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# Shedding light through the smoke on the germination of Mediterranean Basin flora

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## ABSTRACT

Natural and anthropogenic fires have historically been part of the Mediterranean Basin (MB). As a result of this long exposure to fires, MB flora has developed various strategies to persist under recurrent fires. One of these strategies is to survive and regenerate by resprouting and another is through post-fire seedling recruitment. For species that recruit seedlings after a fire there is evidence that fire-related germination cues are implicated in the process of triggering seedling emergence from soil-stored seeds. In some species, particularly in Cistaceae and Fabaceae, germination is stimulated by heat. Fire-related chemical germination cues, such as smoke, charcoal, and nitrogenous compounds (collectively termed 'smoke'), have also been recognised as promoting seed germination in MB flora. However, the role of smoke in the germination of MB plant species has received less attention and recognition than the role of heat-shock and is generally seen as having a relatively limited role as a post-fire germination cue in MB flora.

Our hypothesis is that this is due to the fact that research on smoke-stimulated germination in the MB region has been limited and poorly addressed, and this is biasing our view on the role of smoke in MB flora.

Using available literature, we first analyse the role of smoke in the germination of MB flora and discuss some of the issues that limit our understanding of its role on the flora of the region. We point to gaps in the literature and provide suggestions for further research.

We conclude that differences in evidence of smoke-stimulated germination in MB flora when compared with other Mediterranean regions may not reflect real ecological or evolutionary differences among these regions but are likely the consequence of the limited number of well-designed studies in the MB.

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## 1. Introduction

Most plant communities in the Mediterranean Basin (MB) are highly fire-prone and subject to recurrent fires (Pausas, 2004). Although currently most fires have an anthropogenic origin, fires remain a natural phenomenon in the region (Keeley et al., 2012). In fact, there is increasing evidence that fire has played an important role in shaping fire-related plant traits (He et al., 2012; Pausas et al., 2012) and in driving plant community assembly (Verdú and Pausas, 2007; Pausas and Verdú, 2008). As in other fire-prone ecosystems, many MB plant species have developed several traits to cope with recurrent fires, such as those related to resprouting and post-fire seedling recruitment (Pausas et al., 2004).

Resprouting is a widespread mechanism enabling individual plants to persist after fire; it is widespread in MB flora and many of the resprouting species of this region do not show post-fire seedling emergence or fire-stimulated germination (Paula and Pausas, 2008; Paula et al., 2009). The other mechanism is the accumulation of persistence seed banks in the canopy (serotiny; *Pinus halepensis, P. brutia, P. pinaster;* Hernández-Serrano et al., 2013) or soil. Seedling recruitment from soil-stored seedbanks is enhanced by fire because it acts as a germination cue, triggering emergence when resources are highly available. The most important fire-related germination cues are heat, and the chemical products resulting from biomass combustion during fire such as: smoke; charred wood; or nitrogenous compounds (for simplicity, all of these fire-derived chemicals are here termed 'smoke', e.g., smoke-stimulated germination).

In the MB, fire-stimulated germination and post-fire seedling emergence is common and has been extensively studied, especially in woody species with physical seed dormancy, such as in Fabaceae and Cistaceae (Paula et al., 2009). Seeds of these species have specialised structures in the seed coat (e.g., the strophiole in Fabaceae and the chalazal plug in Cistaceae) that move, or become disrupted, as a response to external factors, and so enable water to pass through the impermeable seed coat layer(s) (Baskin et al., 2000; Baskin and Baskin, 2014). In the MB, the regulation of dormancy release in these species with physical seed dormancy is typically linked to heat-shock resulting from fire (Herranz et al., 1998, 1999; Moreira and Pausas, 2012) and is similar to that which occurs in other fire-prone regions with Mediterranean-type climate (MTC), such as South Africa, California and southern Australia (Bell et al., 1993; Keeley, 1995; Keeley and Bond, 1997). However, post-fire seedling recruitment in MB flora is not limited to species with physical dormancy and is also widespread in species with water-permeable seed coats – such as Lamiaceae, Ericaceae, Asteraceae, and Poaceae (Paula et al., 2009). The role of fire in germination and post-fire seedling recruitment of these species is less clear; however, there is now evidence that both fire-related heat-shock and smoke are important germination cues (Moreira et al., 2010).

Despite recent efforts, the role of smoke in the germination of MB plant species has received less attention than the role of heat-shock (Paula and Pausas, 2008; Paula et al., 2009). It was assumed, based on a small sample size, that smoke-induced germination is less frequent in the MB than in other MTC regions; and that a substantial amount of post-fire recruitment is probably the result of opportunistic colonising species that use other (not strictly fire-related) cues to signal general disturbances, such as light and temperature. This would suggest that fire-stimulated germination results from seed characteristics that are not specialised for fire (Keeley and Baer-Keeley, 1999). Indeed, smoke is generally seen as having a relatively limited role as a post-fire germination cue in MB flora (Buhk and Hensen, 2006; Ne'eman et al., 2012). Our hypothesis is that this view is due to the fact that research on smoke-stimulated germination in the MB region has been limited and not properly addressed, and this is biasing our view on the role of smoke in MB flora. Here we summarise the role of smoke in the germination of the MB flora while highlighting some of the issues that limit our understanding in this topic. We also offer suggestions for further research. For this review, we included sites outside the region with typical mediterranean climate (sensu Olson and Dinerstein, 1998; Fig. 1) because of the local similarity in the flora (particularly, the studied species are also present in the mediterranean climate region) and in the local environment, and because fires are also frequent.

## 2. Methodological issues

Up to now, 24 studies have been published that perform germination (laboratory) experiments with fire-derived products on MB species; five used liquid smoke (Moreira et al., 2010; Tavşanoğlu, 2011; Çatav et al., 2012; Moreira et al., 2012; Çatav et al., 2014); six used aerosol smoke (Crosti et al., 2006; Reyes and Casal, 2006a;

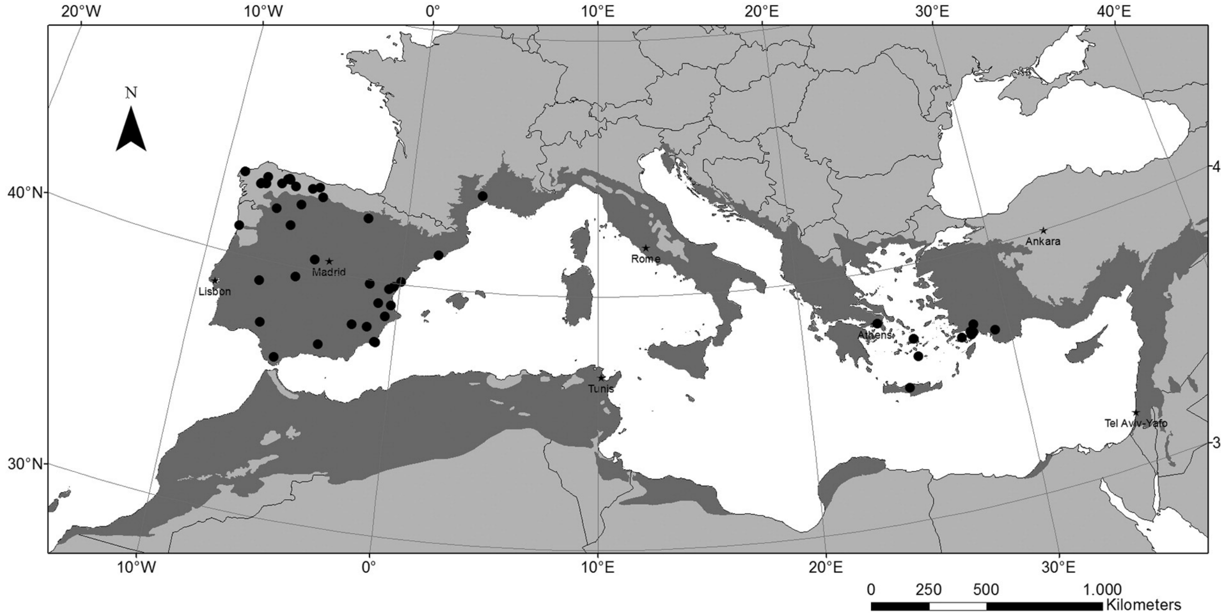

**Fig. 1.** Distribution of the sites where smoke-stimulated germination has been studied (black dots) in the Mediterranean Basin. Dark grey area represents the region with mediterranean-type climate (Olson and Dinerstein, 1998). Few sites outside of this region were also considered because of the local similarity in the flora (particularly, the studied species are present in the mediterranean climate region) and environment, and because fires are also frequent.

Rivas et al., 2006a, 2006b; Reyes and Trabaud, 2009; Iglesia Rodríguez, 2010); two used charred wood (Salvador and Lloret, 1995; Keeley and Baer-Keeley, 1999); six used nitrogenous compounds (Doussi and Thanos, 1997; Rodríguez-Echeverría and Pérez-Fernández, 2001; Cruz et al., 2003; Pérez-Fernández et al., 2006; Luna and Moreno, 2009; Oliva et al., 2009) and five used several of these products (Pérez-Fernández and Rodríguez-Echeverría, 2003; Buhk and Hensen, 2006; Reyes and Casal, 2006b; Çatav et al., 2015; Martínez-Baniela et al., 2016). In addition, one study evaluated the effect of smoke on seedling emergence from the soil seed bank (Tormo et al., 2014). These studies include examples of both eastern and western parts of the MB, although the distribution is far from homogeneous across the region (see Fig. 1). A total of 180 species (33 plant families and 108 genera, Table 1) were tested (in the 24 laboratory studies), with 67 species (37% of the total) showing evidence of smoke stimulation (significant and >10%) in response to at least one of the treatments applied (considering different studies, experimental conditions, and chemical treatments). However, the selection criteria for the tested species were often poor and, in many cases, a priori we would not expect a smoke response; this has the consequence of inflating the negative results. Below, we summarise the main pitfalls in the smoke-germination studies made in the MB.

### *2.1. Species with water-impermeable seeds (physical dormancy)*

Of the 180 species tested, 53 (29%) are from families typically presenting seeds with physical dormancy, particularly Cistaceae (24 species studied) and Fabaceae (29 species studied; Tables 1 and 2). These species are typically stimulated by heat and the lack of response to smoke is expected and likely to result from the water-impermeability of the seed coat (Keeley and Fotheringham, 2000; Moreira et al., 2010). In agreement with this, only six Cistaceae and three Fabaceae showed some positive response and, in some cases, after a pre-treatment of scarification or boiling water (Rodríguez-Echeverría and Pérez-Fernández, 2001; Pérez-Fernández and Rodríguez-Echeverría, 2003; Pérez-Fernández et al., 2006; Martínez-Baniela et al., 2016). All other studies conducted with MB flora failed to find clear evidence of smoke-stimulated germination in these families (e.g., Keeley and Baer-Keeley, 1999; Buhk and Hensen, 2006; Rivas et al., 2006b; Reyes and Trabaud, 2009; Moreira et al., 2010; Tavşanoğlu, 2011; Çatav et al., 2012; Moreira et al., 2012). Certainly, exposure to both heat-shock and smoke could interact in promoting seed germination of some species with water-impermeable seed coats; that is, smoke may enhance germination once physical dormancy has been broken by heat-shock, although there is no evidence for this in the MB (Tavşanoğlu, 2011; Moreira et al., 2012). In addition, care must be taken in such combined studies because charred wood and smoke solutions are typically acidic (pH < 5.5; Keeley and Baer-Keeley, 1999; Pérez-Fernández and Rodríguez-Echeverría, 2003; Moreira et al., 2010) and could potentially affect seed coat permeability, particularly if the coats were previously weakened by heat-shock. This could provide ecologically unrealistic results, because these solutions are likely to be more acidic than in natural conditions – where fire increases soil pH as a result of the complete combustion of fuel at high temperatures (Certini, 2005).

**Table 1**
Summary of the role of fire-derived chemicals in the germination of MB species based on experiments performed with smoke, charred wood, or nitrogenous compounds. For each family, we provide the number of species and genera tested, the number of species with a significant positive response (#Stim), and the mean and maximum increase in germination above the control value (Stimulation, in %).

| Family | Genus | Species | #Stim | Stimulation (%) | |
|---|---|---|---|---|---|
| | | | | Mean | Maximum |
| Anacardiaceae | 1 | 1 | 0 | – | – |
| Apiaceae | 5 | 6 | 3 | 17 | 20 |
| Arecaceae | 1 | 1 | 0 | – | – |
| Asteraceae | 18 | 20 | 10 | 34 | 53 |
| Betulaceae | 1 | 1 | 0 | – | – |
| Brassicaceae | 1 | 3 | 1 | 42 | 42 |
| Caprifoliaceae | 1 | 1 | 0 | – | – |
| Caryophyllaceae | 3 | 3 | 1 | 24 | 24 |
| Cistaceae | 6 | 24 | 6 | 30 | 32 |
| Clusiaceae | 1 | 3 | 1 | 23 | 23 |
| Convolvulaceae | 1 | 1 | 0 | – | – |
| Cupressaceae | 1 | 1 | 0 | – | – |
| Ericaceae | 2 | 13 | 12 | 28 | 55 |
| Fabaceae | 19 | 29 | 3 | 15 | 16 |
| Fagaceae | 1 | 3 | 2 | 18 | 19 |
| Lamiaceae | 14 | 29 | 14 | 26 | 50 |
| Liliaceae | 3 | 4 | 0 | – | – |
| Linaceae | 1 | 2 | 0 | – | – |
| Malvaceae | 1 | 1 | 0 | – | – |
| Oleaceae | 2 | 2 | 0 | – | – |
| Onagraceae | 1 | 1 | 0 | – | – |
| Papaveraceae | 1 | 1 | 0 | – | – |
| Pinaceae | 1 | 3 | 0 | – | – |
| Plantaginaceae | 2 | 3 | 0 | – | – |
| Poaceae | 8 | 9 | 4 | 34 | 40 |
| Polygonaceae | 1 | 1 | 1 | 31 | 31 |
| Primulaceae | 1 | 1 | 1 | 34 | 34 |
| Ranunculaceae | 1 | 2 | 1 | 12 | 12 |
| Resedaceae | 1 | 2 | 2 | 24 | 30 |
| Rhamnaceae | 1 | 1 | 1 | 13 | 13 |
| Rosaceae | 1 | 1 | 1 | 53 | 53 |
| Rubiaceae | 2 | 2 | 1 | 30 | 30 |
| Scrophulariaceae | 4 | 5 | 2 | 43 | 68 |
| Total: 33 | 108 | 180 | 67 | 28 | 34 |

**Table 2**
Summary of the role of fire-derived chemicals in the germination of MB species based on experiments performed with smoke, charred wood or nitrogenous compounds. Species are divided in four groups based on seed coat water-permeability (WI: water-impermeable; WP: water-permeable) and field evidence of post-fire seedling emergence (S-: no evidence; S+: evidence; S?: no or ambiguous data). For each group, we provide the number of species and genera tested, the number of species with a significant positive response (#Stim) and the mean and maximum increase in germination above the control value (Stimulation, in %).

| Group | Genus | Species | #Stim | Stimulation (%) | |
|---|---|---|---|---|---|
| | | | | Mean | Maximum |
| WI | 25 | 53 | 9 | 26 | 65 |
| WP S- | 34 | 41 | 12 | 24 | 40 |
| WP S? | 43 | 46 | 20 | 28 | 68 |
| WP S+ | 32 | 40 | 26 | 33 | 55 |
| Overall | 134 | 180 | 67 | 27 | 57 |

### *2.2. Species without field evidence of post-fire seedling emergence*

From the remaining families (127 species), there is evidence in 41 species of a lack of post-fire seedling emergence in the field (Paula et al., 2009), and so they are not appropriate candidates to test the role of smoke in germination. Indeed, 16 of these species (39%) presented null or reduced dormancy (lower than 25%). Nevertheless, 12 species (29%) exhibited some degree of smoke-stimulated germination (Table 2). Similarly, there is evidence from other regions of smoke-stimulated germination in species that are not typical in fire-prone ecosystems, or that do not recruit seedlings after fire. However, although studying the role of fire-related cues in these species may be interesting from the physiological or applied points of view (particularly for horticulturally important species, Brown et al., 2003; Kulkarni et al., 2011, or for ecological restoration/conservation, Roche et al., 1997a), the ecological relevance is limited (Keeley and Pausas, 2016).

Additionally, there is no available information about post-fire seedling emergence for 46 of the species considered (Paula et al., 2009). Some of these species may recruit after fire, but not all. Indeed, 11 species (24%) presented null or reduced dormancy, while 12 species

(43% of the total) exhibited some degree of smoke-stimulated germination (Table 2).

### *2.3. Species with water-permeable seeds and evidence of post-fire seedling emergence in the field*

The species in which we would expect smoke to be most relevant are those that have water-permeable seeds and show post-fire seedling emergence (post-fire seeders). However, relatively few of these species have been tested (40, i.e., 23% of the total species assayed). There is evidence of smoke-stimulated germination in 26 (65%) of these species, and the effect of smoke is particularly obvious in Lamiaceae and Ericaceae, but also in some Asteraceae, Poaceae, Primulaceae and Rosaceae (Table 1). For many of these taxa, a response to just heat-shock or smoke has been documented but is often variable; and in many cases, both cues may stimulate germination. There are also a number of taxa that, despite having high levels of seed dormancy, show no response to any of the cues. Many of these observations reflect the fact that we do not fully understand the complexity of seed dormancy and the germination requirements of the species. There are many sources of variation from other MTC regions that have been little considered in MB flora, including: intraspecific variation in the germination response to smoke (e.g., Baldwin and Morse, 1994; Baldwin et al., 1994; Tieu et al., 2001a; Thomas et al., 2007); the effect of seed after ripening (Tieu et al., 2001b; Baker et al., 2005a; Downes et al., 2015); the requirement of a pre-treatment for scarification (Baker et al., 2005b); the effect of the dormancy cycle during burial (Roche et al., 1997b; Keeley and Fotheringham, 1998; Tieu et al., 2001b; Baker et al., 2005a, 2005b); or the effect of light regimes and incubation temperatures on smoke response (Keeley, 1987; Bell et al., 1993; Keith, 1997; Baker et al., 2005a, 2005c; Tsuyuzaki and Miyoshi, 2009; Thomas et al., 2010). Indeed, the few studies performed on MB flora that controlled for some of these variables found evidence that seed dormancy and germination response to smoke (particularly in Lamiaceae and Ericaceae) is affected by: light (Doussi and Thanos, 1997; Keeley and Baer-Keeley, 1999; Luna and Moreno, 2009); seed after-ripening (Iglesia Rodríguez, 2010); and intraspecific variability among populations (Cruz et al., 2003).

### *2.4. A bias towards perennial species*

Most smoke-germination experiments in the MB have been performed with woody species (61%) and a few in non-woody perennials (27%, Table 3). In California and South Africa, smoke-stimulated germination is very common in annuals (Keeley and Bond, 1997). However, few of these species have been tested for the effect of smoke in their germination in the MB, despite annual plants being very important in the early phases of post-fire dynamics (Naveh, 1974; Garcia-Novo, 1977; Papanastasis, 1977; Arianoutsou and Margaris, 1981; Arianoutsou, 1984; Trabaud, 1994; Ferrandis et al., 1999; Naveh, 1999). In fact, a recent field study suggests that smoke-stimulated germination might be more important for post-fire regeneration of MB annual flora than previously thought (Tormo et al., 2014).

**Table 3**
Summary of the role of fire-derived chemicals in the germination of MB species based on experiments performed with smoke, charred wood or nitrogenous compounds. Species are divided by growth form. For each growth form, we provide the number of species and genera tested, the number of species with a significant positive response (#Stim) and the mean and maximum increase in germination above the control value (Stimulation, in %).

| Growth Form | Genus | Species | #Stim | Stimulation (%) | |
|---|---|---|---|---|---|
| | | | | Mean | Maximum |
| Annuals (incl. biennials) | 21 | 21 | 9 | 26 | 45 |
| Non-woody perennials (incl. geophytes) | 46 | 49 | 18 | 29 | 68 |
| Subshrubs | 31 | 54 | 16 | 28 | 53 |
| Shrubs | 15 | 41 | 20 | 33 | 65 |
| Trees (incl. large shrubs) | 11 | 15 | 4 | 16 | 19 |
| Overall | 124 | 180 | 67 | 26 | 50 |

## 3. Further research

More studies are needed to generate a better understanding of smoke-stimulated germination of MB flora. However, it is important that these studies are carefully designed so that they can clearly elicit the ecological role of smoke. Below we suggest some points that need to be considered when designing an experiment for testing the role of smoke as a germination cue.

### *3.1. Species selection*

It is important to perform a careful and informed species selection in order to study the effect of smoke, and the first information needed is the dormancy class of the species (Baskin and Baskin, 2004). For instance, it is probably worthless performing germination experiments in species that lack seed dormancy, that is, in species that germinate very well under a wide range of incubation temperatures or light regimes. Species from families that typically present seeds with physical dormancy (such as Cistaceae and Fabaceae) and prolific post-fire seedling emergence are stimulated by heat – and so smoke is likely to have little relevance (Keeley and Fotheringham, 2000; Moreira et al., 2010).

It is also important to move the focus away from perennial species and concentrate on fire ephemerals. These short-lived herbaceous species are an important but understudied component of post-fire dynamics in the MB. These species may be expected to germinate in unburnt nutrient-rich areas, as suggested by the stimulated germination in response to the nitrogenous compounds observed in some of these species. However, in burnt areas, fire-related cues probably play an important role in the germination of these species from the soil seedbank. This is because MB fire ephemerals germinate massively from soil seedbanks after fire and are normally replaced within five years of fire by woody vegetation (Naveh, 1974; Garcia-Novo, 1977; Papanastasis, 1977; Arianoutsou and Margaris, 1981; Arianoutsou, 1984; Trabaud, 1994; Arianoutsou, 1998; Ferrandis et al., 1999; Naveh, 1999). While the germination requirements of this group of species have been extensively studied in other MTC regions such as in California, South Africa, or southern Australia (Keeley and Keeley, 1987; Keeley and Bond, 1997; Keeley and Fotheringham, 1998; Baker et al., 2005a, 2005b, 2005c) this is not the case for the MB.

It is also imperative to achieve a better knowledge of post-fire regeneration mechanisms in MB flora, particularly regarding post-fire seedling emergence. Despite recent efforts to gather this information (Paula et al., 2009), field evidence of post-fire seedling emergence (or lack of it) is unavailable (or ambiguous) for many species, and particularly for herbaceous species. Too few studies provide quantitative values based on experiments that enable the ability of the species to recruit after fire to be confidently inferred.

### *3.2. Understanding germination requirements and the complexity of dormancy*

Future studies should first understand germination requirements and the complexity of the dormancy of the studied species. Globally, most species exhibiting smoke-stimulated germination have seeds with water-permeable coats and probably present physiological dormancy (Brown, 1993; Dixon et al., 1995; Keeley and Bond, 1997; Keeley and Fotheringham, 1998; Keeley and Fotheringham, 2000; Moreira et al., 2010). Indeed, the most common type of dormancy in MTC ecosystems is physiological dormancy (Baskin and Baskin, 2014). Temperature is one of the main factors that regulates germination in seeds with physiological dormancy (Baskin and Baskin, 2014) and physiologically dormant seeds may require particular incubation temperatures for germination, independent of fire cues (Bell et al., 1993; Baker et al., 2005a, 2005c; Thomas et al., 2010). Light regimes may also affect germination (Keeley, 1987; Keith, 1997; Baker et al., 2005a, 2005c; Tsuyuzaki and Miyoshi, 2009). Indeed, some species may germinate in laboratory conditions, although germination in the field may be inhibited by darkness because seeds are buried in soil. Therefore, it is important to test germination response over a range of germination temperatures and light regimes (light, alternating light/darkness, and darkness). In many cases, seeds have been incubated in dark conditions, but the germination counts were made under light (e.g., Moreira et al., 2010; Çatav et al., 2012, 2014), and a small amount of light exposure may be sufficient to overcome dark-imposed seed dormancy (Doussi and Thanos, 1997). It is important to perform the germination experiments with freshly collected seed (Baskin et al., 2006) and understand the effects of after-ripening during dry storage (frequent in many species with non-deep physiological seed dormancy; Baskin and Baskin, 2004). In most studies performed in the MB, seeds were dry-stored for some (variable) period at room temperature prior to germination testing. In these cases, it is possible that the (physiological) dormancy status of the seeds, and thus their response to smoke, may have changed as a result of after-ripening following seed collection. This may render results inconclusive and prevent an appropriate ecological interpretation. For many species that recruit seedlings after fire, germination in response to smoke is enhanced by a period of burial (Roche et al., 1997b; Keeley and Fotheringham, 1998; Tieu et al., 2001b; Baker et al., 2005b; Downes et al., 2015). Various factors may be responsible for enhanced germination in response to smoke following burial – such as increased permeability of the seed coat, or exposure to temperature and moisture fluctuations. During burial, seeds may go through periods in which dormancy is alleviated, so increasing the range of conditions (e.g., temperature and/or light) for germination and/or enabling responsiveness to external cues (such as smoke); and periods in which dormancy is re-imposed (Baker et al., 2005a). Thus, species showing limited germination in response to smoke when freshly collected, or after laboratory storage, may not necessarily lack a smoke response. To date, no study has ascertained the effect of seed burial and how response to smoke may change due to dormancy cycling in MB species. A better understanding of the species germination requirements (e.g., incubation temperatures and light regimes, stratification, after-ripening, and burial) prior to smoke exposure will ensure a proper ecological interpretation of the results.

### *3.3. The importance of testing over a large concentration gradient of chemical solutions*

Most studies in the MB have been performed without testing species germination response to a concentration/dilution gradient, and so they are prone to type II error. Studies should conduct experiments over a large concentration gradient of chemical solutions (charred wood, nitrogenous compounds, and smoke) because the concentration of aqueous solutions that enhance germination is often species-specific (e.g., Moreira et al., 2010; Çatav et al., 2014) and likely to be highly variable under natural conditions. Additionally, to date, no study on the MB flora has investigated the effects of specific smoke-derived compounds (e.g., Nelson et al. 2012). We should not expect differences in the response of MB flora to these compounds when compared with other MTC regions. However, since there are probably many different compounds responsible for stimulating germination (Keeley and Pausas, 2016), a study of their effects in a large biogeographical region (MB) could provide clues for improving our understanding of the evolution of smoke-stimulated germination. Experimentation with smoke-derived germination compounds could also enable us to properly specify the concentrations used, and thus make more accurate comparisons among studies and species.

### *3.4. Combined effect of heat and smoke on germination*

Germination in species with physical seed dormancy is typically stimulated by heat and the lack of response to smoke is likely to result from the water-impermeability of the seed coat (Keeley and Fotheringham, 2000; Moreira et al., 2010). The interaction between heat and smoke on the germination of species from families that typically present seeds with physical dormancy may only be worth testing on species in which the rupture of the water-impermeable seed coat by heat-shock (i.e., despite seeds freely imbibing water) is insufficient to trigger germination (after testing a range of heat treatments). Regarding species with physiological dormant seeds, heat can interact with smoke to promote, slow or inhibit germination (Keeley, 1987; Keith, 1997; Tieu et al., 2001c; Thomas et al., 2003, 2007; Tsuyuzaki and Miyoshi, 2009). To date, only two studies (Tavşanoğlu, 2011; Moreira et al., 2012) have tested the combined treatment of heat and smoke on MB species, encompassing a total of three species and several populations (two species with water-impermeable seeds and one with water-permeable seeds). Generally, the combined treatment of heat plus smoke does not show any statistically significant positive effect on germination, compared to the independent treatment that shows the maximum stimulation; none of the species required the combination of both cues in order to germinate (unitive effect, sensu Thomas et al., 2003). A more comprehensive study testing the combined treatment of heat and smoke on MB species would allow a better understanding of the interaction between these fire-related germination cues.

## 4. Conclusions

Evidence of smoke-stimulated germination in the MB flora is, in general, more limited than for other MTC regions such as California, South Africa, or southern Australia. We propose that these differences do not necessarily reflect real ecological or evolutionary differences among regions, but are the consequence of a limited number of well-designed studies in the MB. These limitations are related mainly to: (1) bias towards perennial species; (2) inappropriate species selection (e.g. species with physical dormancy, with non-dormant seeds, with limited evidence of post-fire seedling emergence); and (3) a limited knowledge/understanding of germination requirements, and a complexity of dormancy in the studied species. Despite these limitations, there are many species with evidence of smoke-stimulated germination (Table 1), and in some cases, germination is strongly dependent on

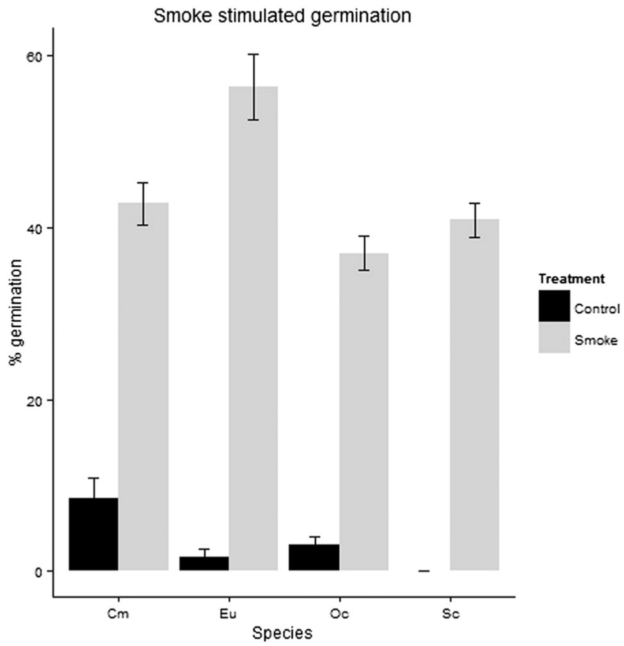

**Fig. 2.** Example of four MB species from different families in which germination is strongly dependent on smoke (mean ± se). Cm: *Coris monspeliensis* (Primulaceae), *Eu: Erica umbellata* (Ericaceae), Oc: *Onopordum caricum* (Asteraceae), *Sc: Stachys cretica* (Lamiaceae). Data from Moreira et al. (2010), and Çatav et al. (2014, 2015).

smoke (Fig. 2). Overcoming these limitations would enable this region to be better placed in context among other MTC regions, as well enabling a proper understanding of the evolutionary role of smoke in the MB to be gained.

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