

Patterns of smoke germination in south-eastern Australian plants

Final Report for the Australian Flora Foundation



Photo: The Australian Plant Bank at the Australian Botanic Garden, Mount Annan. Credit:
<http://www.plantbank.org.au/>

NB: the work reported here is accepted for publication in the *Journal of Vegetation Science*.

Written for the Australian Flora Foundation Grant awarded to the
Principal Researcher, Professor Michelle R. Leishman for 2015.

Prepared by Dr Alexandra J. R. Carthey

May 2018

Department of Biological Sciences, Macquarie University, North Ryde, NSW 2109, Australia

Abstract

Fire is a key part of the abiotic environment in many ecosystems, with adaptive responses to heat and smoke evident in plant taxa worldwide. We have known since the 1990's that smoke treatment promotes seed germination in some species. Smoke can also affect the timing and success of germination, as well as seedling vigour, in a range of species. Most of the research on smoke-promoted germination has been conducted in Mediterranean, Western Australian, South African fynbos, and Californian chaparral ecosystems, but we know little about germination responses to smoke for eastern Australian species. We also know very little about how phylogeny, growth form, vegetation type and fire response strategies (obligate seeders *versus* resprouters) might relate to patterns of smoke-promoted germination. We compiled data from existing literature and digitised paper records of germination trials in the Australian Plant Bank at Mount Annan, New South Wales. We then investigated patterns of smoke-promoted germination in eastern Australian flora to examine relationships between smoke response, taxonomy and phylogeny, growth form, fire response strategies and vegetation type. We also examined the effect of different methodological approaches on the likelihood of finding smoke-responsive germination. Smoke-responsive germination occurs at a somewhat lower rate in south-eastern Australian flora than it does in fynbos and Western Australian floras, with about 41% of tested species showing germination enhanced by smoke treatment. Species from dry habitats were more likely to respond to smoke than were species from other habitats. Obligate-seeding species were more likely to respond, Leguminaceae and Rubiaceae were less likely to respond (although we question the generality of these results), and Poaceae were more likely to respond to smoke. Finally, studies using aerosol smoke, and studies conducted *in-situ* were most likely to find smoke-promoted germination. Obligate seeders and Poaceae may be selected for in habitats with higher fire frequencies, consistent with literature suggesting that short inter-fire intervals favour grasslands over forests. These findings may be particular to south-eastern Australia, or more widely applicable; more broadscale comparative research will reveal the answer. We have broadened our understanding of smoke promoted germination in non-Mediterranean floras. Knowledge of germination and regeneration strategies in response to fire is invaluable for restoration ecologists and conservation practitioners working in fire-prone habitats, such as those common to SE Australia, and which may become more widespread under climate change. We have added the south-eastern Australian data into a larger, global dataset that will be used to examine similar patterns at the global scale, for future research.

Introduction

An understanding of regeneration responses to smoke is critical for management of threatened plant species and vegetation communities under changing climate, including increased fire frequency. Species whose seeds germinate in response to the products of burning vegetation (including smoke, ash, charcoal and their constituents, and hereafter referred to as smoke) may therefore benefit from the post-fire environment (Whelan 1995; Pausas & Keeley 2014). Germination responses to smoke treatments have received increasing research interest over the last 20 years, with large advances made in the identification of the compounds responsible for both enhancing (e.g. karrikinolide, Flematti et al. 2004; van Staden et al. 2004) and inhibiting (e.g. Adkins & Peters 2001) smoke-responsive germination. At the same time, it is clear that the germination response to smoke is a complex one, driven by multiple different compounds (Jefferson et al. 2014). In practical terms, a variety of methods have been used to test a broad array of plant species for smoke-promoted germination, such as smoke water, aerosol smoke, karrikinolide, ash/charate mixes, amongst others (Brown & van Staden 1997; Enright et al. 1997; Dixon et al. 2009). The literature on smoke-promoted germination has also been focused on a limited number of ecosystems – the Mediterranean Basin (e.g. Crosti et al. 2006, Moreira and Pausas 2016), fynbos in the Cape Floristic Region of South Africa (e.g. Brown et al. 2003b), Californian chaparral in the Californian Floristic Region of the United States of America (e.g. Keeley & Fotheringham 1998), and South-western Floristic Region of Australia (e.g. Dixon et al. 1995). These areas share a similar, Mediterranean-type climate that has been thought to drive the evolution of fire-adapted traits such as smoke-promoted germination – they are all fire-prone ecosystems with seasonal rainfall (Keeley 1995b; Keeley & Fotheringham 1998; Brown et al. 2003a).

The proportion of tested species in South African and Western Australian floras showing germination responses to smoke are both close to 50% (Dixon et al. 1995; Brown et al. 2003a), and somewhat lower at approximately 37 % in Mediterranean Basin flora, though the Mediterranean Basin figure is likely to be an underestimate (Moreira & Pausas 2016). No such estimate is available for chaparral. In general, there is little available evidence for links between smoke-responsive germination and plant traits such as life history strategies or growth forms. However, it has been reported that smoke responsive germination is almost unknown in geophytes (Californian chaparral: Keeley & Bond 1997; South African fynbos: Brown et al. 2003a), and that the majority of annuals in both Californian chaparral and South African fynbos respond to smoke (Keeley & Bond 1997).

It seems likely that patterns of smoke-related germination may be linked to the resprouting strategies of plants (e.g. Ne'eman et al. 2009); i.e. plants that resprout from protected buds after complete leaf scorch (resprouters), and those that do not (non-resprouters, or obligate seeders) (Gill 1981; Ne'eman et al. 2009). Given that resprouters do not rely on seed germination to recover post-fire, we might expect that they are less likely to show smoke-responsive germination than obligate seeders. On the other hand, resprouters might still benefit from germinating quickly after a fire, if it allows greater total recruitment of new individuals. Finally, some authors indicate that smoke-responsive germination is phylogenetically widespread (Western Australia: Roche et al. 1997b; Californian chaparral: Keeley & Fotheringham 1998; South African fynbos: Brown et al. 2003b), whereas others suggest it appears to be phylogenetically patterned, with most species within a lineage typically responding to either heat or to smoke chemicals (Keeley 2012). Resprouting and heat-resistant seed coats enabling propagule persistence are phylogenetically patterned in the flora of the Mediterranean Basin (Pausas & Verdú 2005). Therefore, the question of whether smoke-responsive germination is linked to phylogeny remains open.

There have been comparatively few studies of smoke-responsive germination in the south-eastern Australian flora. South-eastern Australia does not fit the Mediterranean-type climate classification; it has a temperate climate with rainfall throughout the year, meaning that seed germination is potentially possible all year, rather than being restricted to the wetter months as it is in Mediterranean-type climates (Mackenzie et al. 2016). As with much of the Australian continent, however, south-eastern Australia is considered fire-prone (Thomas et al. 2007).

In this study, we ask whether there are broad patterns of smoke-promoted germination in relation to life history strategies, post-fire response strategies and phylogeny in the south-eastern Australian flora. Additionally, we ask whether some smoke application methods are better than others at inducing smoke-responsive germination in south-eastern Australian plants. We compiled data comprising 303 entries on germination responses to smoke in 233 south-eastern Australian plant species, from 33 different sources. We digitised hard copy records of germination trials conducted at the Australian Botanic Garden, Mount Annan, and combined these records with an extensive literature search for studies conducted in south-eastern Australia (which we defined as occurring in New South Wales and/or Victoria, the two most south-eastern states). Our aim was to investigate patterns of smoke responsive germination in south-eastern Australian plants, across habitat types, growth forms, fire

response strategies, taxonomic levels, and smoke application methods (including whether tests were made *in-situ* or *ex-situ*). Briefly, we expected that smoke-responsive germination would be:

- (1) more likely in drier, more fire-prone habitats than in wetter, less fire-prone habitats
- (2) unrelated to growth form, but unlikely in geophytes
- (3) more likely in obligate seeders than resprouters
- (4) more effectively induced by aerosol smoke than other methods of smoke application, based on suggestions in previous papers (e.g. Rokich & Dixon 2007)
- (5) more commonly observed in *in-situ* than *ex-situ* tests, as *in-situ* tests provide contextual cues that may enhance seed responsiveness to smoke, and finally,
- (6) may or may not be related to phylogeny and/or taxonomic levels.

Our overall aim was to investigate patterns of germination responses to smoke in the south-eastern Australian flora, broadening our global understanding of germination responses to fire cues across vegetation types and climates, and beyond the better-studied Mediterranean Basin, South African fynbos, Californian chaparral, and Western Australian floras. We intend that this research will enable the incorporation of smoke-promoted regeneration in vegetation management in SE Australia, and promote the cultivation of SE Australian plants.

Methods

Data compilation for this project took place through several main approaches: a literature search, compilation of data contained in Jefferson et al. (2014), digitisation of germination records from the Australian PlantBank at the Australian Botanic Garden Mount Annan, and the addition of data from the New South Wales Flora Fire Response Database (NSWFFRD).

Literature search

We first compiled all records of plant germination trials listed in Jefferson et al. (2014) listing species that are recorded to occur in New South Wales and/or Victoria, Australia. This included widespread species that are found Australia-wide and in other countries.

We then searched the wider literature through Google Scholar, with the last search occurring in April 2017. We used the following search term combination ‘smoke AND plant * AND Australia * AND germ * Victoria OR "New South Wales"’. Hits (Google Scholar: n = 4220) were reviewed manually and qualifying papers were added to our database.

Papers qualified for inclusion in our database if they performed a germination test on intact seeds that included a smoke treatment and a control (no smoke treatment). Where another treatment was combined with smoke (e.g. gibberellic acid or heat shock), we only included the data if the combined treatment resulted in no response.

PlantBank Germination Record Digitisation

Information on smoke-promoted germination for SE Australian species is concentrated in the records of the Australian Plant Bank at the Australian Botanic Garden Mount Annan. Researchers based at Mount Annan have been collecting seed material of NSW plant species systematically for many years. When seed material arrives at Mount Annan it is tested for germinability using a variety of techniques, including smoke, heat, gibberellic acid, and cold stratification. We digitised the results from these seed germinability trials, and combined the germination trial entries with data for each species collated from source references, the PlantNET database of New South Wales flora (<http://plantnet.rbgsyd.nsw.gov.au/floraonline.htm>), and the New South Wales Flora Fire Response Database (NSWFFRDv2.1; NSW Office of Environment and Heritage 2014), on growth form, life history strategy, habitat, and fire strategy. These references were also consulted to achieve the highest data coverage possible for all variables for all species in the dataset. For a full list of variables (and the levels of each variable) included in the database, see Table S1. The data are available online at <https://pirel.wordpress.com/datasets/>.

Nomenclature and taxonomic information

Nomenclature was confirmed using The Plant List (www.theplantlist.org), and taxonomic information was extracted from The Angiosperm Phylogeny Website (www.mobot.org). Taxonomic levels included were Level 1 (Lycopodid/Gymnosperm/Angiosperm), Level 2 (Lycopodid/Gymnosperm/Magnoliid/Monocot/Eudicot), Level 3 (Lycopodid/Gymnosperm/Magnoliid/Asterid/Rosid/Other core eudicot/Other eudicot/Commelinid/Other monocot), Level 4 (Family), Level 5 (Genus) and Level 6 (Species).

Statistical methods

Our database contained more than one entry for many species, which we used to test for differences in smoke-responsive germination related to the method of smoke application. However, we also created a subset of this dataset with only one entry per species, which we used to investigate species-level patterns of smoke responsive germination in relation to ecological factors (habitat, growth form, fire response strategy), taxonomy and phylogeny. In 217 of the 233 species in the dataset, germination response to smoke was consistent, appearing in either all cases or no cases. In the remaining 16 species, smoke-promoted germination was only observed in some tests. In these instances, we prioritised keeping entries in which smoke-promoted germination was observed. This created a subset of the dataset that included only a single entry per species.

Analysis of ecological variables

We performed binomial logistic regression to determine how well plant growth form, life history, habitat type, and fire response category predicted whether a species showed smoke-enhanced germination. Species showing either no response or inhibited germination were grouped as “not positive”. For significant variables, we determined which category membership predicted an enhanced germination response using χ^2 tests. Simulated p-values for the χ^2 tests were calculated using 200,000 replicates of a Monte Carlo simulation, a permutation approach used to calculate the probability of this particular contingency table occurring under the null distribution (Hope 1968). We calculated adjusted standardised residuals (ASRs) for cells of the contingency tables, as ASRs $> |2|$ indicate where counts in a category are greater (positive values) or lower (negative values) than would be expected by chance (Agresti 2002).

Analysis of phylogenetic and taxonomic variables

We generated a phylogenetic tree for our data, and then used the *D* metric of Fritz and Purvis (2010) to measure the strength of phylogenetic clustering of smoke-enhanced germination. We then employed χ^2 tests with simulated p-values to identify specific taxonomic groups (see Table S1) in which smoke-promoted germination was more common than would be expected at random. We limited the analysis at family level to families with five or more species in the dataset ($n = 11$ families; Asparagaceae, Compositae, Cyperaceae, Ericaceae, Leguminosae, Myrtaceae, Poaceae, Proteaceae, Rubiaceae, Rutaceae, Xanthorrhoeaceae). See Supplementary Methods for details.

Analysis of methodological variables

We tested the relative effectiveness of methodological variables using the full dataset that included multiple tests for some species. We again used a χ^2 test with simulated p-values and adjusted standardised residuals to determine whether germination responses to smoke were more commonly seen when tests used aerosol smoke, smoke water, or ash/charate smoke treatments. No south-eastern Australian studies tested glyceronitrile as a germination cue, and only two studies used karrikinolide. These treatments were therefore excluded from the χ^2 test. We also tested whether the tests were conducted *in-situ* (in the field) or *ex-situ* (in a laboratory setting) affected the likelihood of a positive germination response to the smoke treatment. In all cases, we excluded categories from analysis which had fewer than 10 species (for example: in analyses including growth form, we excluded the category “climbers”, which had only 4 species). All analyses were performed in R v 3.0.3 (R Core Team 2014).

Results

Dataset

The dataset contains 303 entries and 233 species, with data obtained from 33 different sources (27 peer-reviewed journal articles, 2 theses, 1 *pers. comm.* within a peer-review journal article, 1 report, 1 database, and 17 Mount Annan Plant Bank germination trials). Details of the variables and levels included in the dataset are given in Table S1. Considering the dataset with only one entry per species (Table 1), we achieved 100% data coverage for growth form, 94.4% for habitat, 97.4% for life history strategy, and 71.7% for fire response category. In the full dataset, we achieved 99.1% data coverage for location of study (*in-situ* or *ex-situ*). The majority of species in the database are woody (136 out of 233 species), from dry habitats (190 out of 220 species with data), and/or show a non-resprouting fire response (110 out of 167 species with data)(Table 1). We had complete ecological data for 150 species, and performed the binomial logistic regression using these 150 species only.

Table 1. Number of species in the dataset within each category of each variable

Variable	Count of species
Growth form	
Climber	4
Graminoid	37
Herbaceous	57
Woody	137
Habitat type	
Aquatic/wet areas/damp areas	15
Arid/dry/rocky/sandy	2
Diverse/widespread	1
Grassland/herbfield	2
Rainforest	14
Woodland/forest/shrubland/heath	188
No information	13
Fire response category	
Resprouter	57
Obligate seeder	111
No information	63
Total number of species	235

Each of the 303 entries in the full dataset represents one test of a smoke treatment on a single plant species. Of these 303 tests, 177 used aerosol smoke, 85 used smoke water, 2 used karrikinolide, 33 used ash or charate, and one used smoked filter paper. No studies were found that tested glyceronitrile effect on germination of south-eastern Australian species. Six entries were not allocated a smoke treatment type because they came from sources that did not give treatment details (a thesis [n = 2], database [n = 2], report [n = 1], or *pers. comm.* [n = 1]). Smoke enhanced germination in 40.7 % (95 out of 233) of the species in the dataset.

Ecological predictors of smoke-responsive germination

Smoke-promoted germination was significantly affected by habitat type ($p = 0.020$) and fire response category ($p = 0.026$). An enhanced germination response to smoke was significantly more likely in obligate seeders ($\chi^2 = 6.180$, $p = 0.014$) than would be expected at

random (ASR = +2.5). Although the overall p value for the exact χ^2 test was not significant ($\chi^2 = 3.971$, $p = 0.067$), the ASRs indicated that species from drier habitats showed increased smoke-promoted germination than would be expected at random (ASR = +2.0). The probability of responding to smoke was not significantly influenced by growth form or life history in either the logistic regression or the χ^2 tests.

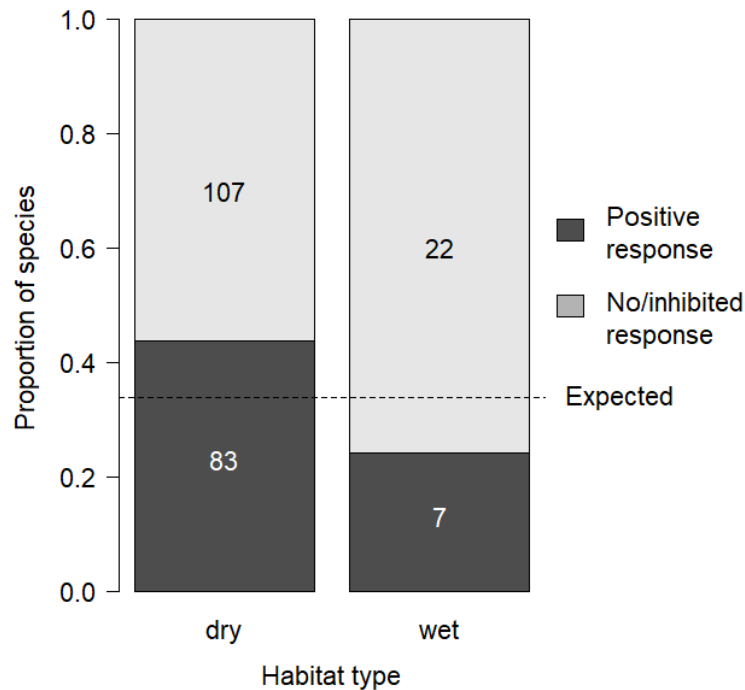


Figure 1. Proportion of species showing a germination response to smoke in each habitat type. Only habitat types with more than two species in the dataset are shown in the figure, although all habitats were included in the statistical analysis. Numbers within the bars show the number of species in each category. If habitat was unrelated to the proportion of germination responses to smoke, then the proportion of responders in each habitat, based on these proportions in the whole dataset, should be approximately equal to 0.46 (dashed line). There are substantially fewer smoke-responders in rainforest habitat (1 out of 14 species, ASR = -3.0), and substantially more smoke-responders in woodland/forest/shrubland/heath habitat (94 out of 188, ASR = 2.5) than would be expected if smoke response was unrelated to habitat. There was no significant difference between observed and expected numbers of smoke responders in the aquatic/wet/damp habitat (ASR = 0.5).

Phylogeny and taxonomy of smoke responsive germination

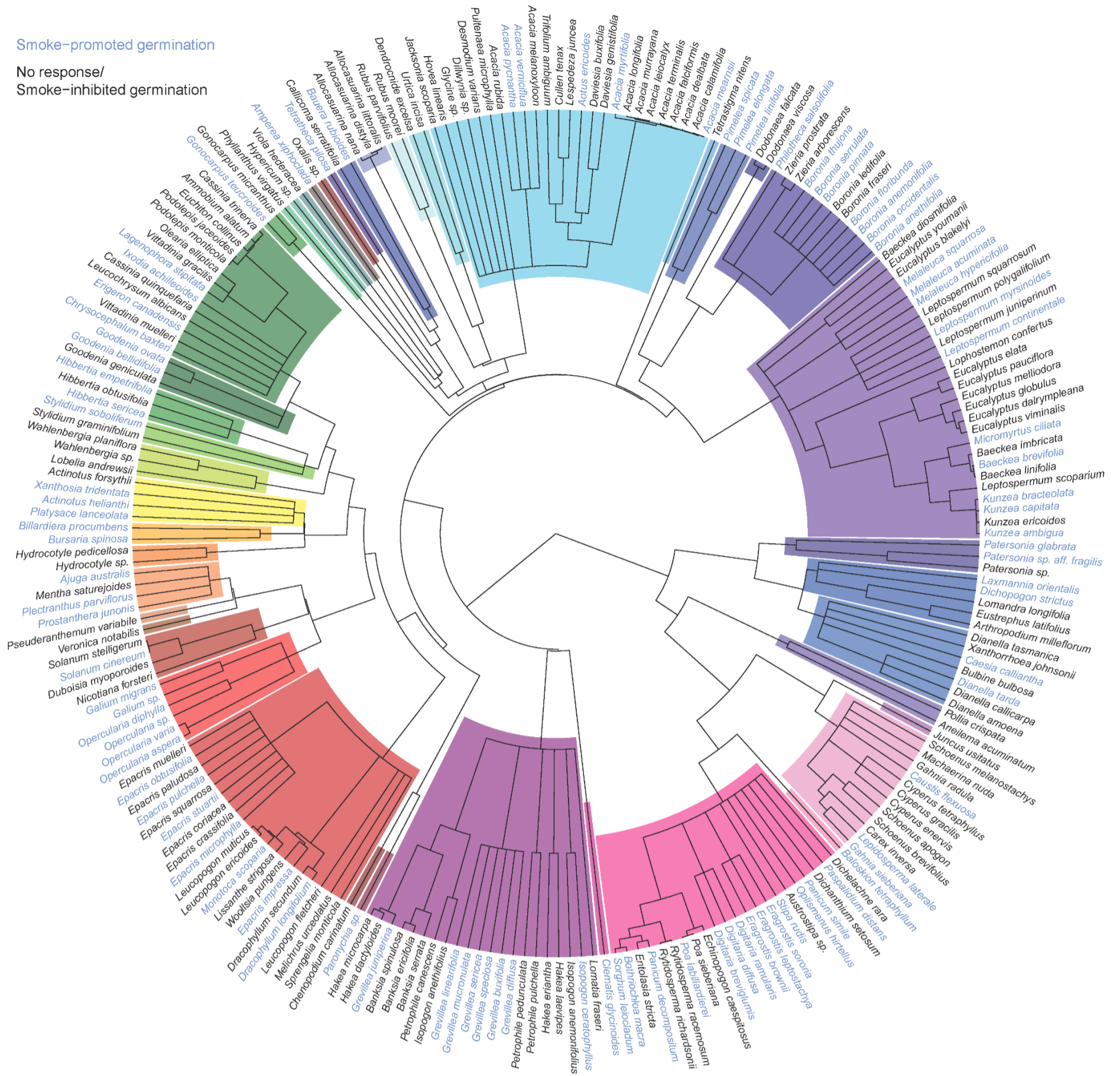
The distribution of smoke-promoted germination response in the 233 species included in this study (Figure 2) was significantly phylogenetically clustered ($D = 0.68$, $p(\text{random}) < 0.001$). While phylogenetic clustering is apparent, the strength of the phylogenetic signal is not as strong as if it depended solely on a Brownian trait distribution. Smoke-promoted germination showed differences at the Family level ($\chi^2 = 24.371$, $p = 0.005$). Enhanced germination responses to smoke were more common in the Poaceae (ASR = +2.4, 14 out of 22 species responded positively) and Rubiaceae (ASR = +3.0, 6 out of 6 species responded positively), but less common in the Leguminaceae (ASR = -2.2, 20 out of 25 species did not respond positively). Germination responses to smoke were not associated with any group at higher taxonomic levels - (eudicots/monocots; $\chi^2 = 0.578$, $p = 0.52$), (asterids/rosids/commelinids/other eudicots/other monocots; $\chi^2 = 2.409$, $p = 0.80$).

Figure 2 (next page). Phylogenetic tree representing the evolutionary relationships between the species included in this study. Smoke-promoted germination is recorded on the diagram where species names are coloured blue. Species with names in black did not show smoke-responsive germination. Families are colour coded according to the key given on the diagram.

Smoke-promoted germination

No response/

Smoke-inhibited germination



Methodological predictors of smoke-responsive germination

The method of smoke application affected the probability of the species tested responding to smoke ($\chi^2 = 12.412$, $df = 2$, $p = 0.002$, Figure 3). Germination responses to smoke were significantly more likely to be found in tests using aerosol smoke (ASR = 3.1), and significantly less likely to be found in tests using ash/charate (ASR = -2.9), than would be predicted if smoke application method had no effect (Figure 3). Whether the test was conducted *in-situ* or *ex-situ* affected the probability of the test species responding to smoke ($\chi^2 = 5.017$, $df = 1$, $p = 0.046$), with tests conducted *in-situ* more likely to find a germination response to smoke (ASR = 2.2) than those conducted *ex-situ* (ASR = -2.2).

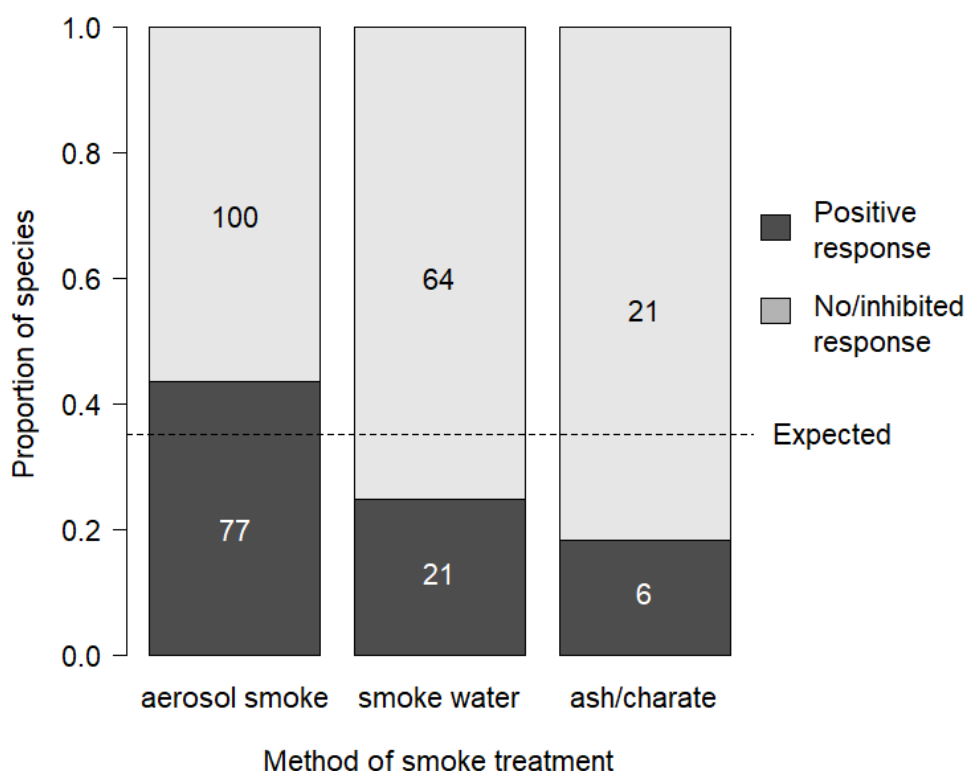


Figure 3. Proportion of tests demonstrating a germination response to smoke, according to the smoke application method used. There were only 2 tests using karrikinolide, hence these entries are not shown. Numbers within the bars are the number of tests for each category. If smoke application method was unrelated to the proportion of germination responses to smoke, then the proportion of responders in each smoke type category, based on these proportions in the whole dataset, should be approximately equal to 0.41 (dashed line). However, tests using aerosol smoke were significantly more likely to find a germination

response to smoke (ASR = 3.0), and tests using ash/charate were significantly less likely to find a germination response to smoke (ASR = -2.9), than predicted if smoke application type was unrelated to smoke response.

Discussion

Here we present the first synthesis of smoke-responsive germination in south-eastern Australian plant species. Approximately 41 % of the species tested ($n = 95/233$ species) showed a germination response to smoke. Somewhat higher proportions of smoke-responsive species have been found in other floras, for example, 49.8 % of 301 South African fynbos species (Brown et al. 2003a), and 50 % of Western Australian species (Dixon et al. 1995) tested showed smoke-promoted germination, although the Mediterranean Basin figure is lower, at 37 %, potentially due to issues such as sampling biases towards perennial plants (Moreira & Pausas 2016), which may also affect sampling in south-eastern Australian flora. Estimates of smoke-responsive germination in global floras have not yet been possible.

As expected due to their having more to gain from rapid germination in the post-fire environment, obligate seeders were more likely to respond positively to smoke than resprouting species. Similarly, non-resprouting Mediterranean Basin species are also more likely to be heat-resistant and to show heat-promoted germination than resprouters (Paula and Pausas 2008). On the other hand, Ne'eman et al. (2009) tested 13 south-eastern Australian species varying in their degree of resprouting ability for germination responses to smoke and found no relationship between fire response strategy and smoke-responsive germination – these data area included in our dataset. It remains to be seen whether responsiveness to post-fire cues including smoke and heat is related to fire response strategy at the broader, global scale, or if this relationship might differ among floras – we suspect it may be the former.

Our analysis revealed that smoke-responsive germination is more likely within drier habitats in south-eastern Australia, most likely explained by fire-frequency being higher in 'dry' rather than 'wet' habitat types (Figure 1). This is consistent with theory suggesting that increasing fire frequency selects for species that are fire tolerant, and the observation that rainforests in Australia generally grow where fires are more or less absent (Bond & Keeley 2005). For these reasons, a low frequency of fire-responsive traits might be expected in rainforests. However, there were only 13 rainforest species in our dataset, making it difficult to say with certainty whether rainforest species are generally less likely to germinate in response to fire cues such as smoke. Smoke-responsive germination has been documented in

a wide range of species, including those from ecosystems with infrequent or absent fires, and in agricultural cultivars (Dixon et al. 2009). Certain soil bacteria have been shown to release karrikinolide in response to disturbance, and enhanced germination responses can be stimulated by topsoil disturbance. Plants that germinate in response to karrikinolide released by soil disturbance would nevertheless benefit from showing this same response to karrikinolide released by fire (Bradshaw et al. 2011). Therefore, although germination responses to the post-fire environment might be advantageous, smoke-responsive germination may not have evolved directly through selection in fire-prone environments (Bradshaw et al. 2011) and should also be investigated in communities that are not fire-prone, such as rainforests. Yet to our knowledge no other study has looked at whether smoke-responsive germination is less prevalent in rainforest habitats, and a global comparative analysis would directly address the question of whether smoke-responsive germination is associated with certain habitat types.

Smoke-responsive germination was highly phylogenetically clustered, being less likely among the Leguminaceae, and more likely in Rubiaceae and Poaceae (Figure 2). Leguminaceae include many species whose seeds are physiologically dormant, requiring heat shock before germination (Baskin & Baskin 2014). Heat-shocked seeds may also respond to smoke. It is possible that this result is an artefact of dormant seeds being treated with smoke, rather than true absence of a smoke response. It is also possible that Leguminaceae invest in physiological dormancy as an alternative to smoke-responsiveness. Further examination of these two hypotheses would be fruitful avenues of investigation. By contrast, Rubiaceae and Poaceae were more likely to show smoke-induced germination than other families. However, the result for Rubiaceae rests on only six species, of which two were only identified to Genus level. Poaceae showed greater smoke-responsive germination than expected at random, with 14 out of 22 species showing a positive response. Poaceae entries came from six different studies, excluding the possibility of methodological artefact. A link between smoke-responsive germination and the Poaceae has not, to our knowledge, been reported from other floras. Read et al. (2000) reported that 7 out of 9 south-eastern Australian Poaceae showed positive germination response to smoke – these data are included in our database. Cox et al. (2017) report that smoke influences germination of eight grassland species from the southern High Plains region in Texas, USA, but do not compare frequency of smoke-responsive germination between grasses and other families, or grassland floras and other habitats. Grasses tend to support fire and be common in fire-prone environments such as open

woodlands and grasslands, and rare in rainforest (Prior et al. 2017), meaning the pattern found for Poaceae may derive from the habitats they are associated with. The lack of phylogenetic patterning of smoke-responsive germination at the higher taxonomic levels (eudicots *vs.* monocots, and asterids, rosids, commelinids, other eudicots, other monocots) suggests that smoke-responsive germination has likely arisen multiple times at the tips of the phylogeny, rather than deep in the evolutionary tree (Figure 2). This pattern would be consistent with smoke-responsive germination having resulted from convergent evolution associated with habitat types. Alternatively, Poaceae may be prone to fire-responsive germination, and so be selected for in high fire-frequencies habitats. A larger dataset of smoke-responsive germination studies across plant Families and habitat types could address this question.

Our analysis evaluated the effectiveness of different smoke application methods used to test for smoke-responsiveness. Aerosol smoke was significantly more likely to induce a germination response to smoke than smoke water, or ash/charate (Figure 2). This is consistent with results for Western Australian species (Roche et al. 1997a; Lloyd et al. 2000; Rokich et al. 2002), but methodological comparisons were not available for other flora. Studies conducted *in-situ* were also significantly more likely to find smoke-responsive germination. Our dataset only included 10 tests conducted *in-situ*, but this finding may reflect the greater contextual complexity of field tests relative to laboratory germination trials. A test was categorised as *in-situ* only if it was conducted in the field, by applying treatments to the soil and its constituent soil seed bank. Therefore, a greater chance of a positive germination response to smoke may arise due to interactions with other important environmental cues for seed germination, such as seed burial, ageing, or seasonal cold temperatures (Clarke et al. 2000; Downes et al. 2015). Determining which of these variables related to *in-situ* seed germination experiments interact most strongly with smoke treatments would be a useful avenue of further investigation. While there is a literature investigating individual factors associated with seed germination in the field such as burial time and ageing (e.g. Roche et al. 1997; Downes et al. 2015), to our knowledge a more comprehensive analysis comparing these variables across *in-situ* and *ex-situ* studies has not been attempted.

Consistent with our expectations, smoke-responsive germination was unrelated to growth form. However, our dataset was dominated by woody species (58%) and contained no geophytic growth forms, meaning we could not test our data for agreement with previous findings that geophytes are much less likely to show smoke-responsive germination (Keeley

& Bond 1997; Brown et al. 2003a). While 33 data sources were found, and these covered 233 different species, our south-eastern Australia dataset clearly reflects a bias towards testing for smoke-responsive germination in woody, perennial plants from woodlands, forests, shrublands, and heaths. This may be due to prior research focus on Mediterranean type climates and vegetation (dominated by woody perennial plants) in the literature (Keeley 1995a, 2012), or that woody, perennial plant-dominated vegetation types are the most common vegetation types in south-eastern Australia. A broader selection of plant growth forms, life histories, and habitat types is desirable to tease out the ecological underpinnings of smoke-responsive germination at the global scale.

Conclusions

Here we have synthesised the available data on smoke-responsive germination in south-eastern Australian plants. While almost half of the species tested show smoke-responsive germination, there are clear patterns within this data. Species with obligate seeding fire responses, and from drier habitats were more likely to be smoke responders. At the same time, clear phylogenetic clustering was present, with Leguminaceae less likely to respond, and Poaceae and Rubiaceae more likely to respond to smoke as a germination cue. These species-level patterns in germination responses to fire may affect vegetation communities via several key mechanisms. It has been suggested that fires are consumers of vegetation, much like herbivores, and as such exert top-down control on vegetation communities. Both processes may affect a community's species composition, as well as vegetation structure, by removing plant biomass and increasing the importance of tolerance to consumption relative to competitive ability (Bond & Keeley 2005). Thus, fires may influence vegetation composition towards species that best tolerate selection pressure from fires, and away from those best suited to competition. Species tolerant of higher fire frequencies are likely to include those that germinate in response to fire-related cues, resulting in habitats with higher fire frequencies having more smoke-responsive germinators. Resprouters also tolerate fire but can only survive where inter-fire intervals are sufficient to allow regrowth and seed set (Pausas & Keeley 2014). Our results suggest that this would mean more obligate seeders (generally annual species) and more Poaceae in areas with the highest fire frequencies. These findings are consistent with the ecological literature suggesting that frequent fires suppress tree saplings and influence ecosystems towards savannahs and grasslands rather than forests (Bond et al. 2005). Investigating these patterns at the global

scale would reveal whether such patterns in smoke-responsive germination are particular to the south-eastern Australian flora or are more broadly applicable. We suspect that, given their alignment with our understanding of the effects of fire on ecosystems as outlined above (Bond & Keeley 2005, Bond et al. 2005), that these patterns are likely to apply broadly. By providing a better understanding of patterns of smoke-responsive germination in south-eastern Australian plants, our results contribute to the global understanding of how smoke-responsive germination is distributed across different floras and climatic zones.

Acknowledgements

We would like to thank C. Laws for her work on compiling the database, Dr C. Offord and Dr P. Cuneo from the Australian Botanic Garden Mount Annan for early discussions, and L. Von Richter from the Australian Botanic Garden Mount Annan for assistance accessing records at the Australian Seed Bank. This research was funded by an Australian Flora Foundation Research Grant.

Research outputs from this project

Peer-reviewed journal articles

1. **Carthy, AJR, and Leishman, MR.** (2016) How do germination responses to smoke relate to phylogeny, growth form, fire response strategies and vegetation type? A focus on eastern Australia. *Australasian Plant Conservation* 25(2): 3-4.
2. **Carthy, AJR, Tims, A., Geedicke, I, and Leishman, MR.** (Accepted) Broad-scale patterns in smoke-responsive germination from the south-eastern Australian flora. *Journal of Vegetation Science*.
3. **Carthy, AJR, Geedicke, I, Tims, A, Alroy, J, and Leishman, MR.** (In prep.) A global comparison of patterns in smoke-responsive germination in plants.

Conference presentations

4. **Carthy, AJR, and Leishman, MR** (2016) How does smoke-promoted germination relate to phylogeny, growth form, fire response strategies and vegetation type? A focus on eastern Australia. Australian National Seed Science Forum, Australian Botanic Gardens, Mount Annan NSW, 14-16th March

Database

1. **Carthy, AJR, and Leishman, MR.** (In prep). A database of global plant germination responses to smoke. *Ecological Archives*.

References

- Adkins, S.W., Davidson, P.J., Matthew, L., Navie, S.C., Wills, D.A., Taylor, I.N. & Bellairs, S.M. 2000. Smoke and Germination of Arable and Rangeland Weeds. In: Black, M., Bradfor, K.J. & Vazquez-Ramos, J. (eds.) *Seed Biology: Advances and Applications. Proceedings of the Sixth International Workshop on Seeds, Mérida, México, 1999*. CABI.
- Adkins, S.W. & Peters, N.C.B. 2001. Smoke derived from burnt vegetation stimulates germination of arable weeds. *Seed Science Research* 11: 213-222.
- Agee, J. K. 1993. *Fire Ecology of Pacific Northwest Forests*. Island Press, Washington DC, US.
- Agresti, A. 2002. *Categorical Data Analysis*. John Wiley & Sons, Inc., Somerset, US.
- Bargmann, T., Maren, I.E. & Vandvik, V. 2014. Life after fire: smoke and ash as germination cues in ericads, herbs and graminoids of northern heathlands. *Applied Vegetation Science* 17: 670-679.
- Baskin, C.C. & Baskin, J.M. 2014. *Seeds Ecology, Biogeography, and Evolution of Dormancy and Germination*. Academic Press Ltd, London, England.
- Bond, W.J. & Keeley, J.E. 2005. Fire as a global 'herbivore': the ecology and evolution of flammable ecosystems. *Trends in Ecology & Evolution* 20: 387-394.
- Bond, W.J., Woodward, F.I. & Keeley, J.E. 2005. The global distribution of ecosystems in a world without fire. *New Phytologist* 165: 525-538.
- Bowman, D. M. J. S. 2000. *Australian Rainforests: Islands of Green in a Land of Fire*. Cambridge University Press, Cambridge, UK.
- Bradshaw, S.D., Dixon, K.W., Hopper, S.D., Lambers, H. & Turner, S.R. 2011. Little evidence for fire-adapted plant traits in Mediterranean climate regions. *Trends in Plant Science* 16: 69-76.
- Brown, N. 1994. First the gas, now instant dehydrated smoke. *Veld & Flora* 80: 72.
- Brown, N. 1992. Seed germination in the fynbos fire ephemeral, *Syncarpha vestita* (L) B-Nord is promoted by smoke, aqueous extracts of smoke and charred wood derived from burning the ericoid-leaved shrub, *Passerina vulgaris* Thoday. *International Journal of Wildland Fire* 3: 203-206.

- Brown, N.A.C. & van Staden, J. 1997. Smoke as a germination cue: a review. *Plant Growth Regulation* 22: 115-124.
- Brown, N.A.C., van Staden, J., Daws, M.I., Johnson, T. & van Wyk, A.E. 2003a. Patterns in the seed germination response to smoke in plants from the Cape Floristic Region, South Africa. *South African Journal of Botany* 69: 514-525.
- Brown, N.A.C., Van Staden, J., Johnson, T. & Daws, M.I. 2003b. A summary of patterns in the seed germination response to smoke in plants from the Cape floral region. In: Smith, R.D., Dickie, J.B., Linington, S.H., Pritchard, H.W. & Probert, R.J. (eds.) *Seed Conservation: Turning Science Into Practice*, pp. 563-574. Kew: Royal Botanic Gardens, Kew.
- Clarke, P.J., Davison, E.A. & Fulloon, L. 2000. Germination and dormancy of grassy woodland and forest species: effects of smoke, heat, darkness and cold. *Australian Journal of Botany* 48: 687-700.
- Cox, R.D., Chou, Y.F. & Wester, D.B. 2017. Smoke water and heat influence emergence of shortgrass prairie species. *Fire Ecology* 13: 138-148.
- Crosti, R., P. Ladd, K. Dixon, and B. Piotto. 2006. Post-fire germination: the effect of smoke on seeds of selected species from the central Mediterranean basin. *Forest Ecology and Management* 221: 306-312
- Daws, M.I., Downes, K.S., Koch, J.M. & Willyams, D. 2014. Is broad-scale smoke-water application always a useful tool for improving seedling emergence in post-mining restoration? Evidence from jarrah forest restoration in Western Australia. *South African Journal of Botany* 90: 109-113.
- De Lange, J.H. & Boucher, C. 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *South African Journal of Botany* 56: 700-703.
- Dixon, K.W., Merritt, D.J., Flematti, G.R. & Ghisalberti, E.L. 2009. Karrikinolide – a phytoreactive compound derived from smoke with applications in horticulture, ecological restoration and agriculture. *Acta Horticulturae* 813: 155-170.
- Dixon, K.W., Roche, S. & Pate, J.S. 1995. The promotive effect of smoke derived from burnt native vegetation on seed-germination of Western Australian plants. *Oecologia* 101: 185-192.

- Downes, K.S., Light, M.E., Posta, M. & van Staden, J. 2015. Fire-related cues and the germination of eight Conostylis (Haemodoraceae) taxa, when freshly collected, after burial and after laboratory storage. *Seed Science Research* 25: 286-298.
- Enright, N.J., Goldblum, D., Ata, P. & Ashton, D.H. 1997. The independent effects of heat, smoke and ash on emergence of seedlings from the soil seed bank of a heathy Eucalyptus woodland in Grampians (Gariwerd) National Park, western Victoria. *Australian Journal of Ecology* 22: 81-88.
- Enright, N.J. & Kintrup, A. 2001. Effects of smoke, heat and charred wood on the germination of dormant soil-stored seeds from a *Eucalyptus baxteri* heathy-woodland in Victoria, SE Australia. *Austral Ecology* 26: 132-141.
- Flematti, G.R., Ghisalberti, E.L., Dixon, K.W. & Trengove, R.D. 2004. A compound from smoke that promotes seed germination. *Science* 305: 977.
- Flematti, G.R., Merritt, D.J., Piggott, M.J., Trengove, R.D., Smith, S.M., Dixon, K.W. & Ghisalberti, E.L. 2011. Burning vegetation produces cyanohydrins that liberate cyanide and stimulate seed germination. *Nature Communications* 2: 360.
- Fritz, S.A. and Purvis, A., 2010. Selectivity in mammalian extinction risk and threat types: a new measure of phylogenetic signal strength in binary traits. *Conservation Biology* 24: 1042-1051.
- Gill, A. 1981. Adaptive responses of Australian vascular plant species to fires. In: Gill, A., Groves, R. & Nobel, I. (eds.) *Fire and the Australian Biota*, pp. 243-271. Australian Academy of Science, Canberra.
- Hope, A. C. C. 1968. A simplified Monte Carlo test procedure. *Journal of the Royal Statistical Society* 30: 582-598.
- Jefferson, L., Pennacchio, M. & Havens-Young, K. 2014. *Ecology of plant-derived smoke: its use in seed germination*. Oxford University Press, Oxford, UK.
- Keeley, J.E. 2012. Fire in mediterranean climate ecosystems - a comparative overview. *Israel Journal of Ecology & Evolution* 58: 123-135.
- Keeley, J.E. 1995a. Seed-germination patterns in fire-prone Mediterranean-climate regions. In: Arroyo, M.T.K., Zedler, P.H. & Fox, M.D. (eds.) *Ecology and Biogeography of Mediterranean Ecosystems in Chile, California, and Australia*. Springer-Verlag, New York.

- Keeley, J.E. 1995b. Seed-Germination Patterns in Fire-Prone Mediterranean-Climate Regions. In: Arroyo, M.T.K., Zedler, P.H. & Fox, M.D. (eds.) *Ecology and Biogeography of Mediterranean Ecosystems in Chile, California, and Australia*, pp. 239-273. Springer New York, New York, NY.
- Keeley, J.E. & Bond, W.J. 1997. Convergent seed germination in South African fynbos and Californian chaparral. *Plant Ecology* 133: 153-167.
- Keeley, J.E. & Fotheringham, C.J. 1998. Smoke-induced seed germination in California chaparral. *Ecology* 79: 2320-2336.
- Keeley, J.E., Pausas, J.G., Rundel, P.W., Bond, W.J. & Bradstock, R.A. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends in Plant Science* 16: 406-411.
- Kulkarni, M.G., Sparg, S.G., Light, M.E. & Van Staden, J. 2006. Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide. *Journal of Agronomy and Crop Science* 192: 395-398.
- Light, M.E., Burger, B.V., Staerk, D., Kohout, L. & Van Staden, J. 2010. Butenolides from plant-derived smoke: natural plant-growth regulators with antagonistic actions on seed germination. *Journal of Natural Products* 73: 267-269.
- Lloyd, M.V., Dixon, K.W. & Sivasithamparam, K. 2000. Comparative effects of different smoke treatments on germination of Australian native plants. *Austral Ecology* 25: 610-615.
- MacArthur, R. & Wilson, E. 1967. *The Theory of Island Biogeography*. The University of Chicago Press, Princeton.
- Mackenzie, B.D.E., Auld, T.D., Keith, D.A., Hui, F.K.C. & Ooi, M.K.J. 2016. The effect of seasonal ambient temperatures on fire-stimulated germination of species with physiological dormancy: a case study using *Boronia* (Rutaceae). *PLoS One* 11: e0156142.
- Maga, J.A. (ed.) 1988. *Smoke in Food Processing*. CRC Press, Florida.
- Miller, J.T., Murphy, D.J., Brown, G.K., Richardson, D.M. & González-Orozco, C.E. 2011. The evolution and phylogenetic placement of invasive Australian *Acacia* species. *Diversity and Distributions* 17: 848-860.

- Moreira, B. & Pausas, J. G. 2016. Shedding light through the smoke on the germination of Mediterranean Basin flora. *South African Journal of Botany*: In press.
- Murphy, D.J., Brown, G.K., Miller, J.T. & Ladiges, P.Y. 2010. Molecular phylogeny of Acacia Mill. (Mimosoideae: Leguminosae): evidence for major clades and informal classification. *Taxon* 59: 7-19.
- Ne'eman, G., Ne'eman, R., Keith, D.A. & Whelan, R.J. 2009. Does post-fire plant regeneration mode affect the germination response to fire-related cues? *Oecologia* 159: 483-492.
- NSW Office of Environment and Heritage 2014. *The NSW Flora Fire Response Database v2.1*. In. Fire Ecology Unit, Office of Environment and Heritage, Hurstville, New South Wales, Australia.
- Paula, S. & Pausas, J.G. 2008. Burning seeds: germinative response to heat treatments in relation to resprouting ability. *Journal of Ecology* 96: 543-552.
- Pausas, J.G. & Keeley, J.E. 2014. Evolutionary ecology of resprouting and seeding in fire-prone ecosystems. *New Phytologist* 204: 55-65.
- Pausas, J.G. & Verdú, M. 2005. Plant persistence traits in fire-prone ecosystems of the Mediterranean basin: a phylogenetic approach. *Oikos* 109: 196-202.
- Prior, L.D., Murphy, B.P., Williamson, G.J., Cochrane, M.A., Jolly, W.M. & Bowman, D. 2017. Does inherent flammability of grass and litter fuels contribute to continental patterns of landscape fire activity? *Journal of Biogeography* 44: 1225-1238.
- R Core Team 2014. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Rambaut, A. 2012. FigTree v1. 4. *Molecular evolution, phylogenetics and epidemiology*. Edinburgh, UK: University of Edinburgh, Institute of Evolutionary Biology.
- Read, T.R., Bellairs, S.M., Mulligan, D.R. & Lamb, D. 2000. Smoke and heat effects on soil seed bank germination for the re-establishment of a native forest community in New South Wales. *Austral Ecology* 25: 48-57.
- Roche, S., Dixon, K.W. & Pate, J.S. 1997a. Seed ageing and smoke: Partner cues in the amelioration of seed dormancy in selected Australian native species. *Australian Journal of Botany* 45: 783-815.

- Roche, S., Koch, J.M. & Dixon, K.W. 1997b. Smoke enhanced seed germination for mine rehabilitation in the southwest of Western Australia. *Restoration Ecology* 5: 191-203.
- Rokich, D.P. & Dixon, K.W. 2007. Recent advances in restoration ecology, with a focus on the Banksia woodland and the smoke germination tool. *Australian Journal of Botany* 55: 375-389.
- Rokich, D.P., Dixon, K.W., Sivasithamparam, K. & Meney, K.A. 2002. Smoke, mulch, and seed broadcasting effects on woodland restoration in Western Australia. *Restoration Ecology* 10: 185-194.
- Stevens, P.F. 2001 onwards. *The Angiosperm Phylogeny Website v.13*, June 2017.
<http://www.mobot.org/MOBOT/research/APweb/>.
- Tang, Y., Boulter, S.L. & Kitching, R.L. 2003. Heat and smoke effects on the germination of seeds from soil seed banks across forest edges between subtropical rainforest and eucalypt forest at Lamington National Park, south-eastern Queensland, Australia. *Australian Journal of Botany* 51: 227-237.
- Thomas, P.B., Morris, E.C. & Auld, T.D. 2007. Response surfaces for the combined effects of heat shock and smoke on germination of 16 species forming soil seed banks in south-east Australia. *Austral Ecology* 32: 605-616.
- van Staden, J., Jager, A.K., Light, M.E. & Burger, B.V. 2004. Isolation of the major germination cue from plant-derived smoke. *South African Journal of Botany* 70: 654-659.
- Webb, C.O. & Donoghue, M.J. 2005. Phylomatic: tree assembly for applied phylogenetics. *Molecular Ecology Resources* 5: 181-183.
- Whelan, R.J. 1995. *The Ecology of Fire*. Cambridge University Press, Cambridge, UK.
- Zanne, A.E., Tank, D.C., Cornwell, W.K., Eastman, J.M., Smith, S.A., FitzJohn, R.G., McGlenn, D.J., O'Meara, B.C., Moles, A.T. & Reich, P.B. 2014. Three keys to the radiation of angiosperms into freezing environments. *Nature* 506: 89-92.