed all experiments and tests; B.B.L. and P.G.B. analysed the data; B.B.L. and R.J.N. wrote the draft manuscript; P.G.B. contributed to drafts and all gave final approval for publication.

Financial support. This work was originally supported by the Australian Research Council (projects DP120013389, DP130103029) and the Bentham-Moxon Trust, Royal Botanic Gardens, Kew, receives grant-in-aid from Defra, UK.

Conflicts of interest. None declared.

Data availability. Data used in this study are given in Table 1, and Supplementary Table S5 of Newton et al. (2021).

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