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Fire persistence mechanisms in Mediterranean plants: ecological and evolutionary consequences

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“Life is not easy for any of us. But what of that? We must have perseverance and above all confidence in ourselves. We must believe that we are gifted for something and that this thing must be attained.”

Marie Curie

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Chapter 1

General introduction

Mediterranean ecosystems, including the Mediterranean Basin, have undergone a long history of fire (Pausas and Keeley 2009, Keeley et al. 2012). This recurrent pressure exerted by fire is a key factor in the evolution of plants and in the structure of plant communities. Many plant species have traits and mechanisms that allow them to persist and regenerate after fires. Thus, these traits have an adaptive value and are essential to understand the evolution and diversity of species in fire prone areas (Bond and van Wilgen 1996, Bond and Midgley 2001, Keeley et al. 2011, 2012).

In Mediterranean shrublands and woodlands, which are typically composed of a relatively dense vegetation, fires are frequently crown fires; i.e., fire affects all the aboveground part of the vegetation (of the different strata; herbaceous, shrub and tree), including the canopies of the dominant growth forms. The intensity and, in general, the severity of these fires is high and much of the vegetation is greatly affected. The two main mechanisms that allow post-fire regeneration of plants, after these fires, are the ability to resprout (R) and the capacity of seedling recruitment (P) after fire. These two post-fire persistence mechanisms occur at two different hierarchical levels, at individual level and population level, respectively (Pausas and Lavorel 2003, Pausas et al. 2004) and allow the recovery of the ecosystem affected by fire. Individuals of a species persist after fire if they have the ability to resprout (R+). If individuals do not persist (R-), populations can persist if these have formed a seed bank with the capacity to withstand fire, allowing the recruitment of new individuals after fire (P+). These two post-fire regeneration mechanisms are not mutually exclusive, and thus species might be classified into four strategies: obligate resprouting species (R+P-), obligate seeding species (R-P+), facultative species (R+P+) and species without any post-fire regeneration mechanism (R-P-).

Plant flammability is also an important mechanism for fire persistence. Flammability-enhancing traits are considered to favour individuals if the elevated flammability confers them fitness benefits. In seeder species, higher flammability could increase the recruitment opportunities for the offspring of the individual with enhanced flammability by increasing the chance of opening spaces and by producing the necessary cues for triggering germination from the seedbank (“kill thy neighbour” hypothesis; Bond and Midgley 1995). In addition,

enhanced flammability (e.g., measured as enhanced fire spread rates) has also been suggested as a mechanism for fire protection in plants (Gagnon et al. 2010) and might be important, particularly for resprouting species.

Despite the importance of these fire persistence mechanisms (post-fire resprouting and seedling recruitment and flammability), both for understanding species evolution and for predicting future ecosystem changes, the current level of knowledge still presents some gaps.

Resprouting

The ability to resprout after a fire, after the elimination of all aboveground biomass, characterizes the *resprouting species* and is important for persisting in fire-prone ecosystems. Resprouting consists of the production of new sprouts from protected buds (e.g., by the soil or by the plant bark). This mechanism confers persistence at individual level (and thus at population level) because part of the individual (at least the below ground part) resists fire and does not die. These species accumulate carbohydrate and nutrient reserves in the belowground organs (that are protected by the soil) and the presence of these reserves has been associated with the resprouting capacity (Bell et al. 1996, Canadell and López-Soria 1998, Bell and Ojeda 1999).

Species have often been classified as resprouters (R+) or non resprouters (R-, e.g., Pausas et al. 2004, Vesik et al. 2004). However, there is evidence of a certain intraspecific variability in this mechanism (Paula et al. 2009). In addition, the information available on the factors driving the ability to resprout, the vigour of resprout, or the overall resprouting success is very low; such information is practically nonexistent for species that, although common, do not dominate the landscape.

Seedling recruitment

Post-fire seedling recruitment characterizes the *seeder species* and is also an important mechanism for persisting in fire-prone ecosystems. Seeder species have several traits that allow the success of this mechanism. Annual seed production is mostly accumulated in a seedbank (in the canopy or in the soil) because seeds are dormant. In addition, seeds resist the heat produced by fire and, frequently, germination is stimulated by fire-related germination cues (e.g., heat and chemical compounds, resulting from the combustion of vegetation during fire and present in the smoke and charred wood).

Seeder species are often classified as such by field evidences of post-fire seedling emergence. In many cases there is a flush of germination after the fire (with the first raining period) and the support for classifying a species as seeder is notorious. However, post-fire seedling recruitment is a complex mechanism and may depend on several abiotic factors (precipitation in the autumn/winter after the fire, fire intensity, etc) and on plant traits. In the Mediterranean Basin, seeder species (P+) have been almost exclusively linked to species with seeds presenting a water impermeable seedcoat (hardcoated seeds, mainly Cistaceae and Fabaceae). It is commonly assumed that it is this type of seedcoat that grants fire resistance and stimulated germination (by heat), allowing persistence at the population level. It has also been proposed that the cumulative effect of summer temperatures in the bare soil (e.g., in vegetation gaps) could have a similar effect to fire (i.e., the total heat dose might be similar and thus have analogous effects on seed dormancy). This has led to the suggestion that the temperature thresholds associated to the release from physical seed dormancy might have evolved independently of fire (Bradshaw et al. 2011).

The effect of the chemical compounds, resulting from the combustion of vegetation during fire, and present in the smoke and charred wood, which is very relevant in other mediterranean ecosystems (e.g., South Africa, California, Keeley and Bond 1997; Keeley and Fotheringham 1998), has been studied very superficially in the Mediterranean Basin. From the panoply of chemical compounds produced during fire, different compounds have already been isolated and shown to stimulated germination (e.g., Karrikins: Flematti et al. 2004, 2005; or Cyanohydrins: Flematti et al. 2011). This fire-related cue can have a significant effect on some species, particularly in species with seeds that have a water permeable seedcoat. That is, fire-stimulated germination and post-fire seedling recruitment is related not only to the weakening (permeabilization) of the seedcoat (physical seed dormancy) but also to the induction of physiological processes (Keeley 1987, Baskin and Baskin 1998). Indeed, there is evidence of post fire seedling emergence in species lacking seeds with water impermeable seedcoat and many of these species are abundant in fire-prone landscapes. However, smoke-stimulated germination might only have an adaptive value in fire-prone ecosystems if seeds also resist to the heat produced by fire.

To understand the mechanisms associated to seed dormancy and post-fire germination and seedling recruitment, experimentation is required (i.e., subjected seeds to the different treatments; summer temperatures, fire temperatures, and smoke) to quantify the extent of seed resistance to the heat and ascertain which cues stimulate germination.

Flammability

There is evidence, both in the Mediterranean Basin and in other mediterranean ecosystems, that seeder species are more present in areas with recurrent fires than in areas with low fire frequency (Pausas et al. 2004, 2006), specifically in areas with high fire recurrence these species are more present than expected from the regional species pool (Verdú and Pausas 2007). Indeed, it has been suggested that, in these species that are favoured by fire (fire increases the population size), not only the post-fire regeneration mechanisms have been selected, but also traits which favour the occurrence of fires, such as flammability-enhancing traits. This hypothesis was first formulated at the community level (Mutch 1970) and reformulated at the individual level (inclusive fitness theory, the “*kill thy neighbour*” hypothesis; Bond and Midgley 1995).

The **general objective** of this thesis is to deepen and broaden our knowledge on the fire persistence mechanisms of mediterranean plants (post-fire resprouting and seedling recruitment, and flammability) and understand the ecological and evolutionary consequences of these mechanisms, particularly in species of the Mediterranean Basin.

The **specific objectives** are to 1) identify the factors that determine the resprouting success of Mediterranean plant species (e.g., carbohydrates, nutrients) and understand the different processes that compose this mechanism; 2) identify the factors that determine the ability to germinate in Mediterranean plant species (e.g., degree of resistance and / or stimulation of seeds by heat and smoke); 3) determine the importance of the seedcoat type (water permeable vs water impermeable seedcoat) in the seed resistance and stimulated germination by fire-related cues (heat and smoke); 4) evaluate the intra-specific variability in traits related to the ability to germinate after the fire (eg, degree of resistance and/or stimulation of seeds by the effect of fire (heat and smoke) and the factors responsible for this variability; 5) evaluate the intra-specific variability in plant flammability and its correlation with the pressure exerted by fire (fire history).

Regardless the importance of fire in the origin of the fire persistence mechanisms, the **general hypothesis** of this thesis is that fire has contributed to shape Mediterranean species and ecosystems through processes of natural selection and ecological filtering (Herrera 1992), and

that the acquisition and/or maintenance of these persistence mechanisms has led to variations in species traits.

The available information on the magnitude and variability (intra- and inter-specific) of plant's post-fire resprouting ability and seedling recruitment, and flammability is very low. Understanding the variability in the traits associated with the mechanisms that confer fire persistence to plant species is essential to understand the evolutionary processes that determine the structure and diversity of mediterranean plant communities, and responses to changes in fire regimes (including global change).

The ability to resprout after a fire, after the elimination of all aboveground biomass, is largely related to the presence of belowground organs containing reserves and buds (Bond and Midgley 2001). There is evidence of differences in the carbohydrate reserves in the belowground organs between resprouting and non-resprouting species and that this is a major factor for the resprouting capacity (Bell et al. 1996, Bell and Ojeda 1999). There is also evidence for other factors that may influence the resprouting ability, as the amount of belowground mineral macronutrients reserves (Canadell and López-Soria 1998). Consequently, the amount of these reserves (carbohydrates and nutrients) might also be responsible for the intra-specific variability in the ability to resprout of adult plants of resprouting species (**Hypothesis 1; Chapter 2**).

It is commonly assumed that, in the Mediterranean Basin, heat stimulates post-fire germination, particularly in species with seeds that have a water impermeable seedcoat (hardcoated seeds; i.e., Cistaceae and Fabaceae). On the contrary, it is assumed that smoke has a less relevant role, in the stimulation of germination and post-fire seedling recruitment, compared with other mediterranean ecosystems, which could suggest that stimulation of germination by fire is less relevant in the Mediterranean Basin comparing with other mediterranean ecosystems (e.g., California). Indeed, it has been suggested that even the physical dormancy (in seeds with water impermeable seedcoat) exhibited by many mediterranean species may be an adaptation to summer conditions of the mediterranean ecosystems rather than to fire. However, recent studies show that the structure of mediterranean plant communities is strongly conditioned by the ability to germinate after fires (P). Consequently, it is likely that the physical seed dormancy exhibited by mediterranean species is best explained as an adaptation to fire than to the mediterranean summer conditions (**Hypothesis 2, Chapter 3**) and that both heat and smoke associated to fire have a significant

effect on the recruitment process following fires of the Mediterranean Basin species (e.g., stimulation of germination and early growth and establishment of seedlings) (**Hypothesis 3; Chapter 4 and Chapter 5**). Furthermore, the germination response, of the different species, to heat and smoke might be influenced by the type of seed coat; i.e., seeds with water permeable seedcoat respond to smoke, while seeds with water impermeable seedcoat respond to heat (**Hypothesis 4; Chapter 7**).

Despite the fact that post-fire germination in Mediterranean Basin flora might be stimulated both by the heat and by the chemical compounds in smoke (hypothesis 3) and fire might have played a different role shaping germination in different species and even different populations within species (hypothesis 4), it is likely that the pressure exerted by fire in the Mediterranean Basin has not select for germination in response to the obligatory combination of both cues (**Hypothesis 5; Chapter 6**). This is because the ecological significance of a species having exclusively seeds that germinate when exposed simultaneously to both factors would only be understandable to 1) avoid germination related to very low intensity fires in which conditions for seedling establishment are not improved (e.g., fire does not produce gaps) and/or to 2) avoid dormancy release by summer temperatures. However, 1) it is likely that these fires are not very common in Mediterranean shrublands and 2) Mediterranean species have other mechanisms that minimize the loss of seed dormancy by summer temperatures (hypothesis 2).

In the Mediterranean Basin, Pausas and Verdú (2005) found a negative evolutionary correlation between the ability to resprout (R) and the ability to recruit new individuals after fire (P), which suggests an evolutionary selective pressure towards species with one of the two persistence mechanisms. The possession of both mechanisms (R+P+, facultative species) does not seem to have been favoured along the evolution in the Mediterranean Basin (although it has been under different fire regimes; Pausas et al. 2004, 2006). This may be due to the fact that by presenting both persistence mechanisms, the selective pressure on each of them decreases. As a result, post-fire seed persistence and stimulated germination of facultative species (R+P+) should be less efficient than in obligate seeders (R-P+) (Paula and Pausas 2008; **Hypothesis 6; Chapter 7**).

Fires produce a high dynamics and heterogeneity on the landscape and Mediterranean ecosystems can be seen as a mosaic with different fire history and in different stages of succession (e.g., with different fire recurrence, severity and time since last fire). At a local scale, the heterogeneity produced by fire, along with other factors of local importance as

species interactions and heterogeneity in the availability of resources, should generate a large variability in traits related to regeneration (e.g., seed size, degree of seed dormancy, and magnitude of stimulated germination by fire-related cues). This variability should be especially pronounced in species which depend on post-fire germination as the only mechanism of post-fire persistence (R-P+, obligate seeders). As a result, obligate seeders must respond to the spatiotemporal heterogeneity of mediterranean fire-prone ecosystems, possessing a variability in these regeneration traits relatively large at local scale (within regions), so that exceeds the variability at larger scales (biogeographic). That is, in fire-prone areas, fire and other local processes that generate variability should be more important than biogeographic processes in structuring biodiversity (**Hypothesis 7; Chapter 8**).

As seeder species are favoured by fire (fire increases the population size), it has been suggested that these species should tend to be more flammable (Schwilk and Ackerly 2001, Saura-Mas et al. 2010). This relationship between germination and flammability has been formulated both between species within the community (Mutch 1970) as between individuals at the population level (Bond and Midgley 1995), although in both cases very few studies have clearly demonstrated this relationship. If natural selection favours traits related to flammability, individuals of seeder species, in recurrently burnt populations, should have greater flammability than individuals of the same species in populations with low fire pressure (**Hypothesis 8; Chapter 9 and Chapter 10**).

These hypotheses will be validated by resprouting experiments, germination experiments, and flammability experiments. The aim is to study the variability in three different fire persistence mechanisms, the resprouting ability, the post-fire germination and seedling recruitment and flammability. The thesis focus mainly on woody species, given their importance and dominance in mediterranean ecosystems, and because it allows for a homogeneous study from the point of view of the sampling techniques. Currently available data have many limitations, thus it is necessary to perform experiments with particular species and locations for obtaining rigorous, quantitative and comparable data.

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Chapter 2

To resprout or not to resprout: factors driving intraspecific variability in resprouting

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Abstract

Resprouting is a mechanism that allows individual plants to persist in disturbance-prone ecosystems. It is often considered a binary trait, defining species as resprouters or non-resprouters. Although this dichotomous classification accounts for a high proportion of the interspecific variability in resprouting, it does not account for the intraspecific variability, as not all individuals of resprouting species successfully resprout, even if they are subject to a similar disturbance. To ascertain the causes of the intraspecific variability in resprouting we propose a conceptual model that disaggregates the process of resprouting into three sequential steps: initial ability to resprout, resprouting vigour and post-resprouting survival. To test this model, we clipped 151 plants of three resprouting species (*Anthyllis cytisoides*, *Globularia alypum* and *Linum suffruticosum*) and, for each individual, we analysed the carbohydrate (starch and soluble sugars) and nutrient (N and P) concentrations in the roots at the moment of the disturbance (clipping all aboveground biomass). We then monitored initial ability to resprout, resprouting vigour and post-resprouting survival in each individual during a one-year period. *Anthyllis* showed high initial ability to resprout and high post-resprouting survival. *Globularia* and *Linum* had low final resprouting success, mainly due to their low post-resprouting survival, but also to the low initial ability to resprout in *Linum*. All three species showed variable resprouting vigour. Our results suggest that resprouting success is limited by different phases of the process of resprouting, depending on the species. Intraspecific variability in resprouting supported the importance of: a) the pre-disturbance state of the plant (i.e. plant size and stored resources) on the initial ability to resprout and on the resprouting vigour, and b) the initial post-disturbance capacity to acquire resources (i.e. resprouting vigour) on the post-resprouting survival. The proposed three-step model of resprouting provides a mechanistic description of the factors driving intraspecific variability in resprouting.

Introduction

Resprouting is a mechanism that allows individual plants to regenerate after the elimination of the aboveground biomass and persist in ecosystems with recurrent disturbances (Bond and Midgley 2001). In such ecosystems, intraspecific variability in resprouting might affect the dynamics of the community by determining to what extent species persist or decline under high disturbance recurrence. Thus, understanding this persistence mechanism is essential for explaining species diversity and evolution (Bond and Midgley 2003) and for predicting vegetation shifts in the framework of global change (Bond et al. 2003, Bradley and Pregitzer 2007).

Resprouting has often been considered a binary trait and used to separate species as resprouters or non-resprouters (Pausas et al. 2004, Vesk et al. 2004). However, after a disturbance that eliminates all aboveground plant parts, neither do all individuals of resprouting species survive, nor do all individuals of non-resprouting species unavoidably die. That is, even if this dichotomous classification of species accounts for a high proportion of the interspecific variability in resprouting, it does not account for the intraspecific variability observed in this trait (Gill and Bradstock 1992, Vesk et al. 2004, Paula et al. 2009). Thus, in many cases, a semi-quantitative classification (e.g. no, weak, strong resprouters) could be more adequate than a binary classification (Vesk and Westoby 2004a). While most studies on resprouting have focused on the response of plants to different disturbance regimes (i.e. different disturbance type, recurrence and intensity; Moreno and Oechel 1991, Lloret and López-Soria 1993, Cruz et al. 2003a, Vesk et al. 2004), few have focused on the response of different individuals (of the same species) to a similar disturbance. However, the fact that intraspecific variability in resprouting is observed under disturbances with similar characteristics (Lloret and López-Soria 1993, Canadell and López-Soria 1998, Kabeya and Sakai 2005, Paula and Ojeda 2006) suggests that this variability may be driven by factors related to the state of the individual plants (e.g. non-structural carbohydrates and nutrients levels; Iwasa and Kubo 1997, Canadell and López-Soria 1998). Thus, studying the factors driving the intraspecific variability in resprouting, for a given disturbance, may provide clues for a mechanistic understanding of the process of resprouting.

A model for the resprouting process

Resprouting might be limited by different phases in the resprouting process, depending on the species. Thus, to ascertain the causes of the intraspecific variability in resprouting, we propose a conceptual model that disaggregates the process of resprouting into three sequential steps: initial ability to resprout, resprouting vigour and post-resprouting survival (Fig. 1).

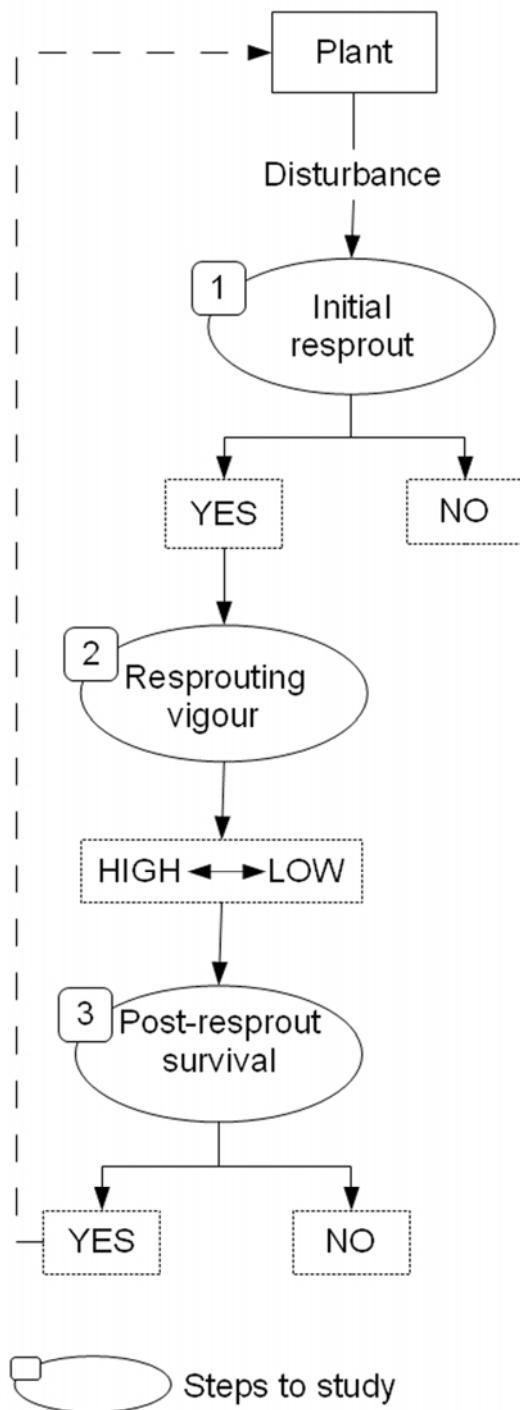


Figure 1. The three-step model of the process of resprouting (step 1: initial resprouting ability, step 2: resprouting vigour, and step 3: post-resprouting survival).

The initial ability to resprout (i.e. whether or not resprouting is initiated after the elimination of aboveground biomass) does not necessarily correspond to the final resprouting success, as many plants do not survive after resprouting is initiated (Moreno and Oechel 1991, Lloret and López-Soria 1993, Cruz et al. 2003a, Catry et al. 2010). A plant can initiate resprouting after its aboveground biomass has been eliminated only if it has a stock of surviving dormant buds (i.e. bud bank; Vesk and Westoby 2004b) from which new sprouts can grow. Moreover, as disturbed plants lose photosynthetic capacity, they need to have sufficient carbohydrates and nutrients stored in their belowground organs to maintain respiration demands and to initiate resprouting (Pate et al. 1990, Bowen and Pate 1993, Canadell and López-Soria 1998, Kabeya and Sakai 2005). Indeed, resprouting species exhibit significantly higher starch levels in their belowground organs than non-resprouting ones (Pate et al. 1990, Bell et al. 1996, Bell and Ojeda 1999, Verdaguer and Ojeda 2002), and these levels decrease during post-disturbance resprouting (Bowen and Pate 1993, Canadell and López-Soria 1998, Kabeya and Sakai 2005). The bud bank should be coordinated with the stored reserves (carbohydrates and nutrients) so that it does not limit resprouting; stored resources would be of limited importance in the absence of buds. Only in severe disturbances (e.g. extremely intense fires) the bud bank might be destroyed preventing plants from initiating the resprouting response regardless of the level of stored reserves (Vesk and Westoby 2004b).

Individuals that resprout can differ quantitatively in their resprouting vigour (e.g. resprouted biomass; Stohlgren et al. 1984, Lloret and López-Soria 1993, Paula and Ojeda 2006). In many cases resprouting vigour is positively correlated with pre-disturbance plant size (Stohlgren et al. 1984, Lloret and López-Soria 1993, Pausas 1997, Vesk et al. 2004, Paula and Ojeda 2006, Vesk 2006) and with the concentration of carbohydrates and nutrients stored in the belowground organs of the plant (Chapin III et al. 1990, Avice et al. 1996, Kabeya and Sakai 2005). However, resprouting vigour is not always correlated with carbohydrates, either because they may be stored in excess (Cruz et al. 2003a, b) or because, once the plant produces photosynthetic biomass, the carbon fixed by the resprouts is used to continue growth (Richards and Caldwell 1985, Chapin III et al. 1990, Avice et al. 1996).

Post-resprouting mortality (i.e. failure to survive after resprouting is initiated) is commonly observed in many species (Moreno and Oechel 1991, Lloret and López-Soria 1993, Cruz et al. 2003a, Catry et al. 2010) and it is not necessarily controlled by the same factors that drive the initial ability to resprout (Lloret and López-Soria 1993), although both processes are often lumped together in a single response (Paula et al. 2009). Resprouting vigour is related to the

capacity of plants to quickly acquire resources for sustaining the maintenance costs and avoiding reserve depletion (Chapin III et al. 1990, Iwasa and Kubo 1997), and thus it should be positively correlated with the probability of plant survival (Moreno and Oechel 1991). Nevertheless, the role of resprouting vigour as a key factor for post-resprouting survival has never been addressed in detail.

To the best of our knowledge, no study has related a plant's resprouting response at individual level to the pre-disturbance stored resources measured in the same individual. Most studies compare levels of stored resources between disturbed and undisturbed individuals (Canadell and López-Soria 1998, Cruz et al. 2003a, b). Thus, the examination of a plant's pre-disturbance state (measured in each individual) should provide a better understanding of the intraspecific variability in the process of resprouting.

Our hypothesis is that there are different steps in the process of resprouting and that each of these steps is limited by different factors. We propose that resprouting can be viewed as a three-step sequential process (Fig. 1) and that studying each of these steps should provide important clues to understand the intraspecific variability and the mechanisms behind resprouting. Specifically, we predict that a plant's initial ability to resprout and its resprouting vigour are determined by the pre-disturbance state of the plant (e.g. plant size and stored reserves), and that post-resprouting survival is related to the plant's resprouting vigour (indicative of the plant's capacity to acquire resources). We tested this model using three species living in **fi re-prone** ecosystems of the eastern Iberian Peninsula (western Mediterranean Basin).

Methods

Species and study area

We selected three shrub species common in eastern Spain: *Anthyllis cytisoides* (Fabaceae), *Globularia alypum* (Plantaginaceae) and *Linum suffruticosum* (Linaceae). These species are relatively short (< 1 m tall) and most aboveground biomass is composed by fine fuel that is consumed during the passage of a fire. *Anthyllis* and *Globularia* typically resprout from the root crown, while *Linum* can also resprout from rhizomes or roots. None of them has a lignotuber (Paula et al. 2009). The three species are facultative resprouters (R+P+ sensu Pausas et al. 2004), that is, after fire they are able to regenerate by resprouting and by recruiting new individuals from seed. We selected facultative resprouters because of the high observed intraspecific variability in resprouting (Paula et al. 2009), as species possessing the

two post-fire regeneration mechanisms might be less efficient in each of them when compared with species that only present one of the mechanisms (Paula and Pausas 2008). Because of the different local abundance, two species (*Linum* and *Globularia*) were sampled at Barranc de la Casella (Alzira, Valencia, Spain; 39°09'N, 00°29'W) and the third (*Anthyllis*) in Serra Calderona (Gátova, Valencia, Spain; 39°74'N, 00°51'W). Barranc de la Casella is located at 700 m a.s.l., the bedrock type is limestone, mean annual precipitation is 633 mm and mean annual temperature is 17.4°C. It is a shrubland dominated by *Linum suffruticosum*, *Globularia alypum*, *Lithodora fruticosa*, *Ulex parviflorus* and *Erica* sp. pl. with an herbaceous layer of *Brachypodium retusum* and with scattered *Pinus halepensis* trees. Serra Calderona is located at 600 m a.s.l., the bedrock type is sandstone, mean annual precipitation is 584 mm and mean annual temperature is 17.7°C. It is an open *Pinus halepensis* stand with a shrubland dominated by *Anthyllis cytisoides*, *Cistus monspeliensis* and *Ulex parviflorus* and an herbaceous layer of *Brachypodium retusum*. In both study areas, precipitation falls mainly in two peaks, a more prominent one in autumn and a weaker one in spring. Summers are warm and dry and wildfires are frequent (Pausas 2004), although they have not affected the study sites for the last 30 years.

Sampling

All plants were clipped in August 2008, i.e. in summer, when physiological activity is low and most fires occur (severe disturbance). For each species we selected ca 50 adult plants, separated by a minimum distance of 10 m, which lacked any sign of recent disturbance (Table 1).

For each individual of each species we measured its height, clipped the aboveground biomass and then fenced it to avoid mammal herbivory. All individuals were clipped at ca 10 mm aboveground without damaging buds below this point (i.e. no variability in the severity of the disturbance) during the same week (i.e. no seasonal variability) and, for each species, on the same site (i.e. low spatial variability). Clipped biomass was oven-dried at 80°C for eight days to obtain the total dry weight. We carefully excavated a few centimetres of the soil close to each individual and extracted a lateral root sample (less than 1 cm in diameter) for carbohydrate and nutrient analyses. The length of the root samples ranged from 13 to 67 cm and was not significantly different between resprouting and non-resprouting plants (*Linum* $p = 0.11$, *Anthyllis* $p = 0.75$, *Globularia* $p = 0.83$). To minimize respiration and loss of carbohydrates during transport, root samples were placed individually in a hermetic bag with silica gel and transported in a cooler (at 0°C) to the laboratory. Root samples were oven-dried

at 80°C for 24 h and because of the high surface/volume ratio of the roots sampled, root temperature was expected to increase very quickly stopping respiration immediately. Samples were conserved frozen at -18°C in hermetic bags with silica gel until analysed (ca one year) for determination of soluble sugars (SS; i.e. sucrose, glucose and fructose), starch, nitrogen (N) and phosphorus (P) concentrations (Agrolab Analítica SL). Soluble sugars and phosphorus were measured through calorimetric reaction and spectrometric analysis (Bertramson 1942, Green et al. 1989). Nitrogen was analyzed following the Kjeldahl method (Nelson and Sommers 1973); and starch was determined by an enzymatic procedure and measured calorimetrically using a coupled glucose oxidase/peroxidase reaction and spectrometric analysis (Rasmussen and Henry 1990). Total amount of carbohydrate and nutrients (i.e. pool size) was estimated multiplying the concentration values by the plant pre-clipping dry weight biomass. Carbohydrate and nutrient were determined in 41, 42 and 35 of the individuals clipped, for *Linum*, *Anthyllis* and *Globularia*, respectively.

Table 1. Total number of plants studied, plant biomass (in g), concentration of stored resources in the roots and responses to clipping for each of the three species studied. Stored resources include concentration (mean and standard deviation; in mg g⁻¹) of nitrogen (N), phosphorus (P), starch and soluble sugars (SS). Responses are the number of plants that failed to resprout, that initiated resprouting but died, or that initiated resprouting and survived, for each of the three species (in parentheses the % of the total plants studied). Different letters (in rows) indicate significant differences among species at $p < 0.03$.

	<i>Linum suffruticosum</i>	<i>Anthyllis cytisoides</i>	<i>Globularia alypum</i>
Number of plants	51	52	48
Plant biomass (g)	46.64 ± 40.82 ^c	121.44 ± 65.04 ^b	142.54 ± 84.72 ^a
Plant stored resources			
N (mg g ⁻¹)	10.21 ± 3.12 ^a	11.55 ± 3.66 ^a	5.34 ± 1.43 ^b
P (mg g ⁻¹)	0.24 ± 0.10 ^b	0.62 ± 0.28 ^a	0.16 ± 0.07 ^c
Starch (mg g ⁻¹)	8.86 ± 5.50 ^b	16.72 ± 10.97 ^a	8.66 ± 7.38 ^b
SS (mg g ⁻¹)	53.26 ± 34.82 ^a	31.80 ± 11.10 ^b	19.96 ± 10.92 ^c
Plant responses			
Failed to resprout	13 (25.5%)	1 (2.0%)	3 (6.0%)
Resprouted and died	25 (49.0%)	4 (8.0%)	33 (69.0%)
Resprouted and survived	13 (25.5%)	47 (90.0%)	12 (25.0%)

Initial ability to resprout, resprouting vigour and post-resprouting survival were monitored 4 and 12 months after clipping (i.e. December 2008 and August 2009). The first survey was after the autumn precipitation peak (213 mm during October 2008, Valencia, AEMET, Spanish Meteorological Service). Plants that initiated resprouting, even if they died later on, were considered to have the initial ability to resprout. Plants that had resprouted and died before the first survey or between surveys were detected by the presence of dead sprouts.

Post-resprouting mortality was considered to have occurred when all resprout shoots within an individual were dead. Final resprouting success refers to plants that initiated resprouting and survived during the study period (one year). In each survey we counted the number of resprouts and measured the maximum height. In the final survey (i.e. 12 months after clipping) we also clipped all resprouted biomass to obtain the dry weight. Although the survival to 12 months might not entirely correspond to the long term success, mortality (attributable to the applied disturbance) in subsequent years is generally low and declines through time (Lloret and López-Soria 1993, Paciorek et al. 2000).

Statistical analysis

For the individuals that resprouted and survived, resprouting vigour was calculated as the product of the number of resprouts and the maximum height four months after clipping (scaled between 0 and 1). This proxy of resprouting vigour was probed to be more closely related to resprout biomass than using resprout mean length obtained from multiple measurements (Cruz et al. 2003a). There was a significant linear correlation between this measure of resprouting vigour and resprouted biomass for the 12-month sampling (*Anthyllis*: $r = 0.59$, $p < 0.001$, $n = 47$; *Globularia*: $r = 0.89$, $p < 0.001$, $n = 12$; *Linum*: $r = 0.59$, $p = 0.032$, $n = 13$), suggesting that it is a good indicator of resprouting vigour. For individuals that resprouted and died by the fourth month, resprouting vigour was not calculated and these individuals were not included either in the analysis of initial resprouting vigour or in the analysis of post-resprouting survival.

In order to evaluate the role of the pre-disturbance state of the plant on its initial ability to resprout (step 1) we studied the probability of resprouting after clipping, in relation to pre-clipping plant biomass (used as a surrogate of plant size) and plant stored reserves (N, P, starch and SS; considering both the concentration and the amount of reserves). Because the resprouting data was binary (yes/no), we used a generalized linear model (GLM) assuming a binomial error distribution. For the individuals that initiated resprouting, we analyzed the role of pre-clipping plant biomass and plant stored reserves in determining resprouting vigour four months after clipping (step 2) using a standard regression model. We also tested the role of pre-clipping plant biomass, plant stored reserves and resprouting vigour in the probability of post-resprouting survival (one year after clipping; step 3) using a GLM with a binomial error distribution (binary data). In the three steps, variables were tested independently and then a multivariate model was built following a forward stepwise procedure and tested with the F-test for Normal distributed data and the χ^2 -test for binomial data. Previous to the analysis,

variables were standardized (subtracted the mean and divided by the standard deviation) for allowing the comparison of regression coefficients as effect sizes. In all cases, interactions between variables were tested and only variables that explained a significant proportion of the variance were retained.

Results

Initial ability to resprout (step 1)

Most individuals of *Anthyllis* and *Globularia* initiated resprouting (98% and 94% of the total individuals, respectively, Table 1). *Linum* showed high intraspecific variability in this step: of the 51 individuals clipped, 38 resprouted while 13 showed no evidence of resprouting. In *Linum*, the probability of initial resprouting after clipping was independent of plant biomass or SS concentration ($p > 0.05$). Similarly, we did not detect any significant effect of the total amount of carbohydrates and nutrients (i.e. pool size). On the other hand, the initial ability to resprout was significantly related to the concentration of nutrients (N and P) and starch both if the three variables were tested independently (nitrogen: $p = 0.006$; phosphorus: $p = 0.012$; starch: $p = 0.008$) or if they were added sequentially (Table 2, Fig. 2); interactions were not significant.

Table 2. Summary of the stepwise regression for the probability of initiating resprouting after clipping for *Linum*, in relation to the concentration (mg g^{-1}) of nitrogen (N), phosphorus (P) and starch in the roots. The final column includes the standardized coefficient (and standard error) of each variable, for the final model. Only variables that explain a significant proportion of the variance are included. Fitted values of this model are presented in Fig. 2.

	Deviance	Resid. DF	Resid. dev	p	Coef. (SE)
NULL		40	51.22		
N	7.68	39	43.54	0.006	1.20 (0.59)
+ P	6.78	38	36.76	0.009	1.48 (0.60)
+ Starch	7.24	37	29.52	0.007	1.76 (0.83)

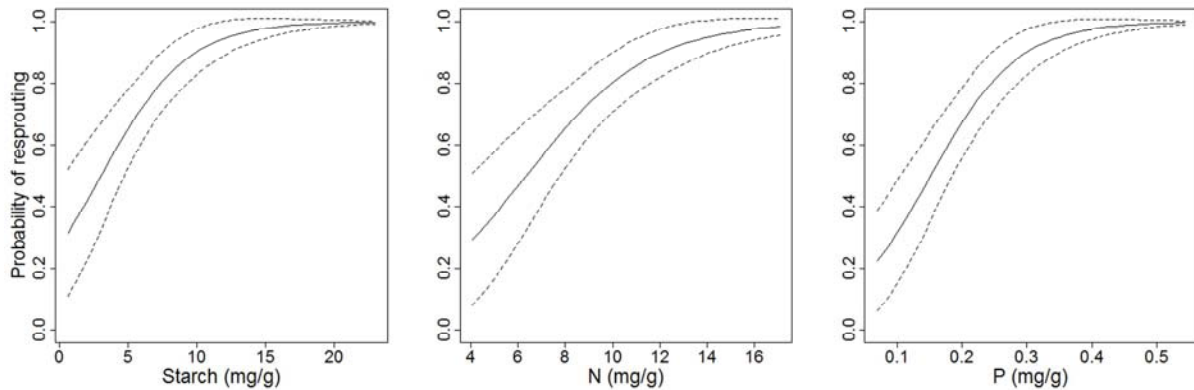


Figure 2. Probability of initiating resprouting (step 1) after clipping for *Linum* in relation to the concentration (mg g^{-1}) of starch, nitrogen (N) and phosphorus (P) in the roots, based on the model presented in Table 2 (using for each variable the median values of the other two variables). Dotted lines refer to SEs.

Resprouting vigour (step 2)

Resprouting vigour at four months after clipping was positively related to pre-clipping plant biomass in *Linum* ($F_{1,14} = 20.44$, $p = 0.0005$) and *Anthyllis* ($F_{1,38} = 8.66$, $p = 0.0055$), but not in *Globularia* ($F_{1,15} = 0.04$, $p = 0.848$). Moreover, for *Anthyllis*, resprouting vigour was also positively related to the concentration of starch and P when tested after plant biomass (Table 3, Fig. 3). The interaction between plant biomass and concentration of reserves was not significant.

Table 3. Summary of the stepwise regression for the analysis of resprouting vigour at 4 months after clipping. Only variables that explain a significant proportion of the variance are included. For *Linum* the significant variable is the pre-clipping plant biomass (in g); and for *Anthyllis* the variables are the pre-clipping plant biomass (g) and the concentration (mg g^{-1}) of phosphorus (P) and starch. The final column is the standardized coefficient (and standard error) of each variable for the final model. Fitted values of these models are presented in Fig. 3.

		SS	Resid. DF	RSS	F	p	Coef. (SE)
<i>Linum</i>	NULL		15	255 699			
	Plant biomass	151766	14	103 933	20.44	<0.001	100.59 (22.25)
<i>Anthyllis</i>	NULL		39	102 549			
	Plant biomass	19042	38	83 507	12.52	0.001	22.63 (6.2)
	+ P	9589	37	73 918	6.31	0.017	8.30 (6.6)
	+ Starch	19189	36	54 729	12.62	0.001	23.38 (6.6)

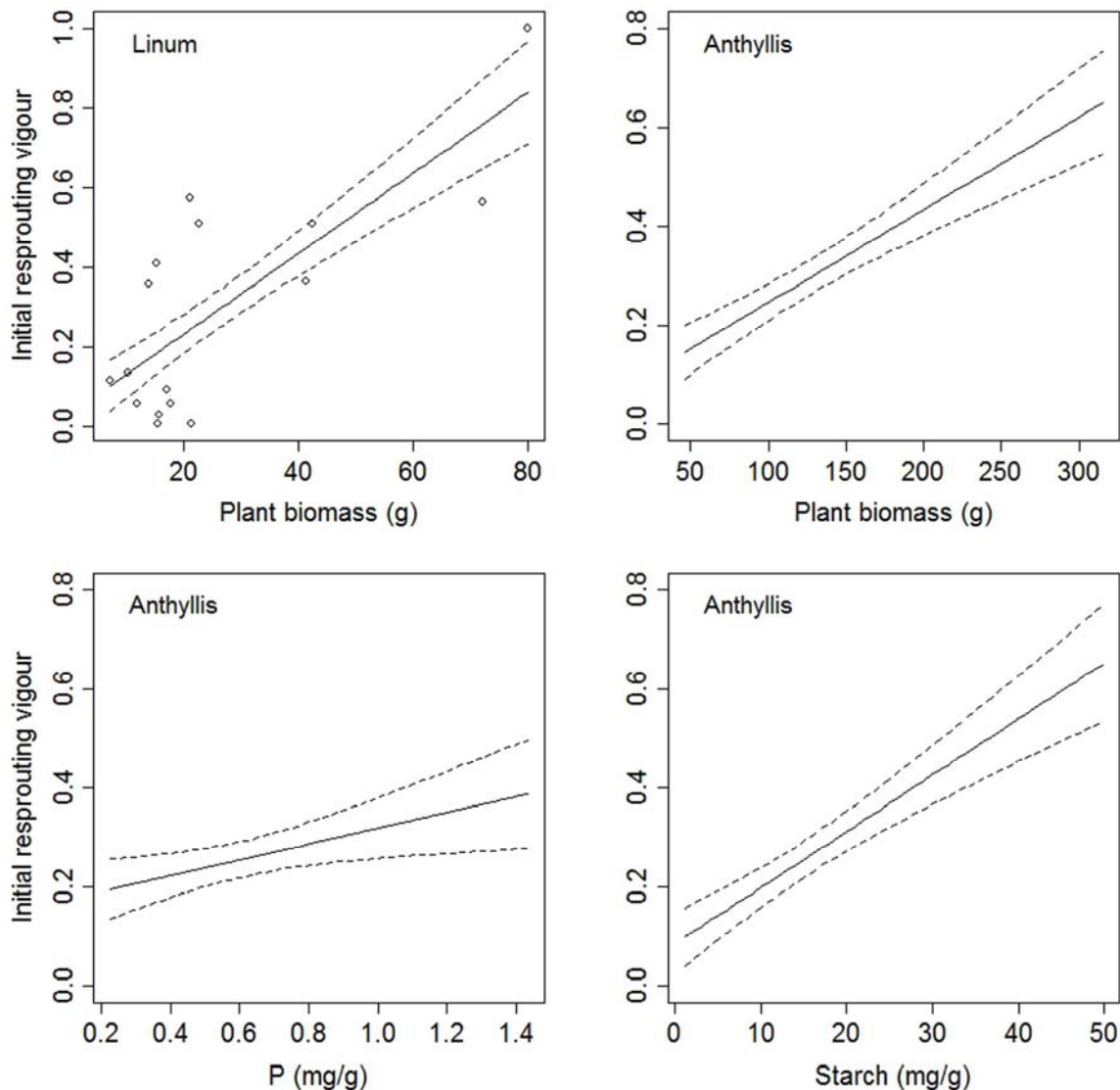


Figure 3. Resprouting vigour at 4 months after clipping (scaled between 0 and 1) for *Linum* in relation to pre-clipping plant biomass (g), and for *Anthyllis* in relation to pre-clipping plant biomass (g), concentration of phosphorus (P, mg g^{-1}) and concentration of starch (mg g^{-1}). For *Anthyllis* we used for each variable in the model the median values of the other two variables (see Table 3 for details). Dotted lines refer to SEs.

Post-resprouting survival (step 3)

At the end of the experiment most of the *Anthyllis* individuals that had initially resprouted (step 1) were found to have survived (47 of the 51 individuals that initiated resprouting; Table 1). In contrast, of the 38 individuals of *Linum* that resprouted (step 1), 25 failed to survive (Table 1). Individuals of *Globularia* exhibited the lowest values of post-resprouting survival; only 12 from the 45 plants that initiated resprouting finally survived (Table 1). The probability of post-resprouting survival was not related to any of the variables associated to the pre-clipping state of the plant (in all cases $p > 0.05$); however, it was positively related to the resprouting vigour in all three species (Fig. 4).

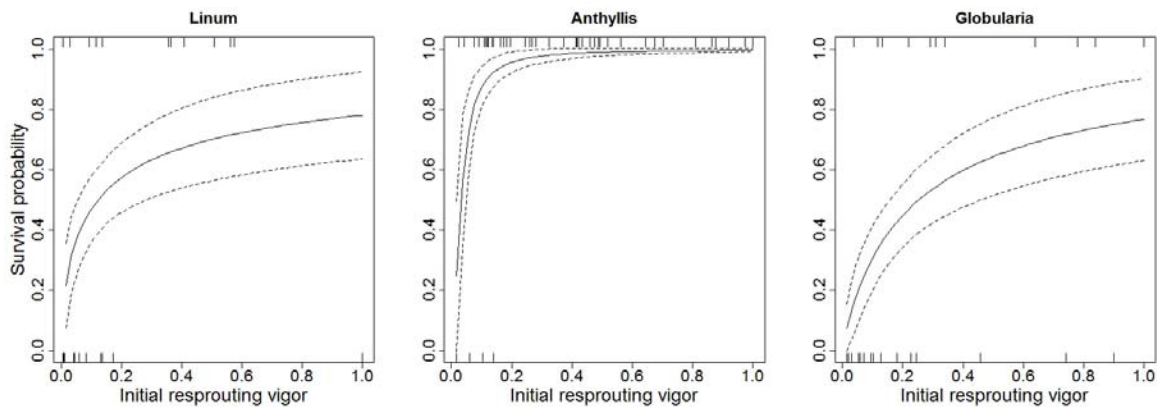


Figure 4. Probability of post-resprouting survival (step 3) one year after clipping in relation to the resprouting vigour at 4 months after clipping (scaled between 0 and 1), considering only the plants that initiated resprouting and had survived in the first survey (GLM, *Linum* $p = 0.0463$, *Anthyllis* $p = 0.0034$, *Globularia* $p = 0.0102$). Dotted lines refer to SEs. Ticks on the top and bottom axis indicate the values on post-resprouting survival and mortality, respectively.

Final resprouting success

The three studied species showed different responses to the elimination of their aboveground biomass. Most individuals ($> 90\%$) of *Anthyllis* and *Globularia* exhibited initial ability to resprout, while only 74% of *Linum* individuals were able to initiate resprouting (step 1). Of the individuals presenting an initial ability to resprout, 65% of *Linum* and 73% of *Globularia* died during the first year after the disturbance (step 3, Table 1). Thus, considering all the plants clipped, the total number of plants that successfully resprouted (i.e., that initiated resprouting and survived for the year) after the disturbance was 47 in *Anthyllis* (90% of total), 13 in *Linum* (25% of total) and 12 in *Globularia* (25% of total, Table 1).

Discussion

Our results suggest that the overall resprouting success is the outcome of the variability in different steps of the resprouting process (i.e. initial resprouting ability, resprouting vigour and post-resprouting survival) and that, depending on the species, each of the steps might limit the overall resprouting success differently. Resprouting success might be limited by the initial ability to resprout (step 1; e.g. 26% of *Linum* plants failed to initiate resprouting). Moreover, resprouted plants exhibit variable resprouting vigour (step 2), which drives the probability of post-resprouting survival (step 3). Indeed, post-resprouting survival might be even more important for the overall resprouting success than the initial ability to resprout (e.g. post-resprouting mortality was 49% in *Linum* and 69% in *Globularia*). The 3-step model (Fig. 1) provides a mechanistic description of the process of resprouting and suggests that the limiting step for resprouting success differs among species. Our results support that the initial

ability to resprout (step 1) might be related to the pre-disturbance state of the plant (e.g. *Linum*). That is, plants with higher levels of starch and nutrients have a higher probability of initiating resprouting. The initial resprouting vigour (step 2) might be driven by the pre-disturbance state of the plant with respect to plant biomass (e.g. *Linum* and *Anthyllis*) or to the concentration of starch and nutrients (e.g. *Anthyllis*). Of a total of 33 plants of *Globularia* that resprouted and died (Table 1), 14 died before the four-month sampling and thus were not used in the analysis of resprouting vigour. These individuals had lower starch concentration ($p = 0.08$), lower SS ($p = 0.01$) and thus lower TNC ($p < 0.01$) than the individuals that survived the first 4 months. Giving the influence of initial resprouting vigour in the probability of survival, it is plausible to consider that these individuals had low resprouting vigour. Thus the lack of a significant predictor for resprouting vigour in *Globularia* might be due to this early death.

Our results emphasize the importance of plant size (Stohlgren et al. 1984, Lloret and López-Soria 1993, Pausas 1997) and stored reserves (Bowen and Pate 1993, Canadell and López-Soria 1998, Kabeya and Sakai 2005) for initiating resprouting vigorously. The effect of plant size (i.e. larger plants resprouted more vigorously than smaller ones) on resprouting vigour may be explained by the positive correlation between size and the capacity of the belowground parts of the plant to mobilize resources after the disturbance; i.e. to use stored resources and uptake water and nutrients from the soil (Rundel et al. 1987, Lloret and López-Soria 1993). Although larger plants might have higher total amount of reserves, they also have higher maintenance costs (the absolute of respiration increases with the amount of root). Thus, for the range of plant sizes considered, we can speculate that the gain resulting from the higher storage capacity and the increase in the volume of soil explored by larger plants (and thus the increased capacity of resource acquisition) is greater than the higher maintenance costs associated with the increase in biomass. Post-resprouting survival (step 3) was not directly related to the pre-disturbance state of the plant but to the initial amount of biomass that resprouting plants could produce in the early stages of regeneration (i.e. resprouting vigour). Higher resprouting vigour allows plants to obtain more resources that can be allocated to sustain plant requirements (for respiration and growth) and restore reserves, thus increasing the probability of post-resprouting survival. This suggests that in resprouting plants living in disturbance-prone ecosystems, the photosynthetic capacity of resprouts must be under strong selective pressure towards high photosynthetic efficiency and/or high photosynthetic biomass. Indeed, leaves on resprouting shoots tend: (a) to be larger than leaves on normal shoots, (b) to have more chlorophyll on a leaf dry weight basis and, consequently,

(c) to have increased photosynthesis efficiency (Arianoutsou and Margaris 1981, Castell et al. 1994, Fleck et al. 1995, Schutz et al. 2009). It should be pointed out that our model is based on shrubs subject to clipping, that is, the disturbance applied eliminates all above ground biomass without the destruction of buds. Applying our model to post-fire resprouting, and especially under high intensity fires, one should consider that the bud bank might also be affected by the disturbance (Vesk and Westoby 2004b). For other growth forms (e.g. trees) other traits like protective structures (e.g. bark thickness) might also become relevant (Pausas 1997).

Despite the fact that the dichotomous classification of species into resprouters and non-resprouters accounts for a high proportion of the interspecific variability in resprouting, it overrides the great intraspecific variability in this trait. Different studies have related resprouting variability to factors such as disturbance regime, and spatial, environmental or ecotypic variability (Keith 2002, Cruz et al. 2003a). However, for a given disturbance and environmental condition, we found that intraspecific variability in resprouting is determined by both the stored (pre-disturbance) resources, and the initial (post-disturbance) resource acquisition capacity. Stored reserves are important for initiating resprout and for the initial growth (first for maintaining root and buds alive and subsequently for producing new leaves and shoots). However, once plants have resprouted (using the stored reserves), they rely on the initial post-disturbance capacity to acquire resources (i.e. resprouting vigour) for surviving.

Intraspecific variability is of paramount importance for predicting species dynamics (Bolnick et al. 2011, Moreira et al. 2012) and the response to environmental changes (Ramírez-Valiente et al. 2010), as well as to understand evolutionary processes (Pausas et al. 2012). This is especially evident in fire-prone environments where the regime of a strong disturbance such as fire is very sensitive to global change drivers (Dubinin et al. 2011, Pausas and Fernández-Muñoz 2012). In such ecosystems, intraspecific variability in resprouting might play a key role in determining the fate of plant species in a world with continuous changes in the disturbance regime. Thus we argue that studying individual resprouting variability in disturbance-prone ecosystems should be encouraged.

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Chapter 3

Tanned or burned: The role of fire in shaping physical seed dormancy

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Abstract

Plant species with physical seed dormancy are common in mediterranean fire-prone ecosystems. Because fire breaks seed dormancy and enhances the recruitment of many species, this trait might be considered adaptive in fire-prone environments. However, to what extent the temperature thresholds that break physical seed dormancy have been shaped by fire (i.e., for post-fire recruitment) or by summer temperatures in the bare soil (i.e., for recruitment in fire-independent gaps) remains unknown. Our hypothesis is that the temperature thresholds that break physical seed dormancy have been shaped by fire and thus we predict higher dormancy lost in response to fire than in response to summer temperatures. We tested this hypothesis in six woody species with physical seed dormancy occurring in fire-prone areas across the Mediterranean Basin. Seeds from different populations of each species were subject to heat treatments simulating fire (i.e., a single high temperature peak of 100°C, 120°C or 150°C for 5 minutes) and heat treatments simulating summer (i.e., temperature fluctuations; 30 daily cycles of 3 hours at 31°C, 4 hours at 43°C, 3 hours at 33°C and 14 hours at 18°C). Fire treatments broke dormancy and stimulated germination in all populations of all species. In contrast, summer treatments had no effect over the seed dormancy for most species and only enhanced the germination in *Ulex parviflorus*, although less than the fire treatments. Our results suggest that in Mediterranean species with physical dormancy, the temperature thresholds necessary to trigger seed germination are better explained as a response to fire than as a response to summer temperatures. The high level of dormancy release by the heat produced by fire might enforce most recruitment to be capitalized into a single post-fire pulse when the most favorable conditions occur. This supports the important role of fire in shaping seed traits.

Introduction

Plant species living in fire-prone ecosystems have several traits to cope with recurrent fires such as those related to resprouting and fire-stimulated recruitment. These two traits define the resprouter and seeder life histories, respectively [1]. Despite the fact that these traits increase plant fitness and thus are adaptive in fire-prone environments [2], to what extent they have been shaped by fire remains debatable [2,3]. While resprouting enhances the persistence of individuals, fire-stimulated recruitment acts on a critical life stage for the persistence of the populations [1]. The consequence of fire-dependent germination is that the bulk of recruitment occurs post-fire and, because recurrent fires shorten the generation time of seeders (i.e., increase population turnover), they provide more opportunities for natural selection to act. Indeed, there is evidence that recurrent fires generate phenotypic [4] and genetic [5] differentiation in seeders. Thus, in fire-prone ecosystems, traits related to post-fire seedling recruitment are a likely target of strong selection.

One trait tightly related to post-fire seedling recruitment is seed dormancy, which allows species to maintain a persistent soil seed bank and delay germination until the conditions are optimal for seedling establishment. Seed dormancy is often broken by specific external stimuli that are coupled with those favorable conditions, allowing species to increase the probability of successful establishment. In mediterranean fire-prone ecosystems, the heat shock produced by wildfires has been shown to break seed dormancy and trigger germination in many species with physical dormancy (heat-stimulated germination, [6,7]), particularly in Fabaceae and Cistaceae [8-11]. Seeds have specialized structures in the seedcoat (e.g., the strophiole in Fabaceae and the chalazal plug in Cistaceae) that move or become disrupted as a response to external factors, and thus allowing the water to surpass the impermeable seed coat layer(s) [12]. In some species, chemicals from the combustion might also enhance germination once physical dormancy has been broken by the heat shock (e.g. [13,14]); however, in most species with impermeable seed coats, the embryo is nondormant [12] and breaking physical dormancy is enough for stimulating germination. Thus, physical seed dormancy is undoubtedly a trait that provides fitness benefits in fire-prone environments.

Although, in mediterranean ecosystems, the regulation of dormancy release in species with physical seed dormancy is typically linked to fire, it could however be a response to the high summer temperatures in the bare soil (e.g., in vegetation gaps unrelated to fire). Because seeds in the seed bank are mostly located in the upper soil layer (seed density might be 2 to 5

times higher in the 0-2.5 cm layer than in the subsequent 2.5–5 cm layer [15]), they are exposed to temperature variations. Indeed, daily maximum summer temperatures registered in the bare soil can be relatively high (e.g., up to 50°C on the soil surface of fire breaks in eastern Spain [16]; up to 40-60°C in south-eastern Australia [17]). Although temperatures registered during a fire might be much higher (e.g., in a spring burn, up to 150°C, 100°C and 50°C at 1 cm, 2.5 cm and 5 cm depth, respectively [18]), the cumulative effect throughout the summer could have a similar effect to a fire; i.e., the total heat dose might be similar and thus have analogous effects on seed dormancy. This has led to the suggestion that the temperature thresholds associated to the release from physical seed dormancy might have evolved independently of fire [3,19,20] and that the cumulative effect of soil temperatures during the summer might be more beneficial than the direct fire cues. This argument is based on the idea that cumulative summer temperatures should stimulate germination at the end of the summer (i.e., when conditions are less stressful) while seedling emergence resulting from the direct effect of fire could occur during the unfavorable conditions for recruitment of summer [21,22]. However, even when dormancy has been broken, germination only occurs when favorable conditions are attained [12,23,24]. In addition, there is some evidence (e.g., comparing results by [22] and by [25]) that for similar maximum temperatures, a single heat dose (i.e., fire scenario) might be as much or even more efficient in breaking dormancy than the cumulative heat doses (i.e., summer scenario). This suggests that the release of physical dormancy might be determined by the maximum temperature experienced rather than by the amplitude of temperature fluctuations.

The summer hypothesis for the physical seed dormancy proposes that it is an adaptation for recruiting in gaps, independently of the origin of the gap (i.e., seedling recruitment from the soil seedbank is not necessarily coupled with fire). One underlying premise of the summer hypothesis is the traditional belief that fire is a relatively new phenomenon (i.e., linked to human activities) and thus traits for post-fire persistence (such as physical seed dormancy) must be linked to another and earlier evolutionary pressure [3]. However, there is now a bulk of information suggesting that fire is an ancient phenomena in terrestrial ecosystems [26,27] and that it has had a prominent role in shaping plant traits [2,28-31]. In addition, there is no evidence that current summer conditions appeared earlier than fires in the evolutionary history and, in fact, predictable warm summers in most mediterranean ecosystems would also imply predictable fires (with the exception of central Chile where the Andes block summer storms reducing lightning and fire ignitions [23]). Thus, to what extent the temperature thresholds that

break physical seed dormancy of species living in fire-prone mediterranean ecosystems have been shaped by fire or by summer temperatures remains unknown [2].

Previous research is ambiguous in disentangling the role of summer and fire in the regulation of dormancy release in species with physical seed dormancy due to the lack of an appropriate experimental approach [19-22]. Indeed, there is no single study simultaneously testing the two alternative hypotheses (fire *versus* summer) on species with physical seed dormancy. Our hypothesis is that fire shaped the temperature thresholds that break the physical seed dormancy in species from mediterranean fire-prone ecosystems. Thus, we predict that, in Mediterranean species with physical dormancy, fire produces a higher increase in the chances of recruitment (i.e., a higher increase in germination) than summer temperatures. To test this prediction we performed a germination experiment using different populations of six post-fire seeder species (Fabaceae and Cistaceae) distributed across the Mediterranean Basin. The experiment included treatments simulating both the temperatures registered in the soil during a fire and the temperature fluctuations observed in the bare soil during summer. Given that seed dormancy is heritable [32,33], demonstrating that it provides higher chances of recruitment (i.e., higher potential fitness benefits) in response to fire than in response to summer temperatures would suggest this trait to be a good candidate for an adaptation to fire.

Methods

Ethics statement

This work did not involve collecting animals or plants; only seeds were collected (from wild populations). None of species studies are endangered or protected, and all necessary permits for seed collection were obtained. Specifically, two of the sites are protected, and written permissions for seed collection were obtained from the corresponding authorities ("*Parque Natural del Carrascal de la Font Roja*" and "*Fundación Caja Castellón-Bancaja*"). For the remaining sites (non-protected), written permission for seed collection was not required, and the corresponding local authorities were properly notified.

Species and seed collection

We selected six woody species with physical seed dormancy (i.e., water-impermeable seeds, Table 1) occurring in fire-prone areas of the Mediterranean Basin. All six species show evidence of post-fire seedling emergence [34]. We included one Fabaceae (*Ulex parviflorus* Pourr.), a family (and species) which had previously been shown to have germination

stimulated by summer temperatures [16,22] and five Cistaceae for which germination response to summer temperatures is unknown. The six species encompass the most abundant post-fire seeder species with physical seed dormancy in the Mediterranean Basin. Three species are abundant in the western part of Basin (*Fumana thymifolia* (L.) Spach, *Cistus albidus* L. and *U. parviflorus*) and seeds were collected in eastern Iberia (Spain), two are typical of the eastern part (*Cistus creticus* L. and *Cistus parviflorus* Lam.) and seeds were collected in south western Anatolia (Turkey) and one (*Cistus salviifolius* L.) occurs across all the basin and seeds were collected at both the eastern and the western sides (Turkey and Spain, [35]). For most species we sampled several populations, totaling 21 populations for the six species (Table 1 and Table S1). All seeds were collected from ripe fruits in wild populations during the dispersal period of each species (Table 1) from several individuals (> 20 individuals for each species and population) spatially dispersed (>10 m from each other). Seeds were cleaned based on their density (i.e., excluding low density seeds that could be empty) using a seed cleaning equipment (vacuum aspirator). Seeds from the same species and population were pooled together for the germination experiments and were placed in aluminum pockets (ca. 50 apparently viable seeds per pocket; i.e., non predated seeds and with any perceptible damage). For each population and species, four aluminum pockets (four replicates) were randomly allocated to each treatment (see below).

Table 1. List of species, number of populations studied per species (#Pop), location (Country) and date (month/year) of the seed collection, and seed age at the time of the experiment (in months). Specific location of each population is given in Table S1.

Family	Species	# Pop	Country	Collection date	Seed age (months)
<i>Cistaceae</i>	<i>Fumana thymifolia</i>	2	Spain	07/2008	3
	<i>Cistus salviifolius</i>	6	Spain, Turkey	07/2009	1
	<i>Cistus albidus</i>	4	Spain	07/2008	3
	<i>Cistus parviflorus</i>	1	Turkey	07/2009	1
	<i>Cistus creticus</i>	3	Turkey	07/2009	1
<i>Fabaceae</i>	<i>Ulex parviflorus</i>	5	Spain	06/2008	4

Fire and summer treatments

For each species, seeds from the different populations were subject to heat-treatments of high temperatures for a short period of time (i.e., fire treatments) and to treatments of temperature fluctuations at relatively low temperatures for a longer period of time (i.e., summer treatments). Treatments were applied to each replicate separately. The fire treatments consisted of submitting seeds to 100°C, 120°C and 150°C for 5 minutes, while in the summer treatments seeds were enclosed in a germination chamber for 30 days with daily cycles (24 hours) of 3 hours at 31°C, 4 hours at 43°C, 3 hours at 33°C and 14 hours at 18°C. These temperatures correspond to the temperature regime observed at the peak of summer on the soil surface of a Mediterranean environment (e.g., on the soil surface of a fire-break with no slash, Fig. 1; data from Ayora, Valencia, eastern Spain [16], which represents a typical environment where the studied species live). Indeed, this treatment is conservative in respect to our hypothesis as in natural conditions many seeds composing the soil seed bank might be buried and thus temperatures experienced by the seeds would be lower. For two of the species (*U. parviflorus* and *C. albidus*) we tested one additional fire treatment (80°C for 5 minutes) and one additional summer treatment in which seeds were enclosed in a germination chamber with daily cycles (24 hours) of 11 hours at 15°C and 13 hours at 45°C for 5 days. Although the 30 days treatment is more realistic (Fig. 1) we applied this 5-day treatment as it has been shown to successfully break dormancy of species with physical dormancy and thus could be used for comparison [16]. Fire treatments were performed using an electric oven in dark conditions at room moisture; summer treatments were performed in a germination chamber at dark conditions with 30% moisture.

After the respective treatments, seeds were set for germination in Petri dishes containing agar (0.9%) as substrate and incubated at 20°C in darkness. Seeds were put in dark conditions because these conditions are appropriate for the germination of many Mediterranean species [36]. Seed germination was monitored for 90 days, when no germination was recorded for 1 week. Seeds were scored as germinated and removed from the Petri dishes if radicle emergence exceeded 0.5-1 mm. At the end of the experiment, the initial number of seeds sown was corrected before statistical analysis by discarding the empty seeds (lacking embryo and storage tissue) detected during the experiment. The increment in the number of rotten seeds after treatment, in relation to control, was considered as seed mortality due to the treatment.

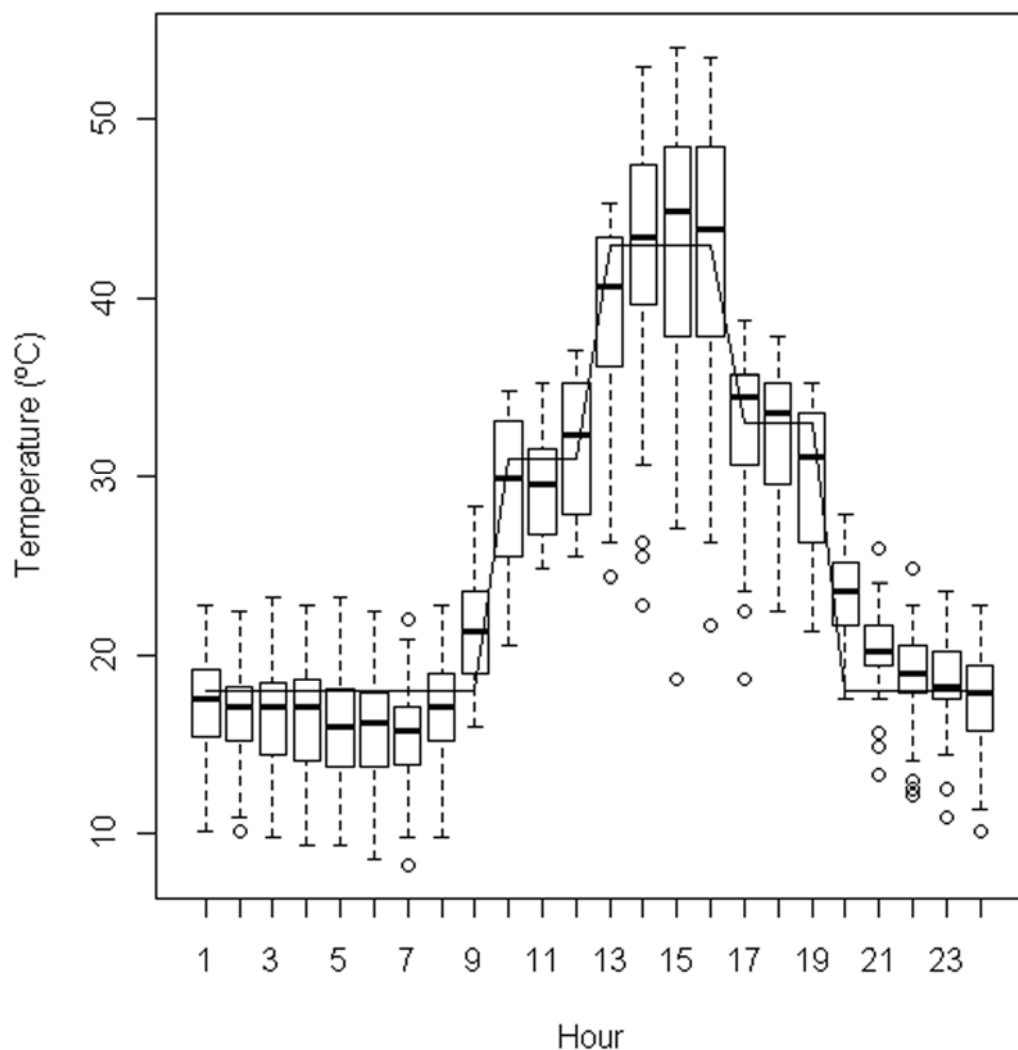


Figure 1. Hourly register of daily temperature (°C) observed during summer on the bare soil surface of a fire-break (with no slash). Data from a typically Mediterranean fire-prone area (August 1998, Valencia, eastern Spain [16]). Boxplots represent daily variability in temperature for each hour ($n = 28$ days). Dashed vertical lines represent the data within 1.5 interquartile range, and open circles are values outside this range (outliers). The continuous line represents the treatment applied to simulate summer temperatures.

Data analysis

We first tested, for each population of each species, whether final germination differed between treatments, using the analysis of deviance (GLM) with binomial error distribution. Then, for each species, we tested the effect of the treatments, accounting for the population variability, by using generalized linear mixed models (GLMM) with binomial error distribution, including treatment as fixed factor and population as random factor. Due to the

large number of pairwise comparisons, we applied the false discovery rate correction [37] to control for the expected proportion of false discoveries amongst the rejected hypotheses; this is a less conservative criterion than the Bonferroni correction [38].

Results

Fire treatments broke dormancy and stimulated germination in all species (Table 2). The summer treatments only enhanced germination of *U. parviflorus* (i.e., they had no significant effect over the germination of any of the Cistaceae species) and the magnitude of the stimulation by these treatments was lower than by the fire treatments (Table 2, Fig. 2 and Figure S1). These results were consistent across all populations (Table 2).

The temperature threshold needed to break dormancy and maximize germination was species-dependent (Table 2); some species maintained a high germination level for a wide range of fire temperatures (e.g., *U. parviflorus* and *C. parviflorus*); others had their maximum germination at the highest heat doses (e.g., *C. creticus*, *C. salviifolius* and *F. thymifolia*); and still others showed seed mortality and consequently reduced germination at the highest heat dose applied (e.g., *C. albidus*). For the two species for which we tested a wider range of treatments (*U. parviflorus* and *C. albidus*), the heat treatment of 80°C for 5 minutes was insufficient to maximize germination (Table 2 and Table 3). In fact this treatment was the most variable among populations and for one of the populations of *U. parviflorus* this treatment was not significantly different from the summer treatments (P12 in Table 2). The two summer treatments were not significantly different for any of the species and populations (Table 2).

Table 2. Germination percentage (mean \pm SE) of untreated seeds (Control), seeds treated with summer temperature fluctuations during 30 days (Summer 30) or 5 days (Summer 5) and heat-treated seeds (Fire treatments; 80°C, 100°C, 120°C or 150°C during 5 minutes), for each species and population (location of the different populations is given in Table S1). Species means consider the variability between populations. For each species and population (Pop.), mean germination values of treatments with the same letter are not significantly different ($P>0.05$), after controlling for the false discovery rate.

Species	Pop.	Summer treatments			Fire treatments				
		Control	Summer 30	Summer 5	80°C	100°C	120°C	150°C	
<i>C. albidus</i>	P1	8 \pm 2.1 a	9 \pm 1.6 a	8 \pm 1.0 a	58 \pm 4.4 b	81 \pm 4.0 c	75 \pm 1.6 c	7 \pm 2.8 a	
	P2	6 \pm 2.8 a	8 \pm 3.2 a	8 \pm 2.8 a	31 \pm 5.0 b	81 \pm 2.0 c	80 \pm 1.7 c	4 \pm 0.9 a	
	P3	7 \pm 2.4 a	8 \pm 2.2 a	8 \pm 2.1 a	53 \pm 5.5 b	78 \pm 4.5 c	70 \pm 3.2 c	0 \pm 0 e	
	P4	3 \pm 1.4 a	3 \pm 2.5 a	3 \pm 0.8 a	73 \pm 6.2 b	93 \pm 1.6 c	94 \pm 0.9 c	56 \pm 2.4 d	
	Mean	6 \pm 1 a	7 \pm 2 a	6 \pm 1 a	54 \pm 9 b	83 \pm 3 c	80 \pm 5 c	17 \pm 13 a	
<i>U. parviflorus</i>	P1	23 \pm 2.7 a	28 \pm 4.6 ab	41 \pm 4.6 bc	58 \pm 3.7 c	86 \pm 3.7 d	96 \pm 1.0 e	87 \pm 2.6 d	
	P12	8 \pm 2.2 a	33 \pm 2.8 b	38 \pm 2.9 b	40 \pm 3.0 b	94 \pm 2.6 d	95 \pm 4.4 d	84 \pm 4 c	
	P2	27 \pm 6.8 a	52 \pm 4.2 b	52 \pm 4.2 b	86 \pm 2.8 c	86 \pm 3.2 c	84 \pm 2.4 c	83 \pm 2.4 c	
	P3	8 \pm 0.8 a	23 \pm 2.8 b	22 \pm 4.4 b	39 \pm 2.8 c	86 \pm 2.9 d	84 \pm 1.7 d	91 \pm 3.8 d	
	P6	8 \pm 1.7 a	23 \pm 7.5 b	31 \pm 2.8 b	52 \pm 4.4 e	90 \pm 1.2 cd	93 \pm 1.9 c	84 \pm 2.6 d	
	Mean	15 \pm 4 a	31 \pm 5 b	37 \pm 5 b	55 \pm 8 c	88 \pm 2 d	90 \pm 3 d	85 \pm 1 d	
<i>C. creticus</i>	P13	4 \pm 0.8 a	2 \pm 1.0 a	-	-	-	12 \pm 9.3 b	34 \pm 5.2 c	73 \pm 15.3 d
	P14	10 \pm 1.9 a	9 \pm 1.1 a	-	-	-	51 \pm 16.3 b	65 \pm 3.9 c	95 \pm 3.3 d
	P16	0 \pm 0.0 a	2 \pm 1.4 a	-	-	-	2 \pm 1.2 a	30 \pm 7.0 b	70 \pm 5.2 c
	Mean	5 \pm 3 a	4 \pm 2 a	-	-	-	22 \pm 15 b	43 \pm 11 c	79 \pm 8 d
<i>C. parviflorus</i>	P16	4 \pm 2.3 a	6 \pm 2.1 a	-	-	-	94 \pm 6.0 b	87 \pm 6.9 b	99 \pm 0.6 c
<i>C. salviifolius</i>	P1	11 \pm 2.3 a	14 \pm 3.5 a	-	-	-	19 \pm 4.0 a	90 \pm 5.2 b	96 \pm 3.1 b
	P13	3 \pm 1.3 a	4 \pm 1.6 ab	-	-	-	10 \pm 4.4 b	52 \pm 7.7 c	98 \pm 1.0 d
	P14	7 \pm 1.3 a	5 \pm 1.1 a	-	-	-	92 \pm 7.0 b	86 \pm 7.9 b	92 \pm 3.7 b
	P16	10 \pm 2.5 a	13 \pm 2.6 a	-	-	-	32 \pm 19.0 b	91 \pm 2.1 c	99 \pm 0.6 d
	P5	3 \pm 1.1 a	9 \pm 2.0 a	-	-	-	28 \pm 17.8 b	84 \pm 2.4 c	97 \pm 1.8 d
	P8	4 \pm 1.8 a	13 \pm 2.0 a	-	-	-	56 \pm 16.7 b	87 \pm 5.3 c	100 \pm 0.0 d
	Mean	6 \pm 2 a	10 \pm 2 a	-	-	-	40 \pm 12 b	82 \pm 6 c	97 \pm 1 d
<i>F. thymifolia</i>	P5	8 \pm 1.2 a	16 \pm 2.2 a	-	-	-	35 \pm 3.2 b	58 \pm 2.7 c	83 \pm 3.1 d
	P6	1 \pm 0.6 a	1 \pm 0.6 a	-	-	-	36 \pm 8.0 b	49 \pm 7.2 b	78 \pm 3.5 c
	Mean	5 \pm 4 a	8 \pm 7 ab	-	-	-	35 \pm 0 b	53 \pm 5 c	80 \pm 2 d

Table 3. Summary of the differences in germination between untreated seeds (Control), seeds subject to a summer treatment (Summer) and seeds subject to the fire treatments, for the six species studied. Fire treatment refers to the seeds submitted to 100°C, 120°C and 150°C for 5 minutes (Fire100, Fire120 and Fire150, respectively). Summer treatment refers to seeds exposed to temperature fluctuations for 30 days (Summer). For *C. albidus* and *U. parviflorus* an additional treatment of 80°C for 5 minutes (Fire80) is also included. The significance of the pairwise comparison between treatments is included (=: not significant; <: $P < 0.05$, <<: $P < 0.01$). For full details of the statistical analysis see Table 2 and S2.

Species	Germination
<i>Fumana thymifolia</i>	Control = Summer = Fire100 << Fire120 << Fire150
<i>Cistus salviifolius</i>	Control = Summer < Fire100 << Fire120 << Fire150
<i>Cistus albidus</i>	Control = Summer = Fire150 < Fire80 << Fire100 = Fire120
<i>Cistus parviflorus</i>	Control = Summer << Fire100 = Fire120 = Fire150
<i>Cistus creticus</i>	Control = Summer << Fire100 < Fire120 << Fire150
<i>Ulex parviflorus</i>	Control << Summer << Fire80 << Fire100 = Fire120 = Fire150

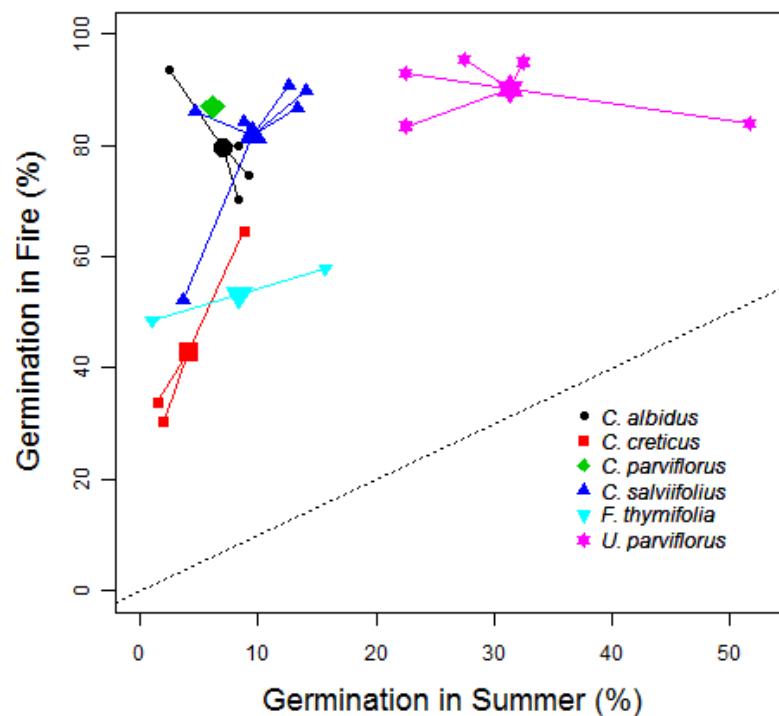


Figure 2. Germination (%) in the fire scenario and germination in the summer scenario. Relationship between the germination (%) after the treatment of 120°C for 5 minutes (Fire scenario, y-axis) and germination after the treatment simulating temperature fluctuations in the soil in open areas during August (Fig. 1; Summer scenario, x-axis). Intraspecific variability (i.e., among populations) is indicated by small symbols (mean population value) emerging from the large symbol (mean species value; *Fumana thymifolia*, n=2 populations; *Cistus salviifolius*, n=6 populations; *Cistus albidus*, n=4 populations; *Cistus parviflorus*, n=1 population; *Cistus creticus*, n=3 populations; *Ulex parviflorus*, n=5 populations). The 1:1 line is also shown (dotted line).

Discussion

Our results suggest that for the species considered, which encompass the main post-fire obligate seeder species with physical dormancy from Mediterranean shrublands, dormancy release is higher in response to fire temperatures than to summer temperatures. That is, fire produces a higher increase in germination and consequently in the chance of recruitment than summer temperatures. These results were consistent for geographically distant populations and for all the studied species.

Many species with heat-stimulated germination have polymorphic seed pools [6]. Thus, while the bulk of the seed bank may respond to fire, a small proportion of the seeds may germinate in the absence of fire (e.g., in response to summer temperatures). Indeed, for some seeder species, especially for Fabaceae, summer temperatures also break physical dormancy of a significant proportion of the seeds (Table 2, [16,21]). However, the magnitude of stimulated germination by summer temperatures is much lower than that resulting from the fire temperatures. Low levels of dormancy loss in response to summer temperatures might be important for the recruitment in fire-independent gaps or for the colonization of new areas, particularly in arid systems (e.g., *U. parviflorus* [39]; but also other species [12]). These species might display a gradual loss of dormancy over time to take advantage of occasional recruitment opportunities but maintain a considerable seed bank as a bet-hedging mechanism. This results in a large commitment of seeds to take advantage of the post-fire environment and a smaller commitment over multiple seasons. A similar pattern of response to summer temperatures may also be observed for other refractory mediterranean species without physical seed dormancy [17]. However, in fire-prone ecosystems, the spatial extent of fire-independent gaps is typically much reduced compared to the extent of gaps generated by fires (i.e., fire-independent gaps are smaller); in addition, in these ecosystems fire intervals are typically shorter than the lifespan of the dominant plants and thus fire-independent gaps (e.g., related to plant mortality) are also less frequent. Consequently, fire-independent recruitment of seeders should be less important and have less evolutionary implications than fire-dependent recruitment. Thus, it is not surprising that for many seeders the effectiveness of summer temperature fluctuations in stimulating germination is very limited (e.g., Cistaceae; Table 3, Fig. 2).

Our results might explain the observed dynamics of seedling establishment in mediterranean shrublands where there is a flush of germination after fire with very little (if any) effective

recruitment between fires [7]. The emergence of species with fire-dependent dormancy release (e.g., *Cistus* sp.) occurs in the post-fire environment, while in species with a significant proportion of seeds with fire-independent dormancy release (e.g., *Ulex parviflorus*) some emergence is also observed in fire-independent gaps ([39,40], Fig. 3). Indeed, for different species of *Cistus*, successful establishment is restricted to the immediate post-fire period [40,41].

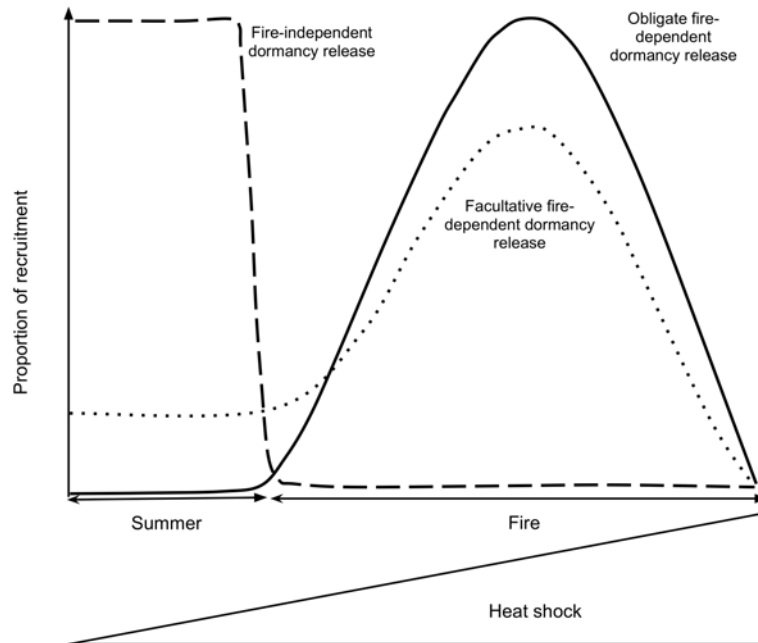


Figure 3. Conceptual model describing recruitment dynamics in mediterranean ecosystems for species with physical dormancy. The x -axis represents an increase in the heat doses reaching the soil (during summer or fires). The y -axis represents the proportion of recruitment associated with dormancy release, for species with different levels of fire-dependent recruitment. For some species, fire is not the main factor shaping seed dormancy and thus they have fire-independent dormancy release and recruitment (dashed line). However, species living in fire prone mediterranean ecosystems have mostly fire-dependent dormancy release, with recruitment strictly related to fire (obligate fire-dependent dormancy release, continuous line) or with a small proportion of recruitment independent of fire (facultative fire-dependent dormancy release, dotted line).

The lower effect of summer temperature fluctuations suggests that cumulative periods of relatively low temperatures (i.e., summer heat dose) are less effective in breaking physical seed dormancy than a single high temperature peak (i.e., fire heat dose). This agrees with the importance of fire intensity at the soil level in fire-prone ecosystems [42]. Fire intensity influences seedling recruitment [43-45] because seeds have different heat tolerance [46] and different temperature thresholds needed for dormancy release (Tables 2 and Table 3; [44,47]). For instance, obligate (non-resprouting) seeders have higher heat tolerance, heat-stimulated

germination and post-fire seedling emergence than facultative (resprouting) seeders and this is particularly evident for high intensity fires (i.e., high heat doses, [48,49]). In addition, in Mediterranean shrublands, there is evidence that obligate seeders have evolved flammability-enhancing traits [4,50] that increase soil temperatures during fires, ensuring that seed dormancy is broken and germination is triggered by fire. Fire intensity also negatively affects the resprouting capacity [51-53] and thus by increasing flammability and the temperature threshold for breaking seed dormancy, seeder species might also be favored in relation to resprouters.

In frequently disturbed mediterranean ecosystems, despite the fact that heat from fire is the main factor driving dormancy release, summer temperatures might also have an important role in shaping physical seed dormancy. That is, physical dormancy might have evolved in such a way that the temperature threshold associated with the dormancy release is above the maximum summer temperatures. Thus, seeds from plants growing in hotter conditions might have dormancy-breaking thresholds higher than those growing in cooler conditions (i.e., dormancy-breaking thresholds might depend of the local climatic conditions). This ensures the maintenance of a persistent seed bank until a fire occurs [54].

Our conclusions are based on the range of summer temperatures tested, which come from field observations in the soil surface (Fig. 1). In fact, using these temperatures is a conservative approach because in natural conditions many seeds in the soil seed bank are buried and thus they experience lower summer temperatures. We cannot discard that extreme heat waves experienced during summer periods can increase soil temperatures above the levels tested; however, we expect that this extreme effects to be rare. The increased frequency of heat waves, due to global warming, might increase dormancy loss, especially in populations growing under cooler conditions [54].

Seed dormancy and germination are key traits in plant evolution, not only because they determine the persistence of populations but also because of the strong selective pressure exerted by the conditions that plants experience for germination. Our results suggest that physical dormancy in mediterranean ecosystems has evolved in the presence of frequent fires in such a way that germination in seeders is mainly capitalized to a single post-fire pulse. That is, the heat from fire produces high levels of dormancy release that might be responsible for the single post-fire germination pulse occurring just after the first post-fire rains. This

semelparity-like strategy [55] allows maximizing germination at the most favorable moment for recruitment (e.g., lower competition and higher resources availability).

The link between fire and dormancy-breaking can also be observed at a biogeographical scale. For instance, the rare fire-dependent recruitment observed in central Chile [7,29], might be explained by the fact that fires are historically rare, compared with the other Mediterranean climate regions where both fires and fire-dependent recruitment are very common [1,29]. In fact, fire-stimulated germination in Chile is mainly observed in alien species and in native annuals, because annuals had time to adapt to anthropogenic fires [56,57].

Despite the fact that some species with physical seed dormancy in fire-prone ecosystems may have a fraction of seeds with fire-independent germination, our results support the hypothesis that temperature thresholds that break physical seed dormancy are better explained as a response to fire than as a response to the summer temperatures. This together with other recent studies (e.g., [30,56]), highlights the role of fire in driving the evolution of seed traits in mediterranean ecosystems.

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Appendix

Table S1. Location and country (TR: south west Turkey; ES: eastern Spain) of the populations used in the study for each species (FTH: *Fumana thymifolia*; CSA: *Cistus salviifolius*; CAL: *Cistus albidus*; CPA: *Cistus parviflorus*; CCR: *Cistus creticus*; UPA: *Ulex parviflorus*).

Code	Location (country)	Coordinates (lat, long)	Species
P1	Serra Espadà (ES)	39.874834, -0.372591	CAL; CSA; UPA
P2	Font Roja (ES)	38.668892, -0.519791	CAL; UPA
P3	Dos Aguas (ES)	39.300432, -0.788097	CAL; UPA
P4	Chiva (ES)	39.530658, -0.809555	CAL
P5	Serra Calderona (ES)	39.753657, -0.494385	CSA; FTH
P6	Ayora (ES)	39.116667, -0.950000	UPA; FTH
P8	Desert de les Palmes (ES)	40.067325, 0.040802	CSA
P12	Barranc dels Horts (ES)	40.401536, -0.086174	UPA
P13	Marmaris (TR)	36.914640, 28.242170	CCR; CSA
P14	Fethiye (TR)	36.725866, 28.990324	CCR; CSA
P16	Datça (TR)	36.681477, 27.404902	CCR; CPA; CSA

Table S2. Statistical analyses (GLMM) of pairwise differences in germination between treatments (Control, Summer and Fire) for each of the six species studied. The significance of the treatment was tested including population as a random factor (ns refers to $p > 0.05$); p-values are those obtained after the false discovery rate correction. For each species, parameter estimates (and S.E.) refer to treatment B in relation to A; that is, positive estimated values indicate higher germination in treatment B while negative estimated values indicate higher germination in treatment A. Control refers to untreated seeds (Control), fire treatment refers to the seeds submitted to 80°C, 100°C, 120°C and 150°C for 5 minutes (Fire 80, Fire 100, Fire 120 and Fire 150, respectively); and the summer treatments refer to seeds exposed to temperature fluctuations for 5 or 30 days (Summer 5 and Summer 30, respectively). A summary of these results is provided in Table 2 (Species mean) and Table 3 of the main text.

Species	A	B	Estimate	S.E.	z value	Pr(> z)
<i>C. albidus</i>	Control	Summer 5	0.0959	0.2724	0.35	ns
		Summer 30	0.1868	0.2698	0.69	ns
		Fire 80	2.9414	0.3795	7.75	< 0.00001
		Fire 100	4.4531	0.3095	14.39	< 0.00001
		Fire 120	4.2650	0.3980	10.71	< 0.00001
		Fire 150	-0.2955	1.3295	-0.22	ns
	Summer 5	Summer 30	-0.0930	0.2587	-0.36	ns
		Fire 80	2.8330	0.3749	7.56	< 0.00001
		Fire 100	4.3448	0.3039	14.29	< 0.00001
		Fire 120	4.1565	0.3937	10.56	< 0.00001
		Fire 150	-0.4039	1.3282	-0.304	ns
	Summer 30	Fire 80	2.7339	0.3711	7.37	< 0.00001
		Fire 100	4.2457	0.2992	14.19	< 0.00001
		Fire 120	4.0574	0.3901	10.40	< 0.00001
		Fire 150	-0.2677	1.3077	-0.20	ns
	Fire 80	Fire 100	1.5095	0.2435	6.20	< 0.00001
		Fire 120	1.3232	0.3145	4.21	< 0.001
		Fire 150	-3.0914	1.0530	-2.94	0.02
	Fire 100	Fire 120	-0.1906	0.1662	-1.15	ns
		Fire 150	-4.3494	0.7838	-5.55	< 0.00001
	Fire 120	Fire 150	-4.2124	0.7391	-5.70	< 0.00001
<i>C. creticus</i>	Control	Summer 30	0.2329	0.5647	0.41	ns
		Fire 100	1.6080	0.3591	4.48	< 0.0001
		Fire 120	3.3047	0.5504	6.00	< 0.00001
		Fire 150	5.2059	0.6044	8.61	< 0.00001
	Summer 30	Fire 100	1.5438	0.4794	3.22	< 0.01
		Fire 120	3.1357	0.2520	12.44	< 0.00001
		Fire 150	4.9889	0.2981	16.73	< 0.00001

	Fire 100	Fire 120	1.5439	0.5179	2.98	0.02
		Fire 150	3.4190	0.4611	7.41	< 0.00001
	Fire 120	Fire 150	1.8604	0.2379	7.82	< 0.00001
<i>C. parviflorus</i>	Control	Summer 30	0.4216	0.4681	0.90	ns
		Fire 100	5.8597	0.4768	12.29	< 0.00001
		Fire 120	5.0531	0.4246	11.90	< 0.00001
		Fire 150	8.2810	1.0660	7.77	< 0.00001
	Summer 30	Fire 100	5.4380	0.4310	12.62	< 0.00001
		Fire 120	4.6315	0.3725	12.43	< 0.00001
		Fire 150	7.8599	1.0463	7.51	< 0.00001
	Fire 100	Fire 120	-0.8066	0.3833	-2.10	ns
		Fire 150	2.4218	1.0501	2.31	ns
	Fire 120	Fire 150	3.2284	1.0275	3.14	0.01
<i>C. salviifolius</i>	Control	Summer 30	0.4483	0.1780	2.52	ns
		Fire 100	2.3844	0.6348	3.76	< 0.01
		Fire 120	4.4691	0.1871	23.89	< 0.00001
		Fire 150	6.5889	0.5106	12.90	< 0.00001
	Summer 30	Fire 100	1.9239	0.6956	2.77	0.04
		Fire 120	4.0255	0.1856	21.69	< 0.00001
		Fire 150	6.1424	0.3811	16.12	< 0.00001
	Fire 100	Fire 120	2.0996	0.5630	3.73	< 0.01
		Fire 150	4.1205	0.8524	4.83	< 0.0001
	Fire 120	Fire 150	2.1017	0.5306	3.96	< 0.001
<i>F. thymifolia</i>	Control	Summer 30	0.3106	0.3699	0.84	ns
		Fire 100	2.7515	0.8592	3.20	< 0.01
		Fire 120	3.4855	0.7390	4.72	< 0.0001
		Fire 150	4.7452	0.7661	6.19	< 0.00001
	Summer 30	Fire 100	2.4790	1.0740	2.31	ns
		Fire 120	3.2071	0.9396	3.41	< 0.01
		Fire 150	4.4708	0.9698	4.61	< 0.0001
	Fire 100	Fire 120	0.7251	0.1678	4.32	< 0.001
		Fire 150	1.9762	0.1683	11.74	< 0.00001

	Fire 120	Fire 150	1.2595	0.1668	7.55	< 0.00001
<i>U. parviflorus</i>	Control	Summer 5	1.3223	0.1772	7.46	< 0.00001
		Summer 30	1.0610	0.2246	4.72	< 0.0001
		Fire 80	2.1264	0.2154	9.87	< 0.00001
		Fire 100	3.9165	0.4085	9.59	< 0.00001
		Fire 120	4.2316	0.4281	9.88	< 0.00001
		Fire 150	3.6527	0.3478	10.50	< 0.00001
	Summer 5	Summer 30	-0.2618	0.1264	-2.071	ns
		Fire 80	0.8106	0.2300	3.525	< 0.01
		Fire 100	2.5983	0.2591	10.030	< 0.00001
		Fire 120	2.9129	0.3314	8.791	< 0.00001
		Fire 150	2.3401	0.2930	7.986	< 0.00001
	Summer 30	Fire 80	1.0745	0.2166	4.96	< 0.00001
		Fire 100	2.8538	0.2835	10.07	< 0.00001
		Fire 120	3.1671	0.4072	7.78	< 0.00001
		Fire 150	2.5911	0.2899	8.94	< 0.00001
Fire 80	Fire 100	1.7824	0.4490	3.97	< 0.001	
	Fire 120	2.0947	0.5314	3.94	< 0.001	
	Fire 150	1.5170	0.4170	3.64	< 0.01	
Fire 100	Fire 120	0.3146	0.2173	1.45	ns	
	Fire 150	-0.2643	0.2189	-1.21	ns	
Fire 120	Fire 150	-0.5785	0.3287	-1.76	ns	

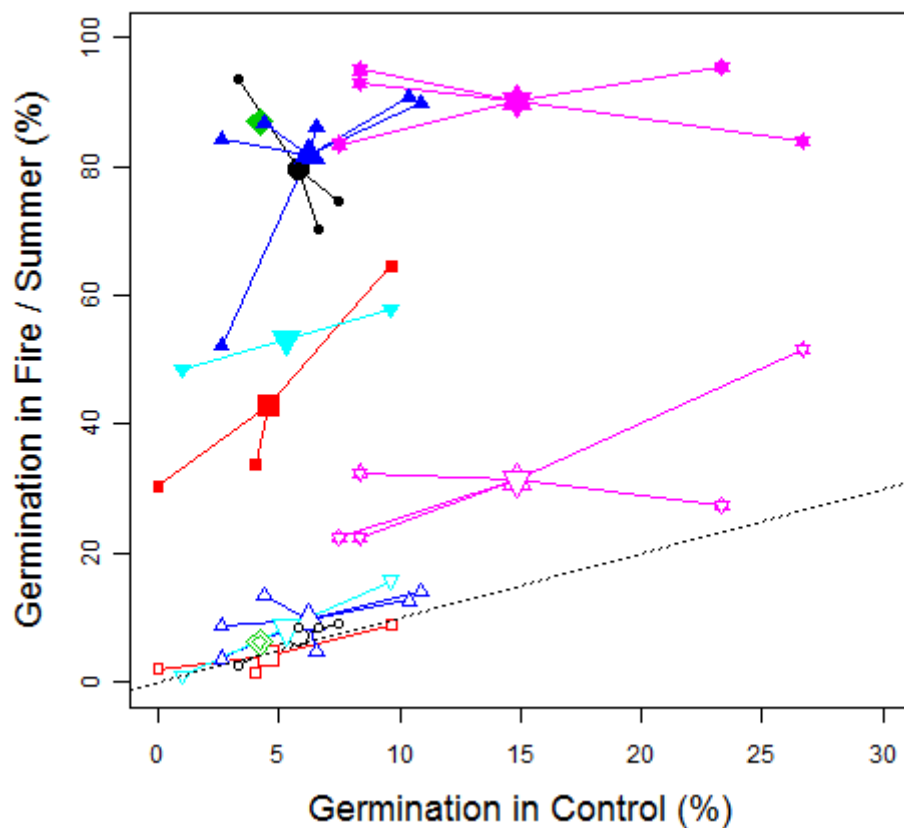


Figure S1. Germination (%) in control conditions (untreated seeds), in the fire scenario and in the summer scenario. Relationship of the germination (%) of untreated seeds (Control) with the germination after the treatment of 120°C for 5 minutes (Fire; filled symbols) and after the treatment simulating temperature fluctuations in the soil (Summer; open symbols). Intraspecific variability (i.e., among populations) is indicated by small symbols (mean population value) emerging from the large symbol (mean species value; *Fumana thymifolia*, n=2 populations; *Cistus salviifolius*, n=6 populations; *Cistus albidus*, n=4 populations; *Cistus parviflorus*, n=1 population; *Cistus creticus*, n=3 populations; *Ulex parviflorus*, n=5 populations). The 1:1 line is also shown (dotted line).

Chapter 4

Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora

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Abstract

The role of fire as a germination cue for Mediterranean Basin (MB) plants is still unclear. The current idea is that heat stimulates germination mainly in Cistaceae and Fabaceae and that smoke has a limited role as a post-fire germination cue, in comparison with other mediterranean-type ecosystems (MTEs), suggesting that fire-stimulated germination is less relevant in the MB than in other MTEs. However, recent studies showed that the assembly of Mediterranean plant communities is strongly driven by post-fire germination, suggesting an important role for fire as a germination cue. We hypothesize that both heat and smoke have important effects on the different post-fire recruitment processes of MB species (e.g. level and rate of germination and initial seedling growth). To ascertain the role of heat and smoke in the post-fire germination response of MB woody plants, a germination experiment was performed with seven heat and two smoke treatments on 30 MB woody species from seven different families, including species with water-permeable seeds and species with water-impermeable seeds. Heat stimulated the germination (probability and rate) of 21 species and smoke in eight species, out of the 30 species studied. In addition, six species showed enhanced initial seedling growth after the smoke treatments. The results suggest that both heat and smoke are important germination cues in a wide range of MB woody species and that fire-cued germination in woody plants of the MB may be as important as in other MTEs.

Keywords: Post-fire germination, heat treatments, Mediterranean Basin, smoke treatments, seedling growth, Cistaceae, Fabaceae, Ericaceae, Lamiaceae, Linaceae, Scrophulariaceae, Primulaceae.

Introduction

Fire-stimulated germination is found in many species from Mediterranean fire-prone ecosystems (Keeley, 1995). Post-fire germination may be triggered by different mechanisms, with heat and smoke being the main fire-related germination cues (Keeley and Fotheringham, 2000; van Staden et al., 2000). Heat shock can trigger germination by accelerating after-ripening in species with a water-permeable seed coat, or by rupturing the seed coat layer, allowing water uptake, in species with water impermeable seeds (Baskin and Baskin, 1998). The smoke produced during fire may also stimulate germination and seedling growth (Blank and Young, 1998; Keeley and Fotheringham, 2000; Light et al., 2009). Smoke-stimulated germination has been attributed to the presence of butenolide molecules (karrikins; e.g. KAR1, 3-methyl-2H-furo[2,3-c]pyran-2-one), a family of structurally related plant growth regulators (Flematti et al., 2004; Nelson et al., 2009).

In the Mediterranean Basin (MB), heat-stimulated germination has been linked to the rupture of the seed coat in species with water-impermeable seeds, mostly Cistaceae and Fabaceae (González-Rabanal and Casal, 1995; Herranz et al., 1998, 1999; Paula and Pausas, 2008). The role of smoke is less clear as most studies performing germination experiments with smoke or charred wood on MB species suggest that smoke has a limited role as a post-fire germination cue (Keeley and Baer-Keeley, 1999; Buhk and Hensen, 2006; Reyes and Casal, 2006; Rivas et al., 2006; Reyes and Trabaud, 2009). However, a few studies have found some evidence of smoke-stimulated germination in MB woody plants, both in species with water-permeable seeds and in species typically having a high percentage of water-impermeable seeds (i.e., Cistaceae and Fabaceae; Pérez-Fernández and Rodríguez-Echevarría, 2003; Crosti et al., 2006). Smoke cannot stimulate germination of seeds that are physically dormant; therefore, smoke stimulation (i.e., increased germination percentage, germination rate or seedling growth) in species with water-impermeable seeds is likely to occur only if seeds are released from physical dormancy (become water permeable) by suitable environmental factors or if smoke acts over the fraction of water-permeable seeds, which in some cases (species or populations) may be high (Thanos et al., 1992).

This limited experimental evidence of fire-cued germination in the MB flora, particularly with regard to smoke, suggests that fire-stimulated germination is less relevant in the MB than in other Mediterranean-type ecosystems (MTEs). Indeed, it has been postulated that smoke-stimulated germination may be an example of the lack of convergent evolution between the MB and other MTEs (Keeley and Baer-Keeley, 1999). On the other hand, recent studies

suggest that fire-stimulated germination is of paramount importance for understanding the evolution of MB flora (Pausas and Verdú, 2005) and the assembly processes of MB communities (Verdú and Pausas, 2007; Pausas and Verdú, 2008; Coca and Pausas, 2009). We suggest that our understanding of the role of fire in the recruitment process of MB species is limited by the availability of well designed experiments, and we hypothesize that both heat and smoke play an important role in the different post-fire recruitment processes (e.g. level and rate of germination and initial seedling growth) of MB species.

To ascertain the role of fire in the post-fire recruitment processes of MB woody plants, a germination experiment was conducted with 30 MB woody species typical of Mediterranean shrublands, in which the same treatments were applied to each species, using a replicated design and subsequently testing the post-treatment seed viability. This experiment included a range of species, from different families, showing evidence of post-fire germination; it also included species both with and without water-impermeable seeds. This experiment allowed an accurate test of the effect of both heat and smoke on the different post-fire recruitment processes (i.e. seed resistance to heat shock, germination and seedling growth stimulation).

Materials and methods

Species and seed collection

Thirty woody species were selected (Table 1), including both shrubs (nanophanerophytes) and scrubs (chamaephytes), occurring in fire-prone areas of the SE Iberian Peninsula in which there was evidence of post-fire seedling emergence (Paula et al., 2009). All seeds were collected from ripe fruits in wild populations during 2007, coinciding with the dispersal period of each species, with the sole exception of the seeds of *Genista umbellata*, which were bought from an accredited commercial collector and collected from wild populations in June 2006. Seeds of each species (except *G. umbellata*) were collected from a minimum of 30 individuals (spatially dispersed) of the same population. Seeds were separated from the fruit tissues that would normally be lost during dispersal and cleaned of unwanted material such as fruit residues. Seeds were then stored in paper bags at controlled temperature (20 °C) and low humidity (40–50 %) until the start of the experiment, in November 2007.

The studied species belong to seven different families; two of them (Cistaceae and Fabaceae) are typically characterized by having water-impermeable seeds (Baskin and Baskin, 1998). Nevertheless, some species of these families may have a high percentage of water-permeable seeds (Baskin et al., 2000) and thus to verify seed permeability ten seeds of each species were

weighed (individually), they were imbibed in water for 24 h and then they were re-weighed. Seeds with a significant increment in weight (>20%) were considered to be water permeable. For *Fumana* species, the presence of mucilage in the seeds invalidated this test and, thus, water permeability was evaluated through a cut test, as water uptake softened the seeds whereas water-impermeable seeds remained hard. Species with at least 20% permeable seeds were considered to have water-permeable seeds despite belonging to families typically with water-impermeable seeds (Table 1).

Treatments

Before applying any treatment, the viability of each seed lot was tested by the cut test method using a sample of 50 intact seeds of each species. Empty seeds or seeds with symptoms of decay were considered non-viable. Seven heat and two smoke treatments, plus two controls, were then performed.

The heat treatments consisted of the following combinations of temperatures and exposure times (heat dose): ‘80 °C for 5 min’ (lowest heat dose), “80°C for 10 min”, “100°C for 5 min”, “100 °C for 10 min”, “120°C for 5 min”, “120°C for 10 min” and “150°C for 5 min” (highest heat dose). A control (untreated seeds) was also included. The heat treatments simulate the range of temperatures and exposure times in the top layer of soil during a fire (Trabaud, 1979) and were carried out using an electric oven. For each heat treatment, we proceeded as follows: seeds were placed in aluminium dishes (one for each species), which were arranged randomly in the centre of the oven tray to avoid the extremes and thus minimize the variation in temperature, and the tray was quickly introduced into the oven. This operation was repeated four times for each treatment and species (four independent replicates). Air temperature inside the oven was monitored using a thermocouple and took between 30 and 65 s to reach the desired temperature. The seeds were then held at this temperature ($\pm 1^\circ\text{C}$) for the corresponding treatment time (5 or 10 min).

Smoke treatments corresponded to two different dilutions of liquid smoke solution: “smoke 1:1” and “smoke 1:10”. To eliminate any effect on the germination caused by the seeds being imbibed in a liquid for 24 h before sowing, we used a ‘water control’ (pH 5.53; seeds immersed in distilled water for 24 h before sowing) and not the dry control used for the heat treatments. Smoke treatments were not performed for *Genista triacanthos* due to the limited availability of seeds. To produce the smoke solution, dry leaves and thin twigs of *Quercus coccifera* were ground in an electric grinder. Four batches (replicates) were separately heated

in an oven for 30 min at 198°C ($\pm 1^\circ\text{C}$) and processed as described in Jager et al. (1996a), to obtain the concentrated smoke solution (smoke 1:1; pH 4.78). For each replicate of the 1:1 smoke solution, a sample was taken to obtain four replicates of diluted smoke solution (smoke 1:10; pH 4.90). Seeds were then incubated in the respective smoke solution for 24 h.

After the respective treatments, seeds were sown in Petri dishes with agar (0.7 %) as substrate and incubated at 20°C ($\pm 1^\circ\text{C}$) in darkness, being exposed to light only during the monitoring periods (see below). Incubation was performed under dark conditions to better ensure homogeneous conditions, given the magnitude of the germination experiment (1320 Petri dishes). The number of seeds sown was adjusted based on the estimation of the viability of the lots for each species (see above), in order to have a minimum of 100 viable seeds per species and treatment, distributed in the four replicates. Seed germination was monitored every 2 days during the first 2 weeks and then once a week until the end of the experiment, 2 months later. At each count, the germinated seeds (those with radicle emergence >1 mm, determined under a magnifying lens) were scored and removed from the Petri dishes. At the end of the experiment, the initial number of seeds sown was corrected by discarding empty seeds and anomalous germinations detected during the experiment.

Post-treatment seed viability

At the end of the experiment, the viability of the non germinated seeds was checked by using the tetrazolium chloride test. A small incision was made in the seed coat of each non-germinated seed in order to facilitate uptake of the stain. Seeds were incubated in a 1% solution of 2,3,5-triphenyl tetrazolium chloride for 24 h at 30°C in dark conditions (International Seed Testing Association, 1999). Seeds were then dissected to check for intact and not discoloured embryo, and classified as viable (most of the embryo stained) or not viable (embryo either barely stained or unstained). A preliminary test with tetrazolium demonstrated that in fresh seeds of *Sideritis angustifolia*, *Lavandula latifolia* and *Lavandula stoechas*, only the radicle became stained; thus, this was the criterion used to classify viability in these species. In the same preliminary test, it was also verified that seeds of *Erica umbellata* and *Erica terminalis* did not become stained with tetrazolium. Therefore, for these species, the viability after treatment was verified through the cut test, and their seeds were classified as viable or non-viable depending on the consistency of the seed.

Initial seedling growth

To test the effect of smoke on the initial seedling growth, germinated seeds from the smoke treatments and water control were transferred to 150 mm diameter Petri dishes with agar (1.5 %) as substrate and placed in a germination chamber at 20°C with a 12/12 h photoperiod. Petri dishes were placed vertically so that seedlings were able to grow, and exposed to similar irradiances. During this period, seedlings were not exposed to smoke. After the seedlings had grown in these conditions for 7–10 days (depending on the species) a digital photo was taken. Shoot and root length was measured with an image analysis program (ImageJ: Rasband, 2005). Only seeds germinated in the first 2 weeks and with radicle emergence >5 mm were used. The number of seeds placed in each of the vertically disposed Petri dishes depended on the number of seeds that fulfilled these requisites. Seedlings that died before accomplishing the monitoring time (7–10 days) were excluded from the analysis.

Statistical analysis

At each count, for each replicate and in all species, seeds were classified as germinated or not germinated following the criterion mentioned above (radicle emergence). For each species, the final germination of each treatment was compared with its respective control using an analysis of deviance (GLM) and assuming a binomial error distribution. Based on the results of post-treatment viability, the seed mortality of each heat treatment was compared with the respective control, and it was ascertained whether mortality was significantly higher than the control for each treatment. Germination rate was calculated by comparing the time to 50 % germination (T50) for each heat treatment with the respective control. Due to the large number of pairwise comparisons in all these analyses (germination percentage, seed mortality and germination rate), the significant level considered was $P < 0.01$, which is a less conservative criterion than the Bonferroni correction (Moran, 2003).

To test the effect of smoke on the initial seedling growth, the final length (shoot, root and total) of seedlings growing from smoke-treated seeds was compared with the final length of seedlings growing from water-treated seeds. Preliminary analysis showed no differences in initial growth between the two smoke treatments and thus they were considered together in the analysis. This analysis of seedling growth was not performed in species with low germination in the smoke treatments or in the distilled water control. Thus, only 18 species were analysed for seedling growth.

Results

Germination percentage

Most species had low germination values (<20 %) in the control, suggesting a high degree of dormancy (Tables 1 and 2). However, some species such as *Digitalis obscura*, *Erica multiflora*, *G. umbellata*, *Linum suffruticosum*, *S. angustifolia*, *Thymus piperella* and *Thymus vulgaris* had high germination values (>75 %) in control conditions, suggesting low dormancy (Tables 1 and 2). Note that the only Fabaceae that had high germination in the control (*G. umbellata*) was the one in which we detected that the seeds were water permeable (Table 1).

Twenty species were stimulated by at least one of the heat treatments (Table 1) and eight species were stimulated by at least one smoke treatment (Table 2). Fifteen species, mainly Cistaceae or Fabaceae, were stimulated by heat and not by smoke treatments (smoke was not tested in *G. triacanthos*). Three species were stimulated strictly by the smoke treatments and five species by both heat and smoke treatments. Thus, 23 of 30 species showed a significant ($P < 0.01$) increase in germination percentage as a result of at least one of the germination treatments studied (Tables 1 and 2). Smoke stimulation was observed in Ericaceae, Lamiaceae and Primulaceae; all species with water-permeable seeds. In some of the species showing low germination in the control (*Anthyllis lagascana*, *Dorycnium pentaphyllum*, *Genista scorpius* and *Helianthemum syriacum*), all treatments failed to trigger germination, which remained at low values. Germination inhibition by heat treatments was observed mainly for the highest heat doses (150°C for 5 min).

Table 1. List of tested species, their family, seed coat type (P, water-permeable, I, water-impermeable; based on the permeability tests described in Materials and methods) and the mean germination percentage in control conditions and after the heat treatments

Species	Family	Seed coat	Control	80 °C	80 °C	100 °C	100 °C	120 °C	120 °C	150 °C
				5 min	10 min	5 min	10 min	5 min	10 min	5 min
<i>Anthyllis cytisoides</i>	Fabaceae	I	1	2ns	2ns	3ns	4ns	1ns	4ns	9**
<i>Anthyllis lagascana</i>	Fabaceae	I	5	5ns	3ns	5ns	8ns	7ns	6ns	0**
<i>Cistus albidus</i>	Cistaceae	I	11	21*	55****	60****	55****	61****	18ns	0****
<i>Cistus monspeliensis</i>	Cistaceae	I	11	19ns	22*	42****	50****	73****	48****	51****
<i>Coris monspeliensis</i>	Primulaceae	P	2	1ns	1ns	4ns	3ns	3ns	7ns	1ns
<i>Coronilla minima</i>	Fabaceae	I	5	12ns	8ns	6ns	20***	20**	0**	0**
<i>Digitalis obscura</i>	Scrophul.	P	90	86ns	83ns	92ns	88ns	94ns	89ns	58****
<i>Dorycnium pentaphyllum</i>	Fabaceae	I	12	11ns	12ns	9ns	17ns	20ns	0****	2***
<i>Erica multiflora</i>	Ericaceae	P	81	83ns	79ns	80ns	90*	87ns	87ns	93***
<i>Erica terminalis</i>	Ericaceae	P	22	25ns	30ns	38*	30ns	31ns	21ns	46***
<i>Erica umbellata</i>	Ericaceae	P	0	0ns	0ns	0ns	0ns	0ns	1ns	1ns
<i>Fumana ericoides</i>	Cistaceae	I	0	2ns	9***	11****	17****	17****	18****	0ns
<i>Fumana thymifolia</i>	Cistaceae	I	8	19*	27***	43****	57****	58****	1**	0****
<i>Genista scorpius</i>	Fabaceae	I	17	10ns	17ns	22ns	24ns	23ns	4**	1***
<i>Genista triacanthos</i>	Fabaceae	I	4	84****	86****	91****	86****	82****	91****	67****
<i>Genista umbellata</i>	Fabaceae	P	78	76ns	72ns	86ns	86ns	92**	83ns	34****
<i>Helianthemum syriacum</i>	Cistaceae	I	12	16ns	13ns	8ns	6ns	6ns	0****	0****
<i>Lavandula latifolia</i>	Lamiaceae	P	13	20**	18ns	32****	25**	30***	2**	1***
<i>Lavandula stoechas</i>	Lamiaceae	P	10	21**	31****	41****	68****	72****	12ns	0****
<i>Linum suffruticosum</i>	Linaceae	P	98	100ns	95ns	95ns	98ns	97ns	94ns	88**
<i>Ononis minutissima</i>	Fabaceae	I	2	1ns	3ns	3ns	29****	21****	1ns	3ns
<i>Rosmarinus officinalis</i>	Lamiaceae	P	19	27ns	37**	46****	26ns	35**	28ns	14ns
<i>Sideritis angustifolia</i>	Lamiaceae	P	87	84ns	82ns	73*	30****	11****	9****	1****
<i>Teucrium capitatum</i>	Lamiaceae	P	17	22ns	39****	33**	25ns	18ns	20ns	1****
<i>Teucrium rosmarineri</i>	Lamiaceae	P	35	36ns	48*	52**	25ns	26ns	18**	1****
<i>Thymus piperella</i>	Lamiaceae	P	78	83ns	81ns	95****	81ns	54****	62**	22****
<i>Thymus vulgaris</i>	Lamiaceae	P	86	93ns	91ns	93ns	87ns	95*	90ns	70**
<i>Ulex borgiae</i>	Fabaceae	I	5	62****	72****	58****	90****	89****	75****	0**
<i>Ulex parviflorus</i>	Fabaceae	I	4	40****	67****	59****	83****	92****	66****	1ns
<i>Xolantha tuberaria</i>	Cistaceae	I	1	5ns	2ns	8*	44****	66****	63****	34****

The significance of the pairwise comparison of each treatment with its corresponding control is also included (ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001).

Table 2. Mean germination percentage, mean germination rate (T50 in days) and mean initial seedling growth (root + shoot length in mm) in the control (C) conditions and after the smoke treatments.

Species	Germination percentage			Germination rate			Total seedling growth	
	C	Smoke 1:1	Smoke 1:10	C	Smoke 1:1	Smoke 1:10	C	Smoke
<i>Anthyllis cytisoides</i>	1	0 ns	2 ns	264	-	154 ns	-	-
<i>Anthyllis lagascana</i>	4	3 ns	2 ns	321	96 ns	69 ns	-	-
<i>Cistus albidus</i>	9	12 ns	6 ns	194	137 ns	136 ns	39.52	44.01 ns
<i>Cistus monspeliensis</i>	18	18 ns	14 ns	105	112 ns	303 ns	27.01	35.05 ns
<i>Coris monspeliensis</i>	95	43 ***	24 **	172	60 ****	113 ****	14.36	36.23 *
<i>Coronilla minima</i>	11	11 ns	12 ns	146	125 ns	140 ns	42.85	63.53 *
<i>Digitalis obscura</i>	94	89 ns	96 ns	6	6 ns	4 *	25.73	26.04 ns
<i>Dorycnium pentaphyllum</i>	9	7 ns	7 ns	216	129 *	162 ns	50.04	45.88 ns
<i>Erica multiflora</i>	82	94 **	83 ns	16	11 **	15 ns	21.63	20.70 ns
<i>Erica terminalis</i>	33	60 ***	53 ****	76	44 ***	54 **	-	-
<i>Erica umbellata</i>	2	56 ***	7 *	195	43 ***	177 ns	-	-
<i>Fumana ericoides</i>	0	2 ns	4 *	-	173	131	-	-
<i>Fumana thymifolia</i>	5	11 ns	11 ns	133	128 ns	114 ns	-	-
<i>Genista scorpius</i>	17	14 ns	17 ns	161	154 ns	169 ns	44.87	58.79 ns
<i>Genista umbellata</i>	70	74.0 ns	77 ns	21	15 ns	11 ns	44.51	55.69 ns
<i>Helianthemum syriacum</i>	8	8 ns	11 ns	189	232 ns	153 ns	-	-
<i>Lavandula latifolia</i>	29	59 ***	47 **	85	35 **	53 *	26.98	48.86 **
<i>Lavandula stoechas</i>	50	100 ***	96 ***	47	4 ****	5 ****	33.22	34.72 ns
<i>Linum suffruticosum</i>	95	99 ns	98 ns	3	2 ns	2 ns	49.02	51.87 ns
<i>Ononis minutissima</i>	0	2 ns	3 *	-	132	496	-	-
<i>Rosmarinus officinalis</i>	26	40 *	42 **	88	70 ns	66 ns	43.88	36.24 ns
<i>Sideritis angustifolia</i>	85	90 ns	90 ns	5	6 ns	6 ns	47.36	54.34 ns
<i>Teucrium capitatum</i>	30	34 ns	30 ns	75	75 ns	87 ns	23.42	18.35 ns
<i>Teucrium ronnigeri</i>	42	55 *	58 *	60	46 ns	45 ns	14.88	26.07 **
<i>Thymus piperella</i>	96	95 ns	83 **	2	2 ns	2 ns	37.12	45.33 **
<i>Thymus vulgaris</i>	88	100 ***	96 ns	4	2 **	2 **	31.59	35.64 *
<i>Ulex borghiae</i>	9	3 ns	3 *	180	319 ns	170 ns	-	-
<i>Ulex parviflorus</i>	2	7 ns	2 ns	171	167 ns	170 ns	-	-
<i>Xolantha tuberaria</i>	4	6 ns	4 ns	462	89 ns	176 ns	-	-

The significance of the pairwise comparison of each treatment with its corresponding control is also included (ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001).
-, not available.

Germination rate

Both heat and smoke treatments affected the germination rate (Figure 1). *Cistus albidus*, *Cistus monspeliensis*, *Fumana thymifolia*, *Xolantha tuberaria*, *Anthyllis cytisoides*, *G. triacanthos*, *Ononis minutissima*, *Ulex borgiae*, *Ulex parviflorus*, *Rosmarinus officinalis* and *Teucrium capitatum* had significantly faster germination in the heat treatments than in the control (Table 3). *Erica umbellata* and *T. vulgare* had significantly faster germination in the smoke treatments than in the control (Table 2). *Coris monspeliensis*, *E. multiflora*, *E. terminalis*, *L. latifolia*, *L. stoechas* and *T. vulgare* had significantly faster germination in both smoke and heat treatments (Tables 2 and 3). Significantly slower germination, in relation to control, occurred mainly for the highest heat doses in *D. obscura*, *G. umbellata*, *L. suffruticosum*, *S. angustifolia*, *T. vulgare* and *T. piperella*.

Seed mortality after heat treatments

For low-moderate heat doses, seed mortality of most species did not change significantly with respect to the control. The heat treatments of 120°C 10 min and 150°C 5 min produced high mortality in most species. However, some species proved to be highly resistant to heat shock and their mortality did not increase significantly even at the highest heat dose (e.g., *E. multiflora* or *L. suffruticosum*).

Effect of smoke in the initial growth of the seedlings

Exposing seeds to smoke significantly increased total initial seedling growth compared with seedlings germinating from untreated seeds in six species out of 18: *Coronilla minima* (Fabaceae), *C. monspeliensis* (Primulaceae), *L. latifolia*, *T. piperella*, *T. vulgare* and *Teucrium rosnigeri* (Lamiaceae, Table 2). For all these species, differences in seedling growth were seen to be greater in the roots than the shoots. None of the species showed any reduction in total seedling length following the smoke treatments, and four species (*D. obscura*, *E. multiflora*, *L. stoechas* and *S. angustifolia*) showed changes in root and/or shoot lengths without significantly changing total length.

Discussion

Effect of heat

All species with water-impermeable seeds and thus physical dormancy (Cistaceae and most Fabaceae) showed low germination in control conditions. Germination was triggered in most species by one or several heat treatments. Nevertheless, stimulation by heat was not exclusive to Cistaceae and Fabaceae, for which heat-stimulated germination is well known (Herranz et

al., 1998, 1999; Paula and Pausas, 2008). Most Ericaceae and Lamiaceae species, having water permeable seeds, were also stimulated by heat (Table 4). Maximum stimulation by heat, in terms of both the number of species stimulated and the magnitude of the stimulation, was observed at 120°C 5 min. The highest heat doses (treatments of 120°C 10 min and 150°C 5 min) were lethal for many species, and thus the stimulation of germination was reduced. In species with a low dormancy level (i.e., species with high germination in the control), germination remained high for almost the full range of heat treatments and was only inhibited in a few species and at high heat doses (heat doses higher than 100°C 10 min in *S. angustifolia*; 120°C 5 min in *T. piperella*; and 150°C 5 min in *D. obscura* and *L. suffruticosum*). Thus, these species with low dormancy resist heat shock, suggesting that they may also germinate and recruit after fire, although with less intensity than the species with higher seed dormancy.

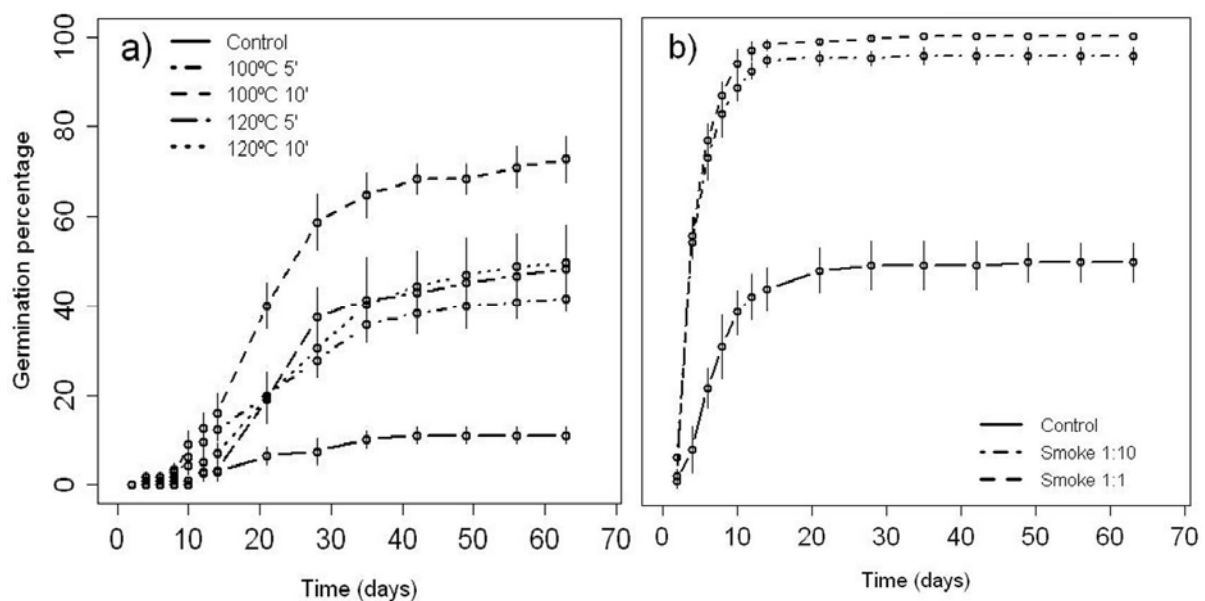


Figure 1. Germination percentage (mean \pm s.e.) in relation to time since sowing (days) for *Cistus monspeliensis* after different heat treatments (A), and for *Lavandula stoechas* after different smoke treatments (B). See text for details of treatments.

Table 3. Mean germination rate (T50 in days) in the control conditions and after the heat treatments (-: not available), and the significance of the pairwise comparison of each treatment with its corresponding control (* p < 0.05; ** p < 0.01; *** p < 0.001; **** p < 0.0001).

Species	Control	80°C		100°C		120°C		150°C
		5'	10'	5'	10'	5'	10'	5'
<i>Anthyllis cytisoides</i>	266	177*	187*	163**	163**	207ns	183*	144**
<i>Anthyllis lagascana</i>	158	110ns	217ns	154ns	178ns	154ns	168ns	-
<i>Cistus albidus</i>	171	92*	54**	48***	50***	47**	95*	-
<i>Cistus monspeliensis</i>	135	107ns	92*	63***	52***	33****	52****	52***
<i>Coris monspeliensis</i>	264	283ns	245ns	211ns	177*	165*	134**	154ns
<i>Coronilla minima</i>	154	179ns	133ns	181ns	111ns	109ns	-	-
<i>Digitalis obscura</i>	7	7ns	6ns	6ns	7ns	10ns	11ns	25****
<i>Dorycnium pentaphyllum</i>	197	251ns	168ns	182ns	133ns	128ns	-	216ns
<i>Erica multiflora</i>	20	17ns	18ns	23ns	14**	14**	14**	13**
<i>Erica terminalis</i>	96	82ns	81ns	59***	74*	77*	89ns	63***
<i>Erica umbellata</i>	-	-	-	-	-	-	279	179
<i>Fumana ericoides</i>	-	-	161	261	125	92	130	-
<i>Fumana thymifolia</i>	146	114ns	89**	61***	46****	45****	165ns	-
<i>Genista scorpius</i>	154	237ns	156ns	152ns	110ns	121ns	170ns	242ns
<i>Genista triacanthos</i>	127	34***	27***	28***	28***	31***	25****	42***
<i>Genista umbellata</i>	9	13ns	18ns	8ns	8ns	7ns	13ns	81****
<i>Helianthemum syriacum</i>	103	122ns	117ns	253ns	223ns	241ns	-	-
<i>Lavandula latifolia</i>	113	64*	109ns	80ns	95ns	85ns	159ns	177ns
<i>Lavandula stoechas</i>	156	113*	94**	66****	24****	22****	145ns	-
<i>Linum suffruticosum</i>	4	4ns	4ns	4ns	4ns	4ns	6***	9***
<i>Ononis minutissima</i>	251	-	189*	207ns	87****	106****	213ns	-
<i>Rosmarinus officinalis</i>	130	89**	74***	56****	92*	76***	99ns	116ns
<i>Sideritis angustifolia</i>	6	7ns	7ns	10ns	52****	64****	65****	83****
<i>Teucrium capitatum</i>	129	103ns	65**	77**	91*	99ns	118ns	172ns
<i>Teucrium ronnigeri</i>	71	69ns	56ns	50ns	87ns	84ns	98*	-
<i>Thymus piperella</i>	3	3ns	5ns	2ns	4ns	23**	18*	54****
<i>Thymus vulgaris</i>	6	6ns	5ns	5ns	5ns	4ns	4ns	16****
<i>Ulex borgiae</i>	198	53***	35***	40***	25***	29***	30***	-
<i>Ulex parviflorus</i>	143	70***	45***	55***	36***	30***	44***	156ns
<i>Xolantha tuberaria</i>	211	191ns	239ns	119*	59***	36***	33***	91**

The significance of the pairwise comparison of each treatment with its corresponding control is also included (ns, not significant; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001).
-, not available.

Table 4. Species whose germination (percentage and/or rate) and initial seedling growth is stimulated by heat and/or smoke.

Family	Species	Germination percentage	Germination rate	Smoke-stimulated growth
Cistaceae	<i>Cistus albidus</i>	Heat	Heat	No
	<i>Cistus monspeliensis</i>	Heat	Heat	No
	<i>Fumana ericoides</i>	Heat		–
	<i>Fumana thymifolia</i>	Heat	Heat	–
	<i>Xolantha tuberaria</i>	Heat	Heat	–
Ericaceae	<i>Erica umbellata</i>	Smoke	Smoke	–
	<i>Erica multiflora</i>	Heat, smoke	Heat, smoke	No
	<i>Erica terminalis</i>	Heat, smoke	Heat, smoke	–
Fabaceae	<i>Anthyllis cytisoides</i>	Heat	Heat	–
	<i>Coronilla minima</i>	Heat		Yes
	<i>Genista triacanthos</i>	Heat	Heat	–
	<i>Genista umbellata</i>	Heat		No
	<i>Ononis minutissima</i>	Heat	Heat	–
	<i>Ulex borgiae</i>	Heat	Heat	–
	<i>Ulex parviflorus</i>	Heat	Heat	–
Lamiaceae	<i>Teucrium capitatum</i>	Heat	Heat	No
	<i>Teucrium ronnigeri</i>	Heat		Yes
	<i>Thymus piperella</i>	Heat	Heat	Yes
	<i>Thymus vulgaris</i>	Smoke	Smoke	Yes
	<i>Lavandula stoechas</i>	Heat, smoke	Heat, smoke	No
	<i>Lavandula latifolia</i>	Heat, smoke	Heat, smoke	Yes
	<i>Rosmarinus officinalis</i>	Heat, smoke	Heat	–
Primulaceae	<i>Coris monspeliensis</i>	Smoke	Heat, smoke	Yes

Stimulation is considered when there was a significant difference from the control at $P < 0.01$ (from Tables 1–3).

Effect of smoke

Previous studies on germination in MB flora had so far found a limited response to smoke or charred wood (Keeley, 1995; Keeley and Baer-Keeley, 1999; Pérez-Fernández and Rodríguez-Echeverría, 2003; Buhk and Hensen, 2006; Reyes and Casal, 2006; Rivas et al., 2006). These results could suggest that MB flora, in contrast to other MTEs (Dixon et al., 1995; Keeley and Bond, 1997; Keeley and Fotheringham, 1998; Brown et al., 2003), has not acquired the capacity to respond to this fire-related germination cue. Indeed, this difference has been interpreted as a lack of convergence between the MB and other MTEs where smoke stimulation is present, as the stimulation by heat presented by Cistaceae and Fabaceae can be considered a homologous character (Keeley and Baer-Keeley, 1999). However, our results suggest that smoke may indeed play an important role in the germination and growth of MB species, especially in species that are not stimulated by heat or in which the effect of heat on

germination is limited. Smoke-stimulated germination was verified in Ericaceae (*E. umbellata*, *E. terminalis* and *E. multiflora*), Lamiaceae (*L. latifolia*, *L. stoechas*, *R. officinalis* and *T. vulgaris*) and Primulaceae (*Coris monspeliensis*; Table 2). The wide taxonomic range of smoke-stimulated species supports the possible ancient origin of this trait (Pausas and Keeley, 2009) rather than the convergent evolution (Keeley and Bond, 1997; Keeley and Baer-Keeley, 1999).

All three of the *Erica* species studied were stimulated by smoke. In *E. umbellata*, germination was triggered exclusively by smoke and not by heat, whereas the germination of *E. multiflora* and *E. terminalis* was stimulated by both smoke and heat. Smoke plays an important role in the germination of this genus in South Africa (Brown, 1993), and our results suggest that it is also an important germination cue for the *Erica* species of the MB.

With respect to the Lamiaceae, *L. stoechas* was highly stimulated by smoke, achieving very high germination values (95–100 %). Keeley and Baer-Keeley (1999) previously observed stimulation by charred wood in this species, although with a lower final germination percentage. Smoke also stimulated germination in three species, *L. latifolia*, *R. officinalis* and *T. vulgaris*, in which smoke and heat produced similar percentages of stimulation. As far as we know, this is the first time that stimulation by smoke has been demonstrated for these species, thus reinforcing the idea of the importance of smoke cued germination in Lamiaceae. This family has also been shown to respond positively to smoke in MTEs of California, where of the 25 species stimulated by smoke in Keeley and Fotheringham (1998), the only shrubs (three species) were Lamiaceae. *Coris monspeliensis* (Primulaceae) germinated exclusively after smoke treatments. Both the control and the heat treatments produced very low germination, while smoke greatly triggered the germination. As far as we know, this is the first time that a fire cue, specifically smoke, has been shown to stimulate germination in this species. Previous observations of smoke stimulation in Primulaceae were from Australia (Read et al., 2000).

Our results, along with those of other recent studies (Crosti et al., 2006), confirm that, at least for woody species with a water-permeable seed coat, smoke stimulates germination in several important MB species. The idea that germination response to smoke in the MB is very limited probably reflects the scarcity of studies on the effect of smoke in this flora. Most of the studies on post-fire germination in the MB have focused on shrub species of Cistaceae and Fabaceae (see the compilation by Paula and Pausas, 2008), whereas the majority of the

species stimulated by smoke in other MTEs, such as California or South Africa, are annuals (Keeley, 1991; Keeley and Bond, 1997; Keeley and Fotheringham, 1998). In addition, some species may respond to the smoke stimulus only after being in the seed bank for a period of time (Keeley and Fotheringham, 1998; Baker et al., 2005a; Keeley et al., 2005) or after the heat shock caused by fire (Keith, 1997; Tieu et al., 2001). Moreover, stimulation by smoke may depend on factors such as seed age, temperature, light levels, hydration (Brown and van Staden, 1997; van Staden et al., 2000) or dormancy cycling during seed burial (Baker et al., 2005b). None of these possibilities has yet been studied for MB flora. Thus, although herein clear evidence of smoke as an important germination cue in woody plants of MB is provided, our knowledge of smoke-stimulated germination in this region is conservative, and future studies will probably demonstrate that smoke plays an even more prominent role in the germination of MB flora. For instance, MB flora lacks evidence of short-lived fire-dependent flora only found after fire, although this strategy is well represented in other Mediterranean ecosystems (e.g. California, South Africa and Australia) where it is largely stimulated by smoke. It remains unknown whether the absence of post-fire specialization by annual plants is due to different historical fire regimes, to different adaptive strategies, to different sorting processes, to the different historical human impacts or to the lack of studies (Pausas et al. 2006). Further studies on post-fire germination of annuals and other short-lived herbaceous plants may provide relevant insights on this topic.

Methodological issues may also lead to confusing results on the response of MB flora to smoke. For instance, in studies where no significant results were obtained using liquid smoke (e.g. Buhk and Hensen, 2006), it is difficult to know whether the concentration of liquid smoke used was adequate. Jager et al. (1996b), using commercial concentrated smoke, obtained maximum germination at 1:10⁵ dilution (0.001%), which decreased at 1:10⁴ dilution (0.01%) and was totally inhibited at 1:10³ dilution (0.1%). The lowest concentration of commercial liquid smoke used by Buhk and Hensen (2006) was 0.01%. Without at least one positive response to validate the effectiveness of the smoke concentration used, it is hard to reach any conclusion. The role of smoke on MB flora is also supported by our results concerning seedling growth. Exposing seeds to smoke significantly increased initial seedling growth in six out of 18 species tested. This higher development in initial seedling growth was more notable in the roots (seven of the 18 species). In some cases, root growth with the smoke treatment was about twice (*C. minima* and *L. latifolia*) or even three times (*C. monspeliensis*) higher than in untreated seeds. The fact that *C. minima* presented increased seedling growth, despite having mainly water-impermeable seeds, suggests that smoke can act over the

proportion of water-permeable seeds. Indeed, we found that approx. 10 % of the seeds of this species were water permeable. This study represents the first time that smoke-stimulated seedling growth has been reported in woody species of MB flora [see Daws et al. (2007) for temperate and sub-tropical arable weeds that include some species also occurring in the MB]. Initial seedling growth is an important factor in the post-fire environment, as seedlings that grow faster and have a more robust root system will have a competitive advantage in the post-fire environment.

In conclusion, our results suggest that both heat and smoke are important germination cues for many MB woody species. The successful post-fire recruitment of these species is due not only to seed resistance to heat shock, but also to the enhanced germination by heat and/or smoke (in both germination percentage and germination rate). Furthermore, seedlings of some species develop faster and thus with greater fitness under post-fire conditions than after other disturbances. To understand fully the role of fire in the germination of MB flora, further studies will need to consider annuals and short lived herbaceous species (as they are very important in the post-fire dynamics of other MTEs), as well as the interaction between heat and smoke. In any case, our results point towards fire-cued germination being an evolutionary response to the frequent fires in the MB as in the other MTEs, and this needs to be considered when addressing evolutionary questions on plant traits at global scale.

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Chapter 5

Field evidence of smoke-stimulated seedling emergence and establishment in the Mediterranean Basin flora

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Abstract

Question: Many plant species from fire-prone ecosystems show smoke-stimulated germination. In most cases, this information comes from experiments performed in laboratory conditions, and thus they do not consider species interaction and density-dependent processes. This is especially true for the Mediterranean Basin (MB) flora where smoke germination response has been assessed through few laboratory experiments. Indeed, while annual species are described as a very important component of the smoke stimulated flora in other fire prone ecosystems, most of the experiments with MB species have excluded annuals. We have tested the following hypothesis (1) seedling emergence and establishment of MB species are enhanced by smoke, and (2) annual species are an important component of this smoke-stimulated flora.

Location: Mediterranean fire prone shrublands in Eastern Spain; Western Mediterranean Basin.

Methods: Here we present a field experiment, in which we applied a smoke treatment and tracked seedling emergence and establishment during one year. Differences between smoke and control sub-plots with respect to seedling emergence and establishment were analyzed at different scales: community, growth form (annual or perennial), family, and species level.

Results: At community level, smoke has a clear role in seedling recruitment, increasing seedling emergence and establishment, particularly of annual species. A positive smoke effect was detected in several families and species.

Conclusions: These results highlight the role of smoke in the regeneration of MB flora and demonstrate the crucial effect of smoke in plant fitness and consequently its role in community assembly.

Keywords: Soil seed bank; annual species; post-fire regeneration; germination cue; regeneration; fitness; fire cue; recruitment.

Introduction

Fire is one of the main drivers of vegetation dynamics in mediterranean type ecosystems (MTE) where plant species have different mechanisms that allow them to persist under recurrent fires (Pausas and Keeley 2009; Keeley et al. 2012). One of these mechanisms is post-fire recruitment from soil or canopy seed banks (Pausas et al. 2004), which allows plants to take advantage of the reduced competition and the higher resource availability in the post-fire conditions. The post-fire flush of seedling emergence has been associated with both the heat and the chemicals produced by the fire (Keeley and Fotheringham 2000). Laboratory experiments have demonstrated that those chemical compounds act as a germination cue in many species (Flematti et al. 2004, 2011), particularly in those from MTE; e.g., South Africa (Brown and Botha 2004), Australia (Dixon et al. 1995), California (Keeley and Fotheringham 1998) and Mediterranean Basin (Moreira et al. 2010). Smoke effect has been demonstrated also in ex situ (Hill and French 2003) and field experiments (Rokich and Dixon 2007).

In the MB, data about seed germination in relation to smoke comes from a limited number of laboratory experiments (Pérez-Fernández and Rodríguez-Echeverría 2003; Moreira et al. 2010). These laboratory experiments have used few species and, in some cases, the species selection criteria are subjective and biased. For example, while annual species are described as a very important component of the smoke stimulated flora in other MTE (e.g., California and South Africa; Keeley and Bond, 1997), most of the experiments performed with MB species have inadvertently excluded this group (Paula et al. 2009). This biased species selection might have led to a one-sided knowledge of vegetation response to fire.

In addition, while these experiments provide valuable information on the potential reproduction, they do not consider species interaction and density-dependent processes and offer a limited insight over the realized plant fitness (i.e., successful seedling establishment). *In situ* field experiments allow monitoring seedling recruitment in natural conditions up to the establishment phase (realized fitness) and thus are an important and reliable source of information to study the role of smoke on plant recruitment. In these *in situ* experiments, species are properly framed in their community (i.e., in an unaltered environment and interacting with coexisting species), and allow to disentangle the effect of smoke on seedling emergence and on establishment (i.e., effective recruitment). The importance of this lies in the fact that previous laboratory experiments demonstrated smoke-stimulated germination in species with no evidence of post-fire seedling recruitment (e.g., Drewes et al. 1995; Pierce et al. 1995). Only by demonstrating the effect of smoke on seedling establishment we can

evaluate its evolutionary role (Keeley et al. 2011).

In order to understand the real effect of smoke on plant recruitment, we performed a field experiment comparing seedling emergence and establishment between smoke-treated and untreated plots in a fire-prone community of the western MB. This experiment allowed us to test the following hypothesis (1) seedling emergence and establishment of MB species are enhanced by smoke, and (2) annual species are an important component of this smoke-stimulated flora.

Materials and Methods

Study area and sampling

The experiment was carried out in two adjacent areas in the eastern Spain (Valencia region, western MB) with a typical Mediterranean climate: Serra Calderona (39.728°N, 0.506°W) and Barranc de la Casella (39.090°N, 0.304°W). We selected two different sites for accounting a higher variability in the species composition sampled. The vegetation in Serra Calderona study site was dominated by the shrubs *Anthyllis cytisoides*, *Cistus albidus*, *Cistus monspeliensis* and *Ulex parviflorus* while in Barranc de la Casella study site was dominated by *Pistacia lentiscus*, *Quercus coccifera*, *Erica scoparia*, *Erica multiflora*, *C. monspeliensis*, *Rosmarinus officinalis* and *U. parviflorus*. In both sites, scattered individuals of *Pinus halepensis* composed the tree layer while *Brachypodium retusum* dominated the herbaceous layer. The mean annual precipitation is 584 mm and 633 mm and mean annual temperature 17.4°C and 17.7°C in Serra Calderona study site and Barranc de la Casella study site respectively. Precipitation distribution peaks in autumn with a secondary peak in spring and a drought period with high temperatures in summer, when fires are common (Pausas 2004).

In Sept. 2007, twenty-one plots (2 x 1 m) were delimited, nine in BC and 12 in SC. In each plot we cut all the standing vegetation and carefully removed the litter without disturbing the soil profile. Within each plot, we further delimited eight sub-plots (30x30 cm). Four of them received a smoke treatment and the remaining four were used as control (see below). Smoke application was performed using a commercial liquid smoke (Reese Hickory Liquid Smoke) diluted at 1:100 in distilled water (see Doherty and Cohn (2000) and Jager (1996) for details about the effectiveness of commercial liquid smoke). This concentration was chosen based on preliminary tests in laboratory with representative species of the studied community (data not shown). In the smoke sub-plots we applied 0.5 l of liquid smoke and in the control ones (interspersed design) 0.5 l of distilled water. This amount of liquid will not significantly

change the natural availability of water in the soil in relation to the natural rainfall pattern. Despite liquid smoke might leach through the soil profile (Roche et al. 1997a), the smoke effect remains for long on seeds of the seed bank (Roche et al. 1997b).

Plots were surveyed ten times during one year, starting one month after the treatments (September, October, November and December 2007, and January, March, April, June, July and September 2008) and, in each survey, we recorded and tracked all seedlings within each sub-plot with the help of a mesh (30x30 cm divided in 5x5 cm cells).

Data analysis

Data on species growth form (perennial or annual) were obtained from Mateo and Crespo (2001) and Paula et al. (2009). Preliminary analysis showed that the effect of smoke was not significantly different between sites ($p=0.95$) and thus, for all analyses the data from the two sites were pooled together. Because it was impossible to properly identify all emerged seedlings, due to the early dead of many of them, seedling emergence was not analysed at growth form, family or species level. Thus, seedling emergence was analysed only at community level, considering all emerged seedlings. Seedling establishment was analysed considering only those seedlings that survived the first summer (in the case of perennial species) or that flowered during the study period (in the case of annual species). In this data set of established seedlings, all seedlings were successfully identified (at least at genus level), which allowed to study the effect of smoke at different levels, including community (all established seedlings), growth form (annual or perennial species), family and species (or genus) level. When analysing a particular subset (community, growth form, family or species), we only considered the plots in which the mean establishment density in that subset was at least equal to one seedling per sub-plot in smoke or in control **sub-plots**. That is, at least four seedlings spread amongst one of the treatments.

We first evaluated whether the proportion of plots with more seedlings emerged in smoke-treated sub-plots was different from the random expectation by means of a one-tail binomial exact test. Then, for each plot, we evaluated the significance of the differences in total seedling emergence between smoke and control sub-plots (replicates) by means of Generalized Lineal Models (GLM). Finally, we performed the same comparison for the whole dataset using Generalized Linear Mixed Models (GLMM), including plot and species as random effects. These same analyses were used for seedling establishment, considering the different levels: community, growth form (annuals and perennials), families and species.

GLM and GLMM analyses assumed a Poisson error distribution which is adequate for count data. When a large number of statistical comparisons were performed (i.e., plot, family and species level analyses) the critical level for significance was considered at $p < 0.01$, which is a less conservative criterion than the Bonferroni correction (Moran 2003). All statistical analyses were carried out by means of R language. Specifically, binomial tests and GLM analyses were performed using the “stats” package (R Development core team 2011) and GLMM analyses were performed using the “lme4” package (Bates et al. 2011).

Results

Seedling emergence

Seedling emergence occurred in all studied plots, ranging from two to 757 seedlings/sub-plot. Overall we scored 14 203 different seedlings. The number of plots in which seedling emergence was greater in smoke than in control was significantly higher than the random expectation (18 out of 21; $p = 0.001$, binomial test; Fig. 1). This increase was significant in 11 plots (ranging from 14 % to 350 % increase due to smoke). In contrast, two plots showed significantly less seedling emergence in smoke than in control sub-plots (38 % and 31 % decrease due to smoke). No significant differences between smoke and control sub-plots were found in eight of the plots (Fig. 1). Analysing all the plots together, showed a significant overall increase in seedling emergence, produced by the smoke treatment (Table 1).

Table 1. Number of emerged and established seedlings and differences in the mean number of seedlings between smoke and control sub-plots (30x30 cm) for the different data subsets (community, annuals and perennials). P-values refer to the GLMM analysis.

<i>Process</i>	<i>Subset</i>	<i>Total seedlings</i>	<i>Mean</i>		<i>p- value</i>
			Smoke	Control	
Emergence	Community	14203	98.90	70.20	<0.001
Establishment	Community	1998	13.88	9.90	<0.001
Establishment	Annuals	971	8.08	4.70	<0.001
Establishment	Perennials	1027	6.57	5.65	0.070

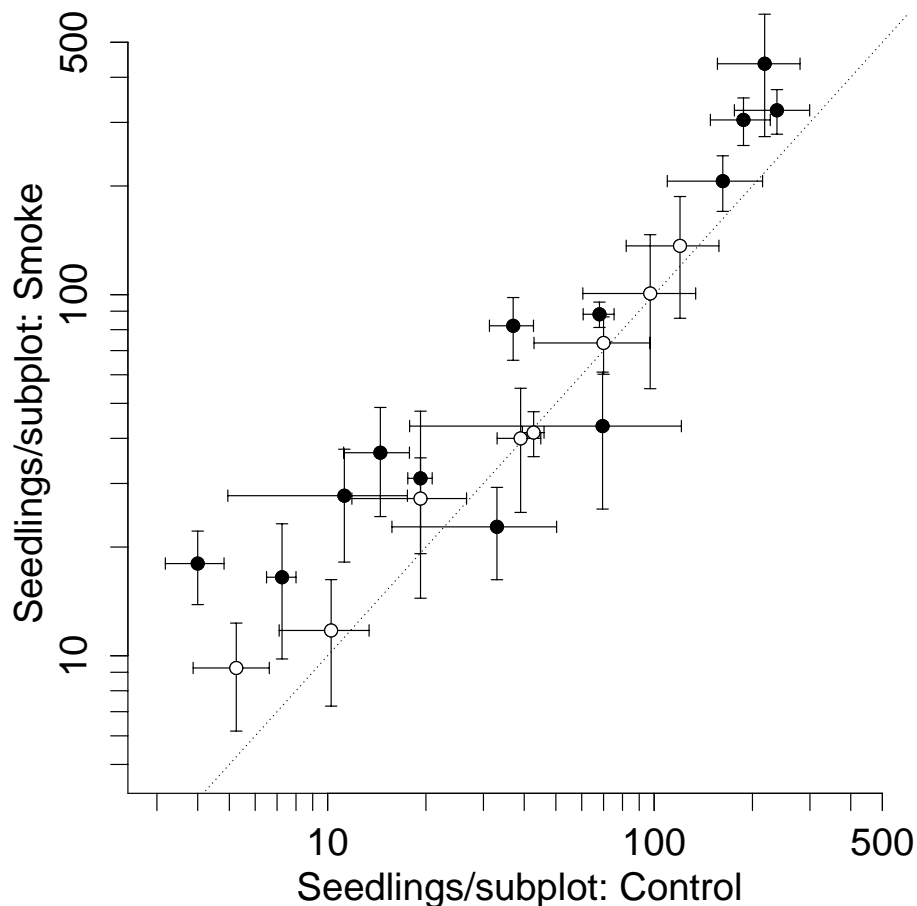


Figure 1. Relation between mean seedling emergence in smoke and control sub-plots (30x30 cm) in the 21 studied plots. Horizontal and vertical bars represent standard errors in smoke and control sub-plots, respectively. Dashed line represents equal seedling emergence in smoke and control (1:1 line); plots above this line had higher emergence in smoke than in control, and plots below this line had higher emergence in control than in smoke. Filled circles represent plots with significantly ($p < 0.05$) different seedling emergence between smoke and control treatments. The x- and y-axes are represented in logarithmic scale.

Seedling establishment

From the total seedlings emerged, only 1998 (14% of the total) successfully established (i.e., survived the first summer in the case of perennial species or reached maturity during the study period in the case of annual species). These seedlings accounted for 59 species in 22 families; 33 species, in ten families, reached the minimum seedling density to be studied (appendix, Table S1 and S2).

Community level: The number of plots in which seedling establishment was greater in smoke than in control was significantly higher than the random expectation (16 out of 20; $p = 0.012$, binomial test, Fig. 2). When analysing plots individually, the increase in seedling establishment due to smoke was significant in three plots (ranging from 51 % to 135 % increase due to smoke). One plot showed significantly lower seedling establishment in smoke

than in control sub-plots while 16 plots showed no significant differences between treatments. In fact, the smoke effect was concentrated in plots with higher density of seedlings (Fig. 2). The combined analysis of all the plots, showed a significant overall increase in seedling establishment due to smoke (GLMM; Table 1).

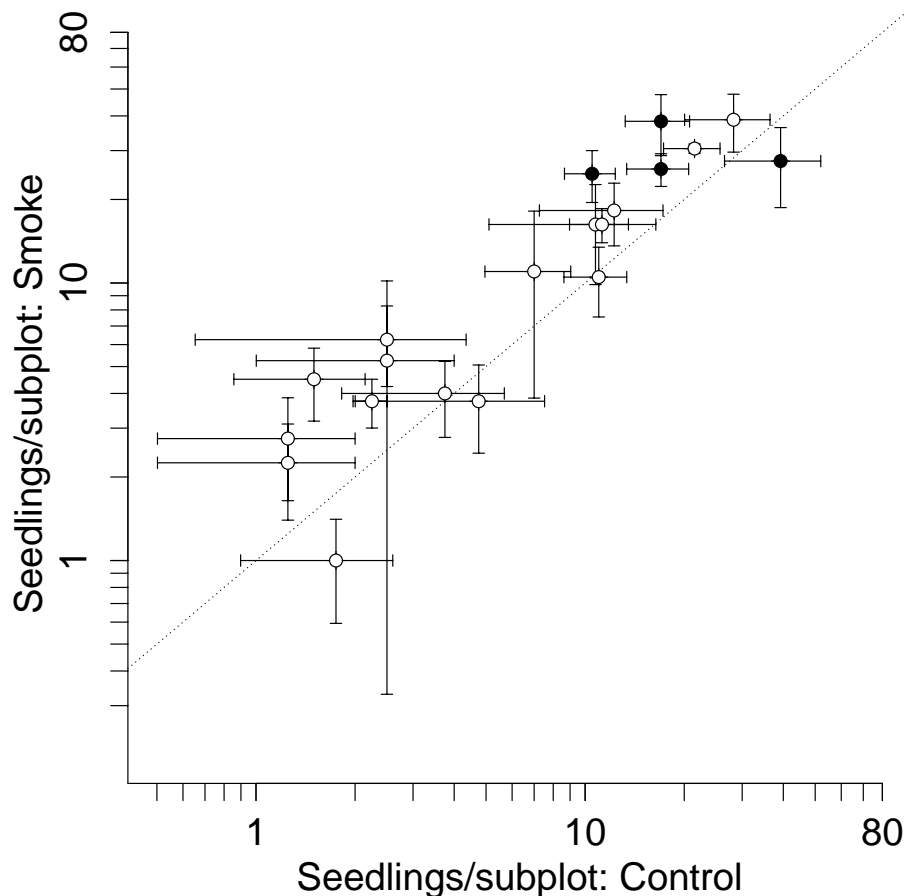


Figure 2. Relation between mean seedling establishment in smoke and control sub-plots (30x30 cm) in the studied plots. Horizontal and vertical bars represent standard errors in smoke and control sub-plots, respectively. Dashed line represents equal seedling establishment in smoke and control (1:1 line); plots above this line had higher establishment in smoke than in control, and plots below this line had higher seedling establishment in control than in smoke. Filled circles represent plots with significant ($p < 0.05$) different seedling establishment between smoke and control treatments. The x- and y-axes are represented in logarithmic scale.

Annual species: Only 13 plots reached the minimum seedling density of annuals to be considered for the analysis. The proportion of plots with more seedling establishment in smoke than in control was significantly higher than the random expectation (12 out of 13, $p = 0.003$; binomial test, Fig. 3). This increase was significant in three plots (ranging from 93 % to 191 % increase due to smoke). In the remaining ten plots there were no significant differences between treatments. As at community level, the smoke effect was concentrated in

plots with higher density of seedlings (Fig. 3). Analysing all the plots together (including plot and species as a random effect), showed a significant increase in seedling establishment due to the smoke treatment (GLMM, Table 1).

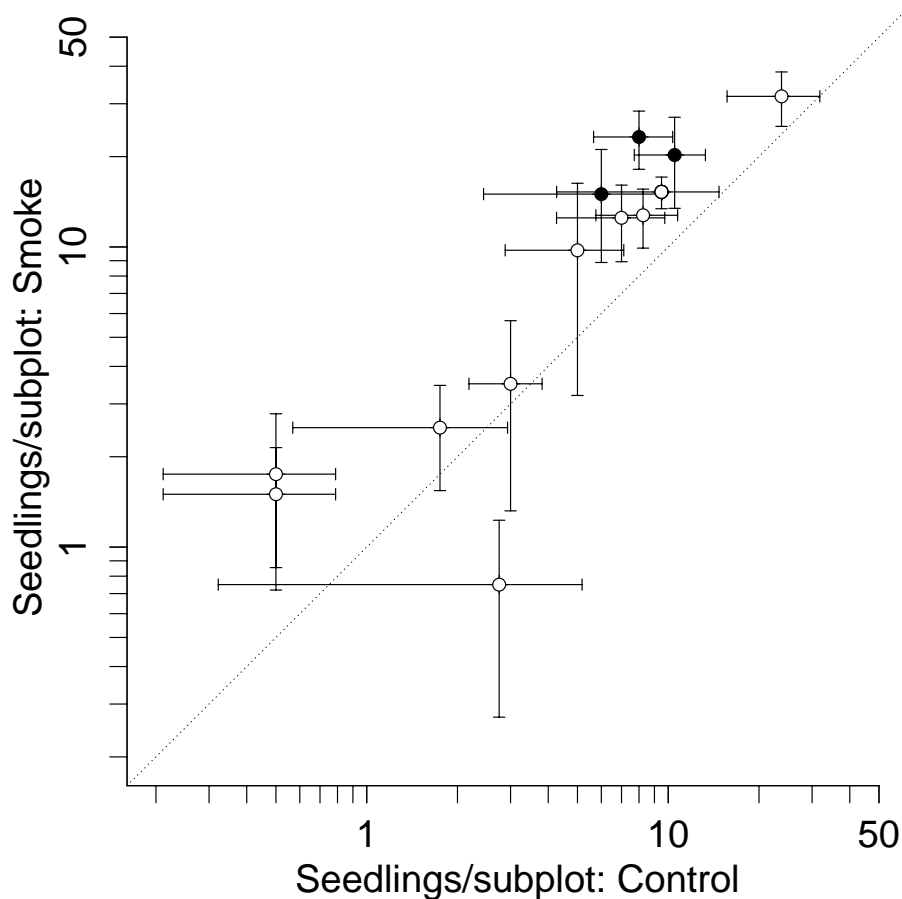


Figure 3. Relation between mean seedling establishment of annual species in smoke and control sub-plots (30x30 cm) in plots with established annuals. Horizontal and vertical bars represent standard errors in smoke and control sub-plots, respectively. Dashed line represents equal seedling establishment in smoke and control (1:1 line); plots above this line had higher establishment in smoke than in control and plots below this line had higher establishment in control than in smoke. Filled circles represent plots with significant ($p < 0.05$) different seedling establishment (of annual species) between smoke and control treatments. The x- and y-axes are represented in logarithmic scale.

Perennial species: All but one plot reached the minimum seedling density of perennials to be studied. However, the proportion of plots with more seedlings established in smoke than in control was not significantly higher from the proportion expected by chance (14 out of 20, $p = 0.115$; binomial test). Seedling establishment of perennial species in smoke-treated sub-plots was significantly higher in one plot (177 % increase) and significantly lower in one plot (33% decrease). In the remaining 18 plots there were no significant differences between treatments.

The combined analysis of all plots, showed a marginal positive effect of smoke on the seedling establishment ($p = 0.081$, Table 1).

Family level: Seedling establishment was higher in smoke than in control sub-plots in all studied families, and thus clearly greater than the proportion expected by chance. Lamiaceae clearly showed a significantly ($p < 0.01$) higher seedling establishment in smoke than in control sub-plots, and three families (Caryophyllaceae, Primulaceae and Scrophulariaceae) showed a marginal ($p < 0.05$) positive effect (appendix, Table S1)

Species level: The proportion of species in which seedling establishment was higher in smoke than in control was significantly greater than the expected by chance (26 out of 33, $p = 0.001$; binomial test). This increase was significant ($p < 0.01$) only in three taxa (*Helianthemum* sp., *Medicago* sp. and *Rosmarinus officinalis*) and marginal ($0.01 < p < 0.05$) in nine taxa (appendix, Table S2).

Discussion

Our results support previous findings suggesting that germination of Mediterranean Basin flora is enhanced by the chemicals contained in smoke (Pérez-Fernández and Rodríguez-Echeverría 2003; Moreira et al. 2010). In addition, our field-based approach allowed accounting for the successful establishment at the whole community scale. Specifically, our results suggest that the chemicals in smoke enhance both seedling emergence and establishment.

When disaggregating the results into growth forms, we found a clear effect of smoke in enhancing seedling establishment of the annual species. This results are in agreement with observations, in fire prone areas of South Africa, Australia and California, where annual species are strongly related to post-fire dynamics (Keeley et al. 1981; Cowling et al. 1996), suggesting that the importance of this group might be a common feature in fire-prone mediterranean ecosystems (Keeley et al. 2012). Most of the emerged annuals were not present in the undisturbed vegetation, indicating the presence of a persistent soil seed bank of fire ephemeral species (Ne'eman and Izhaki 1996; Ferrandis et al. 1999) positively affected by smoke. That is, our results point that, in the Mediterranean Basin flora, smoke-stimulated ephemerals might be more important in the post-fire recruitment than previously thought.

Despite the clear results at community level and that most species and all families had higher seedling establishment in the smoke than in the control sub-plots, there were very few families and species in which the effect of smoke was clearly significant. The lack of significant differences in seedling establishment at family or species level could be due to the seed bank heterogeneity that increased the noise and hindered to detect the smoke effect in our sampling design. Moreover, smoke effect could have been more conspicuous if aerosol in place of liquid smoke had been used because aerosol smoke might produce great stimulatory effect than liquid smoke (van Staden et al. 2000) but we see ourselves forced to use liquid smoke because the use of fire in the wild is prohibited during summer in Spain. Both limitations produced that our methodology is conservative in relation to our hypothesis. In any case, our results are consistent with previous studies in Australian ecosystems in such a way that the clear significant effect of smoke at community level was only reflected in a small proportion of species in the community. For example, Lloyd et al. (2000) found an increase in seedling emergence between 8 and 10 fold, but only two of the 18 studied species had significant increase in emergence due to smoke despite 17 of the 18 species showed higher emergence in smoke than in control. Roche et al. (1997a) found a similar pattern, that is, liquid smoke application produced an increase greater than 10 fold in the total number of emergences. But, despite smoke increased emergence in 63 of the 71 studied species, only in 22 this increase was significant. All these results suggest that the community response to smoke cannot be inferred from individual species because it is the sum of small differences in each species towards the same direction what produces a significant pattern at community scale. This emerging property of the community is often neglected by considering the role of smoke from laboratory experiments only.

Despite our experiment did not try to simulate a real fire (heat shock was not considered), it suggests that, in the Mediterranean Basin flora, the chemicals from fires not only act as a germination cue but also have a positive effect on plant establishment and consequently in the community assembly. It also suggests that the effect of smoke in annual species might be more relevant than previously thought, encouraging further work on the role of annual species for post-fire regeneration in the Mediterranean Basin.

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Appendix

Table S1. Total number of established seedlings and mean number of seedlings per subplot (30 x 30 cm) in control and smoke-treated subplots for each of the studied families. P-values refers to the differences between treatments (smoke vs control) analyzed with GLMM, including plot as random effect. P-values lower than 0.01 are in bold.

Family	Total seedlings		Mean seedlings/subplot		n plots	p-value
	Control	Smoke	Control	Smoke		
Rubiaceae	45	49	1.88	2.04	6	0.8618
Fabaceae	92	123	2.30	3.08	10	0.1511
Cistaceae	99	146	2.06	3.04	12	0.6747
Caryophyllaceae	33	52	4.13	6.50	2	0.0385
Lamiaceae	103	153	3.22	4.78	8	0.0017
Linaceae	13	24	1.63	3	2	0.0684
Primulaceae	115	231	4.79	9.63	6	0.0370
Scrophulariaceae	13	48	1.63	6.00	2	0.0107
Asteraceae	13	22	1.08	1.83	3	0.1260
Plantaginaceae	7	10	0.58	0.83	3	0.465

Table S2. Total (total seedlings) and mean number (seedling/spot) of established seedlings in control and smoke-treated subplots (30 x 30 cm) for each of the studied species and significance of the differences between treatments (p-value, GLMM including plot as random effect). For each species the growth form (GF: a, annual; p, perennial) and family is also shown. P-values lower than 0.01 are in bold.

Species	GF	Family	Total seedlings		Seedling/spot		n plots	p-value
			Control	Smoke	Control	Smoke		
<i>Sisymbrium orientale</i>	a	Apiaceae	0	4	0	1	1	0.019
<i>Centaurea spachii</i>	p	Asteraceae	2	4	0.5	1	1	0.410
<i>Filago pyramidata</i>	a	Asteraceae	1	7	0.25	1.75	1	0.024
<i>Sonchus oleraceus</i>	a	Asteraceae	6	9	1.50	2.25	1	0.437
<i>Echium vulgare</i>	p	Boraginaceae	46	13	5.8	1.6	2	0.038
<i>Herniaria cinerea</i>	a	Caryophyllaceae	5	4	1.25	1.00	1	0.739
<i>Polycarpon tetraphyllum</i>	a	Caryophyllaceae	28	48	7	12	1	0.021
<i>Cistus</i> spp.	p	Cistaceae	43	47	1.34	1.47	8	0.673
<i>Fumana thymifolia</i>	p	Cistaceae	3	10	0.75	2.5	1	0.046
<i>Helianthemum</i> spp.	p	Cistaceae	9	27	0.45	1.35	5	0.002
<i>Xolantha gutatta</i>	a	Cistaceae	37	56	4.63	7.00	2	0.629
<i>Sedum sediforme</i>	p	Crassulaceae	18	9	4.50	2.25	1	0.080
<i>Euphorbia exigua</i>	a	Euphorbiaceae	11	21	1.38	2.63	2	0.075
<i>Anthyllis cytisoides</i>	p	Fabaceae	38	37	2.38	2.31	4	0.542
<i>Bituminaria bituminosa</i>	p	Fabaceae	23	27	5.75	6.75	1	0.571
<i>Medicago</i> spp.	a	Fabaceae	16	35	2	4.38	2	0.007
<i>Ulex parviflorus</i>	p	Fabaceae	6	13	0.50	1.08	3	0.104
<i>Centaurium quadrifolium</i>	a	Gentianaceae	141	75	17.63	9.38	2	0.201
<i>Hypericum perforatum</i>	p	Gutiferae	14	12	1.17	1.00	3	0.695
<i>Lamium</i> sp.	a	Lamiaceae	7	11	1.75	2.75	1	0.344
<i>Rosmarinus officinalis</i>	p	Lamiaceae	34	61	2.13	3.81	4	0.005
<i>Teucrium</i> spp.	p	Lamiaceae	4	13	0.25	1.63	2	0.025
<i>Thymus vulgaris</i>	p	Lamiaceae	50	57	6.25	7.13	2	0.498
<i>Asterolinon linum-stellatum</i>	a	Linaceae	59	86	14.75	21.50	1	0.025
<i>Linum trigyum</i>	a	Linaceae	11	20	1.38	2.5	2	0.068
<i>Desmazeria rigida</i>	a	Poaceae	19	20	4.75	5.00	1	0.873
<i>Anagallis arvensis</i>	a	Primulaceae	54	145	2.70	7.25	5	0.049
<i>Sanguisorba</i> sp.	p	Rosaceae	1	5	0.25	1.25	1	0.088
<i>Asperula</i> sp.	a	Rubiaceae	1	5	0.25	1.25	1	0.088
<i>Galium murale</i>	a	Rubiaceae	29	32	3.63	4.00	2	0.701
<i>Sherardia arvensis</i>	a	Rubiaceae	15	11	1.25	0.92	3	0.432
<i>Anthirrhinum orontium</i>	a	Scrophulariaceae	5	8	0.63	1.00	2	0.403
<i>Linaria arvensis</i>	a	Scrophulariaceae	8	40	2	10	1	0.018

Chapter 6

Combined effect of heat and smoke on the germination of Mediterranean Basin flora

This chapter is in preparation for submission:

Moreira B, Pausas JG (in preparation). Combined effect of heat and smoke on the germination of Mediterranean Basin flora

Introduction

Wildfires are a major disturbance that drives plant communities. Indeed, in many species, adult plants are killed by fire and these species rely on post-fire seedling recruitment to persist in recurrently burnt ecosystems (seeder species, Pausas et al. 2004). These species often maintain a persistent soil seed bank and seedling emergence is enhanced after the passage of fire because germination is triggered by fire-related cues. The most important fire-related germination cues are the heat and the chemicals products of combustion and present in the smoke or charred wood produced during fire. Although, in the different mediterranean ecosystems, both cues have been shown to stimulated germination when applied independently (Keeley and Bond 1997, Bell 1999, Moreira et al. 2010), some species may require the combination of both cues for triggering germination (or at least to maximize germination). This might occur, for instance, if one of the factors breaks dormancy (e.g., heat) and the other factor stimulates germination (e.g., smoke) or if the species has a polymorphic seed pool in which a proportion of the seeds respond to one cue or to the other (but not to both). Different responses in germination have been observed to the combination of heat and smoke (Fig. 1): a) germination might be triggered by heat and smoke independently but, when combined, germination stimulation is higher (1 in Fig. 1; independent and additive effect; e.g., Keith 1997); b) germination might be triggered by only one of the cues when applied independently but when combined the germination is further stimulated (2.1 and 2.2 in Fig. 1, synergistic effect; e.g. Keeley 1987); and c) the strict requirement for both cues (3 in Fig. 1, unitive effect; i.e. germination not triggered by any of the cues when applied independently, and the combined treatment is required to stimulate germination) is more uncommon and usually results in low degree of stimulation (e.g. Thomas et al. 2003, 2007).

In addition, intraspecific variation in the germination response to heat and smoke has been found between different populations of the same species (Tieu et al. 2001; Thomas et al. 2007). This variability suggests that the germination response to fire-related cues might not be widespread within species, but related to different local disturbance history (e.g., fire regime)

and heterogeneity (e.g., burn patterns), or to other environmental factors as species interactions or availability of resources (Moreira et al. 2012).

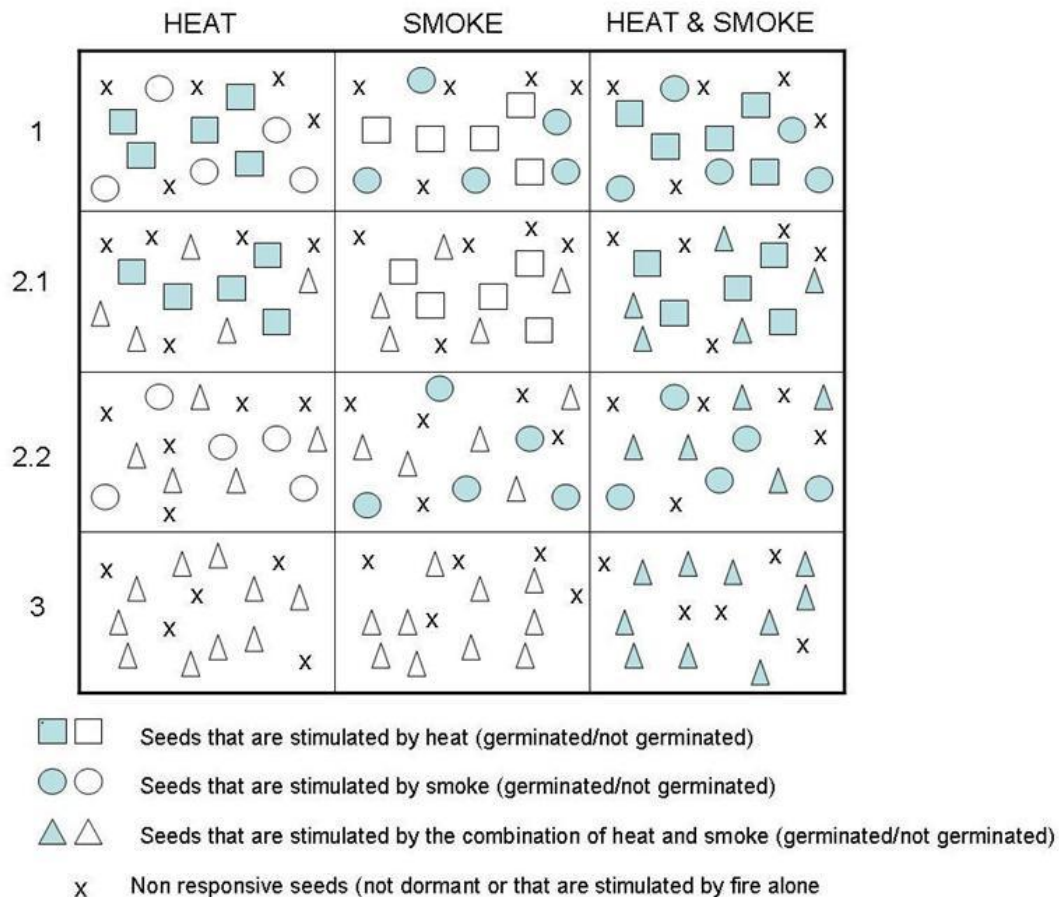


Figure 1. Different responses in germination that have been observed to the combination of heat and smoke: 1) germination might be triggered by heat and smoke independently but, when combined, germination stimulation is higher (independent and additive effect); 2.1 and 2.2) germination might be triggered by only one of the cues when applied independently but when combined the germination is further stimulated (synergistic effect); and 3) the strict requirement for both cues (unitive effect; i.e. germination not triggered by any of the cues when applied independently, and the combined treatment is required to stimulate germination). Filled symbols represent germinated seeds; unfilled symbols represent not germinated seeds.

Our aim is to study the role of heat and smoke in breaking dormancy and/or stimulating germination, ascertaining the intraspecific variability in this response, for a wide variety of Mediterranean Basin species. We address the following questions: (1) does the combination of both fire-related germination cues (i.e., heat and smoke) has an interactive effect on the germination response? and (2) is there intraspecific variability in the germination response, between different populations of the same species?

To answer these questions we performed a germination experiment with woody species from the Mediterranean Basin, showing evidence of post-fire seedling emergence (seeder species).

This experiment included several populations for most species. We tested the role of heat, smoke and the combination of both cues in the germination of 16 species.

Methods

Species and seed collection

We selected 16 woody species with evidence of post-fire seedling emergence (seeder species, Paula et al., 2009), including both shrubs (nanophanerophytes) and scrubs (chamaephytes). These species are common in fire-prone areas of the Mediterranean Basin. For some species, seeds from several different populations were collected (Table 1 and S1). All seeds were collected from ripe fruits in wild populations during the dispersal period of each species (Table 1), from several individuals (spatially dispersed). Seeds from the same species and population were pooled together for the germination experiments and were placed in aluminum pockets (ca. 50 seeds per pocket). For each species and population, four aluminum pockets (four replicates) were randomly allocated to each treatment (see below).

Table 1. List of taxa (Family and Species names), number of populations studied (#pop), collection date (month/year) and seed age (in months) at the time of the experiment. Location of each population is given in Table S1.

<i>Family</i>	<i>Species</i>	<i>#pop</i>	<i>Collection date</i> (month/year)	<i>Seed age</i> (months)
Lamiaceae	<i>Lavandula latifolia</i>	3	09/08	1
	<i>Lavandula stoechas</i>	3	07/09	1
Ericaceae	<i>Erica umbellata</i>	1	10/07	11
	<i>Erica multiflora</i>	3	02/08	7
	<i>Erica arborea</i>	3	07/09	3
	<i>Erica scoparia</i>	1	07/07	14
	<i>Erica manipuliflora</i>	3	02/09	6
	Cistaceae	<i>Helianthemum syriacum</i>	3	07/08
<i>Fumana ericoides</i>		7	07/08	3
<i>Fumana thymifolia</i>		6	07/08	3
<i>Cistus salviifolius</i>		4	07/08	3
<i>Cistus albidus</i>		4	07/08	3
<i>Cistus clusii</i>		4	07/08	3
<i>Cistus parviflorus</i>		1	07/09	1
<i>Cistus creticus</i>		3	07/09	1
Fabaceae		<i>Ulex parviflorus</i>	7	06/08

Treatments

For each species, seeds from the different populations were subject to a heat treatment, a smoke treatment and a combined treatment of heat and smoke. The heat treatment was performed using a temperature-controlled oven, setting the temperature at 100°C for 5 minutes. The aluminum pockets were disposed randomly in the metal tray of the oven. This operation was repeated four times for each population and species to achieve four independent replicates. For the smoke treatment, we prepared four batches (replicates) of smoke solutions by grounding dry leaves and thin twigs of *Quercus coccifera* (a common shrub of the study areas) with an electric grinder following the method described in Jäger et al. (1996). Seeds were incubated in these solutions (four replicates) for 24 hours before sowing in the Petri dishes. To test the effect of the combination of heat and smoke (combined treatment), seeds were first heated at 100 °C for 5 minutes (as above, heat treatment) and then incubated in the liquid smoke solutions for 24 hours (as above, smoke treatment). Two controls were applied: a “dry control” (seeds without any treatment, sown directly into the Petri dishes) and a “watered control” (seeds immersed in distilled water for 24 hours before sowing) to detect effects of seed imbibition.

Germination conditions

After the respective treatments, seeds were set for germination in Petri dishes with agar (0.8±0.1%) as substrate and incubated at 20°C in darkness as these conditions are suitable for the germination of many Mediterranean species (Thanos et al. 1991). Seed germination was monitored every 2 days during the first 2 weeks and then once a week until the end of the experiment, 2 months later. Seed were scored as germinated when radicle emergence was visible and were removed from the Petri dishes. At the end of the experiment, the initial number of seeds sown was corrected by discarding empty seeds (lacking embryo and storage tissue) detected during the experiment.

Data analysis

We tested for each population and species whether final germination differed between treatments using the analysis of deviance (GLM) with binomial error distribution. For each species, differences between treatments were evaluated using generalized linear mixed models (GLMM) with binomial error distribution, including treatment as fixed factor and population as random factor. Due to the large number of pairwise comparisons the significant level considered was $P < 0.01$.

Results

Most species were stimulated by heat while smoke treatments only stimulated germination in Ericaceae and Lamiaceae. *Helianthemum syriacum* was the only species which was stimulated neither by heat nor by smoke treatments (Table 2), in any of the populations (Table S2). For most species (13 of the 16 studied), the combined treatment of heat plus smoke did not show any additional positive effect on germination, compared to the independent treatments that showed the maximum stimulation. The only exception was *Cistus creticus*, in which the combined treatment of heat and smoke showed a positive effect, compared to the treatments using only one of the cues. This positive effect was observed in all populations; with a mean increase of 30.2% respect to the control (heat treatment had an increase of 16.2%, Table S2). However, this may be because the heat treatment applied (100°C for 5 minutes) was insufficient to maximize germination, as a treatment of 120°C for 5 minutes increased the germination further (mean increase of 37.7%; data not shown). In *E. manipuliflora*, heat (in the combined treatment) had a negative effect over smoke-stimulation while in *F. ericoides* smoke (in the combined treatment) had a negative effect over heat-stimulation (Table 2), and this was reproduced in all populations. That is, in *E. manipuliflora* and *F. ericoides* the combination of both cues decreased the germination respect to the treatments with maximum stimulation when applied independently (smoke and heat respectively). In *F. thymifolia* the combined treatment of heat plus smoke had a very variable effect; in two populations this treatment had no additional effect on germination, in one population germination was enhanced and in three populations germination was inhibited (Table S2). Furthermore, for this species, one population out of five was not significantly stimulated by heat, but was stimulated by smoke (Table S2). However, for this species, the overall effect of the combined treatment was not different from the heat treatment (Table 2).

Table 2. Analysis of the differences in germination between untreated seeds (C: Control), heat-treated seeds (H: 100°C 5'), Smoke-treated seeds (S: Smoke) and seed exposed to both treatments (HS: Heat plus Smoke). The significance of the pairwise comparison between treatments is also included (=: not significant; <: p<0.01, <<: p<0.001).

Species	Germination
<i>Lavandula latifolia</i>	C<<H<<S=HS
<i>Lavandula stoechas</i>	C=H<<S=HS
<i>Erica umbellata</i>	C<<H=S=HS
<i>Erica multiflora</i>	C<<H=S=HS
<i>Erica arborea</i>	C<<H=S=HS
<i>Erica scoparia</i>	C<<H<<S=HS
<i>Erica manipuliflora</i>	C<<H<<HS<<S
<i>Helianthemum syriacum</i>	C=S=H=HS
<i>Fumana ericoides</i>	C=S<<HS<<H
<i>Fumana thymifolia</i>	C=S<H=HS
<i>Cistus salviifolius</i>	C=S<<H=HS
<i>Cistus albidus</i>	C=S<<H=HS
<i>Cistus parviflorus</i>	C=S<<H=HS
<i>Cistus creticus</i>	C=S<H<<HS
<i>Cistus clusii</i>	C=S<<H=HS
<i>Ulex parviflorus</i>	C=S<<H=HS

Discussion

Enhanced germination by fire related cues is a common feature in Mediterranean Basin flora. Both heat and smoke generated during wildfires break dormancy and/or stimulate germination in many species (Paula and Pausas 2008; Moreira et al. 2010). In this study we found evidences for smoke-stimulated germination in three *Erica* species that had not been reported before to be smoke responsive (i.e., *E. scoparia*, *E. arborea* and *E. manipuliflora*). This gives further support to the important role of smoke (and heat) as germination cues for this family, likewise in other mediterranean ecosystems (e.g., South Africa, Brown et al. 1993).

For some species and particular populations, the combination of both cues (heat and smoke) had an interactive effect over germination. However this effect was not very marked and was mostly negative (Fig. 2 and Table S2). None of the species required the combination of both cues to germinate (unitive effect).

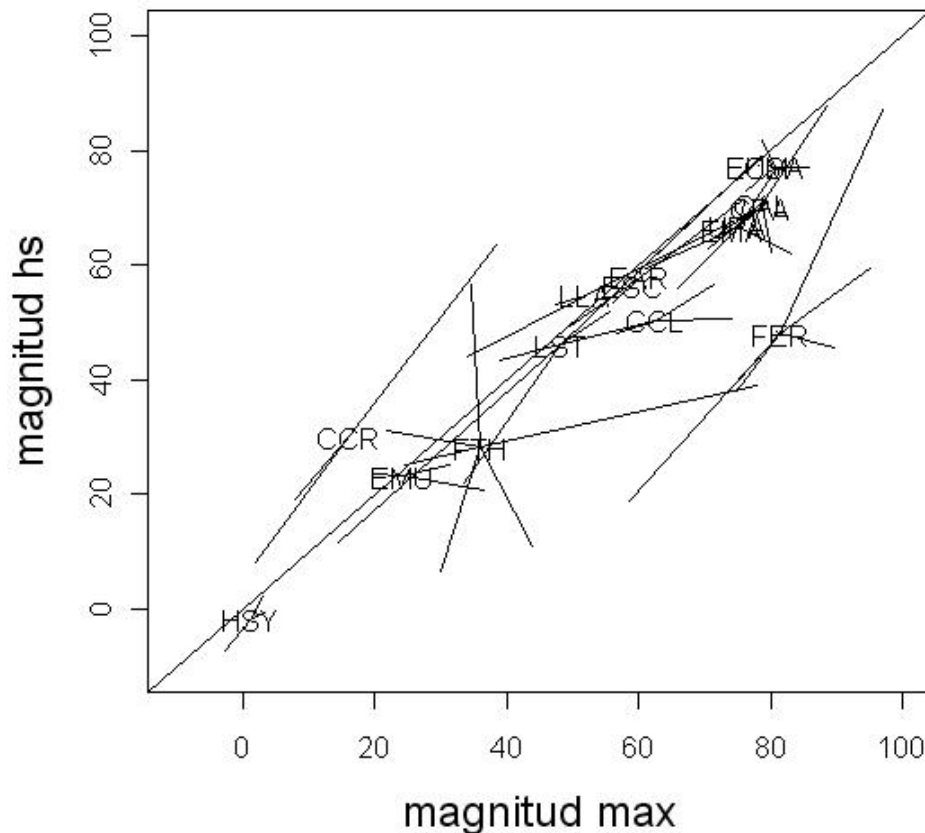


Figure 2. Relationship between germination stimulation by the combined treatment of heat and smoke (y-axis, magnitud hs), and by the treatment that resulted in higher germination when applied independently (heat or smoke; x-axis, magnitud max). Intraspecific variability is indicated by segments emerging from each species code (mean species value). Stimulated germination was measured as the magnitude of stimulation, that is, difference between the maximum germination (%) in treated seeds minus the germination in the control. Species codes are: LLA: *Lavandula latifolia*; LST: *Lavandula stoechas*; EUM: *Erica umbellata*; EMU: *Erica multiflora*; EAR: *Erica arborea*; ESC: *Erica scoparia*; EMA: *Erica manipuliflora*; HSY: *Helianthemum syriacum*; FER: *Fumana ericoides*; FTH: *Fumana thymifolia*; CSA: *Cistus salvifolius*; CAL: *Cistus albidus*; CPA: *Cistus parviflorus*; CCR: *Cistus creticus*; CCL: *Cistus clusii*; UPA: *Ulex parviflorus*.

Some species might have a polymorphic seed pool in which a fraction of the seeds is stimulated to germinate by heat and other fraction stimulated by smoke (independent and additive effect), and/or a proportion of seeds that are only stimulated by the combination of both (synergistic effect). This might ensure that germination is stimulated regardless of the fire intensity but avoid the depletion of the soil seed bank after a single fire event. However, none of the species presented a seed pool requiring the combination of both cues to germinate (unitive effect). The ecological significance of a species having exclusively seeds that germinate when exposed simultaneously to both factors (unitive effect) would only be

understandable to 1) avoid germination related to very low intensity fires in which conditions for seedling establishment are not improved (e.g., fire does not produce gaps); seeds could perceive this by the fact that smoke would be produced but soil heating would be low; and/or to 2) avoid dormancy release by summer temperatures, which could be particularly important for species presenting physical seed dormancy; seeds could perceive this by the fact that heat would be produced but without the presence of the smoke cue.

Fire regimes of very low intensity fires, in which conditions for seedling establishment are not improved, are probably not common in Mediterranean shrublands (e.g., frequent low intensity surface fires or winter fires). In addition, physical seed dormancy might have evolved in such a way that the temperature threshold associated with the dormancy release is above the maximum summer temperatures (Ooi et al. 2012). Indeed, low levels of dormancy loss in response to summer temperatures might be important for the recruitment in fire-independent gaps or for the colonization of new areas. However, the high level of dormancy release by the heat produced by fire enforces most recruitment to be capitalized into a single post-fire pulse when the most favorable conditions occur (Moreira and Pausas *submitted*). Thus, it is not surprising that, in the Mediterranean Basin, the pressure exerted by fire did not select for germination in response to the obligatory combination of both cues. It is unknown whether seeds of species with an obligatory requirement of heat and smoke to germinate are dormant and need one of the factors to break dormancy and the other to stimulate germination or if they are not dormant and require both cues for stimulating germination. Indeed, in some species germination is only stimulated by smoke after burial or stratification (Baker et al. 2005).

Different species, respond to different fire-related germination cues. Apparently, there is an important phylogenetic effect on this response as most Cistaceae and the Fabaceae tested (*U. parviflorus*) had germination triggered by heat-treatments and not by smoke. On the contrary, Ericaceae and Lamiaceae had germination stimulated by both cues; although stimulation by smoke was higher. Our results also show some degree of intraspecific variability in germination response between populations. These differences were mainly in the magnitude of the response but not in the cue that triggered germination. The only exception was *Fumana thymifolia*, which generally had germination stimulated by heat, but a single population had germination stimulated in response to smoke (and not by heat).

In conclusion, our results show that post-fire germination in Mediterranean Basin flora is stimulated both by the heat and by the chemical compounds produced during wildfires. However they also show that for most species one of the cues applied independently maximized germination and none of the species required the combination of both cues to germinate (unitive effect). This suggests that the pressure exerted by fire in the Mediterranean basin did not select for germination in response to the obligatory combination of both cues. Although wildfires are a frequent disturbance in Mediterranean Basin, fire has probably played a different role shaping germination in different species and even different populations within species. Different species respond to different fire-related cues and within species there is variability in the level of germination response between populations.

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Appendix

Table S1. Populations used in the study (LLA: *Lavandula latifolia*; LST: *Lavandula stoechas*; EUM: *Erica umbellata*; EMU: *Erica multiflora*; EAR: *Erica arborea*; ESC: *Erica scoparia*; EMA: *Erica manipuliflora*; HSY: *Helianthemum syriacum*; FER: *Fumana ericoides*; FTH: *Fumana thymifolia*; CSA: *Cistus salviifolius*; CAL: *Cistus albidus*; CPA: *Cistus parviflorus*; CCR: *Cistus creticus*; CCL: *Cistus clusii*; UPA: *Ulex parviflorus*).

cod	Population	Species
P1	Serra Espadà	CAL; CSA; UPA; EAR; LST
P2	Font Roja	CAL; UPA; FER; FTH; LLA; CCL
P3	Dos Aguas	CAL; UPA; FER; FTH; CSA; CCL; HSY
P4	Chiva	CAL; CSA; FER; HSY
P5	Serra Calderona	CSA; FTH; EAR; EMU; LST; UPA
P6	Ayora	UPA; FTH; CCL; FER; FTH; LLA
P7	Alzira	CSA; EMU; ESC; FER; FTH; HSY; UPA
P8	Desert Palmes	EAR; LST
P9	Buñol	EMU
P10	Cadiz	EUM
P11	Villamarxant	FER; FTH
P12	Barranc Horts	UPA; FER; LLA
P13	Marmaris (Tk)	CCR; EMA
P14	Feth (Tk)	CCR; EMA
P15	Koycegiz (Tk)	EMA
P16	Datça (Tk)	CCR; CPA

Table S2. Germination of untreated seeds (CH), seeds imbibed in water for 24 hours (CS), heat-treated seeds for 100°C 5' (Heat), smoke-treated seeds (Smoke) and seeds treated with heat and smoke (Heat and Smoke), for each species and population. For each species and population, treatment germination means with the same letter were not significantly different ($P > 0.01$).

<i>Species</i>	<i>Pop.</i>	<i>CH</i>	<i>CS</i>	<i>Heat</i>	<i>Heat and Smoke</i>	<i>Smoke</i>
<i>C. albidus</i>	P1	5±1.7 a	6±1.5 a	81±4.0 b	74±4.6 b	6±0.8 a
	P2	2±1.0 a	2±1.0 a	81±2.0 c	64±3.1 b	1±0.5 a
	P3	7±2.1 a	9±2.2 a	78±4.5 b	73±3.3 b	4±0.5 a
	P4	4±1.7 a	6±1.0 a	93±1.6 b	93±0.9 b	2±1.4 a
<i>C. clusii</i>	P2	6±0.9 a	8±1.6 a	80±3.7 c	58±3.9 b	4±1.7 a
	P3	3±0.6 a	4±1.4 a	74±3.3 c	60±2.4 b	1±0.5 a
	P5	4±1.5 a	6±1.6 a	53±2.7 b	52±4.0 b	5±1.6 a
	P6	3±1.7 a	3±1.8 a	59±6.7 b	51±2.3 b	3±0.9 a
<i>C. creticus</i>	P13	4±0.8 a	7±1.3 ab	12±9.3 b	26±21.4 c	6±1.3 a
	P14	10±1.9 a	10±1.0 a	48±14.1 b	73±4.4 c	8±1.8 a
	P16	0±0.0 a	1±0.6 a	2±1.2 a	9±4.7 b	3±0.6 a
<i>C. parviflorus</i>	P16	4±2.2 a	4±1.8 a	82±4.8 b	74±7.8 b	4±1.5 a
<i>C. salviifolius</i>	P3	2±0.8 a	3±0.5 a	78±3.8 b	76±2.2 b	7±0.5 a
	P4	5±1.7 a	5±1.8 a	84±1.9 b	87±3.8 b	5±2.0 a
	P5	4±0.8 a	5±1.4 a	90±1.8 b	82±2.5 b	2±1.4 a
	P7	6±2.6 a	7±2.2 a	88±1.6 b	84±1.2 b	4±0.9 a
<i>E. arborea</i>	P1	50±3.4 b	28±5.5 a	78±3.0 c	81±4.1 c	84±1.0 c
	P5	52±4.2 a	44±2.9 a	88±2.8 b	94±1.7 b	94±1.0 b
	P8	26±3.3 b	10±3.7 a	65±6.2 c	82±2.9 d	86±0.0 d
<i>E. manipuliflora</i>	P13	32±5.4 b	20±1.6 a	78±2.2 c	86±1.7 c	95±1.0 d
	P14	32±8.5 b	11±4.7 a	70±4.2 c	81±1.8 cd	89±2.4 d
	P15	42±8.6 b	24±2.4 a	87±0.9 b	87±4.7 b	94±1.0 c
<i>E. multiflora</i>	P5	20±3.0 a	27±3.2 a	32±3.5 a	53±2.8 b	59±3.5 b
	P7	21±2.0 a	41±5.4 b	39±5.5 b	65±2.9 c	61±1.2 c
	P9	39±2.5 a	56±4.4 b	76±3.1 c	77±2.8 c	78±1.3 c
<i>E. scoparia</i>	P7	0±0.0 a	0±0.0 a	32±8.0 b	56±1.5 c	59±11.2 c
<i>E. umbellata</i>	P10	0±0.0 a	0±0.0 a	72±1.6 b	77±3.1 b	78±2.5 b
<i>F. ericoides</i>	P11	1±0.6 a	1±0.6 a	82±2.8 c	50±3.8 b	0±0.0 a
	P12	7±1.3 a	8±1.4 a	82±4.1 c	46±3.7 b	4±1.0 a
	P2	10±3.1 a	10±3.1 a	84±2.9 c	49±5.4 b	7±1.0 a
	P3	1±0.6 a	2±1.0 a	98±1.4 c	88±1.7 b	2±1.0 a
	P4	1±0.6 a	2±1.0 a	90±1.7 c	47±5.8 b	2±0.5 a
	P6	10±2.1 a	11±2.1 a	69±2.5 c	30±3.9 b	13±2.1 a
	P7	0±0.5 a	1±0.5 a	96±1.7 c	60±2.4 b	0±0.5 a
<i>F. thymifolia</i>	P11	2±1.0 a	2±0.0 a	45±3.4 b	13±3.9 c	1±1.0 a
	P2	34±5.3 a	34±4.8 a	38±1.6 a	65±2.4 b	56±4.0 b
	P3	0±0.0 a	0±0.0 a	78±2.2 b	39±3.4 c	2±0.5 a
	P5	8±1.2 a	8±1.2 a	32±3.4 b	33±3.3 b	6±1.3 a
	P6	1±0.6 a	2±1.0 a	36±8.1 b	58±11.4 c	0±0.0 a
	P7	1±0.7 a	1±0.7 a	31±4.4 b	8±2.0 c	0±0.0 a
<i>H. syriacum</i>	P3	5±1.9 a	7±1.8 a	8±4.3 a	7±1.6 a	6±1.4 a
	P4	3±1.2 a	5±1.8 a	6±2.7 a	7±1.9 a	4±2.3 a
	HP7	11±2.8 a	12±2.7 a	9±1.5 a	4±1.7 a	7±2.3 a
<i>L. latifolia</i>	P12	9±1.9 a	20±2.2 b	19±2.6 b	73±9.0 c	68±6.8 c

	P2	5±1.9	a	10±1.2	a	6±2.6	a	54±16.7	b	44±19.9	b
	P6	1±1.0	a	2±0.0	a	10±3.0	b	70±10.8	c	76±10.9	c
<i>L. stoechas</i>	P1	32±3.6	a	30±2.9	a	50±4.2	b	82±1.9	c	86±2.4	c
	P5	52±7.9	a	50±4.8	a	46±3.0	a	93±2.9	b	88±3.8	b
	P8	50±5.3	a	59±3.5	ab	64±3.4	b	81±13.1	c	92±1.9	d
<i>U. parviflorus</i>	P1	10±1.6	a	14±0.0	a	86±3.7	b	85±3.1	b	16±2.9	a
	P12	14±2.1	a	18±2.2	a	94±2.6	b	88±3.3	b	16±2.8	a
	P2	20±1.5	a	24±2.2	a	86±3.2	b	80±0.8	b	24±1.9	a
	P3	6±1.5	a	7±1.5	a	86±2.9	b	78±3.9	b	10±1.7	a
	P5	18±1.0	a	20±1.9	a	78±3.0	b	80±3.7	b	15±2.5	a
	P6	9±3.1	a	11±3.9	a	90±1.2	b	88±0.8	b	9±2.6	a
	P7	10±1.7	a	12±1.4	a	92±1.7	c	74±2.9	b	6±1.9	a

Chapter 7

Heat and smoke stimulated germination: two mechanisms for plant coexistence in fire-prone ecosystems

This chapter is in preparation for submission:

Moreira B, Pausas JG (in preparation). Heat and smoke stimulated germination: two mechanisms for plant coexistence in fire-prone ecosystems

Abstract

In Mediterranean type ecosystems, given the long history of fires, post-fire germination is a trait of paramount importance. The two main mechanisms behind this trait are the heat- and the smoke-stimulated germination. Studies on heat-stimulated germination suggest that this cue acts mainly on species with water-impermeable seeds. However, in fire-prone areas, both species with water-permeable seeds and species with water-impermeable seeds coexist. This suggests that these two groups of species have evolved post-fire germination but in response to different fire-related germination cues (heat and smoke-stimulation) depending on their seedcoat type (permeability). In addition, species with post-fire germination may also have the ability to resprout after fire. However, having one persistence mechanism (resprouting) may reduce the evolutionary pressure over a second persistence mechanism such as post-fire germination. We aim to evaluate the role of seedcoat type and resprouting ability in determining the level of post-fire germination and the differential response to fire-related germination cues (heat- vs smoke- stimulation). For this purpose, we used data from an experimental study of germination response to heat and smoke treatments in 30 woody species with different resprouting ability and seedcoat type. The results show that obligate seeders (i.e., species with no resprouting ability) had higher seed dormancy and higher heat and smoke stimulated germination than facultative species (i.e. species with resprouting ability); but for each fire-related germination cue, the highest stimulation is dependent of the species' seedcoat type: seeders with water-impermeable seeds are mostly stimulated by heat, while those with water-permeable seeds are stimulated by smoke. Different species have developed different solutions to the evolutionary pressure exerted by fire, allowing the coexistence in fire-prone ecosystems of species with different seedcoat type.

Keywords: Mediterranean-type ecosystems, plant traits, post-fire germination, post-fire regeneration, resprouting, seedcoat, seeders

Introduction

Plant traits are the product of constraints and trade-offs along the history of the flora, and the correlation among traits provides the basis for functional type classifications as well as for understanding ecological and historical processes (Herrera 1992; Ackerly 2004; Pausas & Verdú 2005). In Mediterranean type ecosystems (MTEs), given the long history of fires (Pausas & Keeley 2009), post-fire germination (i.e. the ability to germinate after a fire) is a trait of paramount importance that depends on different processes like seed dormancy, seed thermal tolerance and fire-stimulated germination. Selection over these processes allows species that have post-fire germination (named seeder species) to increase their population size after fires and thus to have a fitness advantage in fire-prone ecosystems. Post-fire germination can be driven by the heat from fire or by the chemicals contained in the smoke or charred wood (hereafter, heat- and smoke-stimulated germination, respectively). These two mechanisms are important in most MTEs (Keeley & Fotheringham 2000; Moreira et al. 2010) and both heat- and smoke-stimulated species coexist in fire-prone areas (Keeley 1995). These two mechanisms are often lumped into a single functional trait, post-fire germination (e.g., P trait in Pausas et al. 2004); however, little is known about the factors driving each of these germination cues.

Species which seeds have a strongly lignified and water-impermeable (WI) seedcoat show mainly heat-stimulated germination (Keeley 1991; Bell, Plummer & Baker 1993; Jeffery, Holmes & Rebelo 1988; Paula & Pausas 2008), and reduced smoke-stimulated germination (Pérez-Fernández & Rodríguez-Echevarría 2003; Crosti et al. 2006). On the contrary, the germination of species which seeds have a water-permeable (WP) seedcoat is mostly smoke-stimulated (Dixon, Roche & Pate 1995; Kelley & Bond 1997; Keeley & Fotheringham 1998; Moreira et al. 2010). This suggests that both species with WI seeds and those with WP seeds have evolved post-fire germination but in response to different fire-related germination cues (heat vs smoke-stimulation) and could explain the coexistence of species with WI seeds and species with WP seeds in fire-prone areas. In order to respond to smoke stimuli, seeds must resist the heat shock produced by the fire and thus there should be no differences in seed thermal tolerance between species with WI seeds and species with WP seeds (Bell & Williams 1998). However, it has been suggested that the strongly lignified cell walls in the seedcoat that confer impermeability to WI seeds may also confer them thermal tolerance and prevent embryo death (Thanos & Georghiou 1988; Ferrandis et al. 1999).

Post-fire regeneration traits are not independent and the correlation among these traits may be related to ecological trade-offs as well as to historical contingencies of the flora (Pausas 2001; Pausas et al. 2004; Pausas & Verdú 2005). Resprouting (R) is another trait of paramount importance in post-fire dynamics of MTEs (Bond & Midgley 2001; Pausas et al. 2004). Post-fire germination occurs (P+, seeder species) in both resprouting (R+) and non-resprouting (R-) species. That is, there are species that may regenerate after fire by both germination and resprouting (facultative seeders, R+P+) and species that regenerate only by germination (obligate seeders, R-P+). However, the degree of post-fire germination may depend on resprouting ability since having one persistence mechanism (R+, resprouting) may reduce the evolutionary pressure over a second persistence mechanism such as post-fire germination (P+) (Pausas & Verdú 2005). Thus, post-fire germination should be negatively related to post-fire resprouting. In fact, Paula & Pausas (2008), based on a compilation of published data, showed that the germination response of seeds after heat exposure was greater in obligate (R-P+) than in facultative (R+P+) seeders. However, regarding smoke, some studies have found limited relationship between germination stimulation and resprouting ability (van Staden et al. 2000; Ne'eman et al. 2009). Furthermore, differences between facultative and obligate seeders in seed thermal tolerance are also ambiguous as some authors found higher thermal tolerance in seeds of obligate seeders than in those of facultative seeders (e.g., Moreno & Oechel 1991) while others found no clear differences between these two groups (e.g., Bell & Williams 1998).

Our general hypothesis is that post-fire germination (P+) is associated to other plant traits (Pausas et al. 2004) such as resprouting ability and seedcoat type. Thus we aim to evaluate the role of resprouting ability (R+ vs R-) and seedcoat type (WP vs WI) in determining the post-fire germination response. Specifically, we predict that the level of post-fire germination depends on resprouting ability but not on seedcoat type (i.e. obligate seeders have higher germination response to fire cues than facultative seeders), but seedcoat type (and not resprouting ability) determines the differential response to the different germination cues (i.e., WI are heat-stimulated, while WP are smoke-stimulated), in such a way that heat- and smoke-stimulated germination are negatively related. We evaluated these predictions for woody species common in the post-fire environment of the Iberian Peninsula, in the Mediterranean Basin.

Materials and methods

Data, species, and traits

We used data from an experimental analysis of germination response to fire-related germination cues (heat and smoke) of 30 common woody species (shrubs and scrubs; Fig. 1) occurring in fire-prone areas of the South East Iberian Peninsula (Moreira et al. 2010). The analysis included both resprouting (R+; 16 species) and non-resprouting (R-; 14 species) species showing evidence of post-fire seedling emergence (P+), so that the fire response groups compared (Pausas et al. 2004) were obligate seeders (R-P+, post-fire regeneration relies only on germination) and facultative seeders (R+P+, post-fire regeneration by both resprouting and germination). Data on post-fire regeneration mechanisms was derived from bibliographic references (Paula et al. 2009) and personal observations. Seed permeability was based on experimental evidence (Moreira et al. 2010), and the set of species studied included both species with water-impermeable seeds (WI; 15 species) and species with water-permeable seeds (WP; 15 species).

Seeds collected from several individuals (spatially dispersed) in wild populations were subject to several heat and smoke treatments (each replicated four times). Heat treatment levels were: “80°C for 5 minutes”, “80°C for 10 minutes”, “100°C for 5 minutes”, “100°C for 10 minutes”, “120°C for 5 minutes”, “120°C for 10 minutes” and “150°C for 5 minutes”. Smoke treatment levels corresponded to two different dilutions of liquid smoke solution. Previous results show that the combination of both cues has only a marginal effect on seed germination (chapter 6). Thus, this combined treatment was not considered in the present study. Seeds were set for germination in Petri dishes with agar (0.7%) as substrate and incubated at 20°C in darkness. At the end of the experiment, seed viability of the non-germinated seeds was checked by using the tetrazolium chloride and the cut test, in order to assess seed mortality (Ooi et al. 2004). See Moreira et al. (2010) for further details.

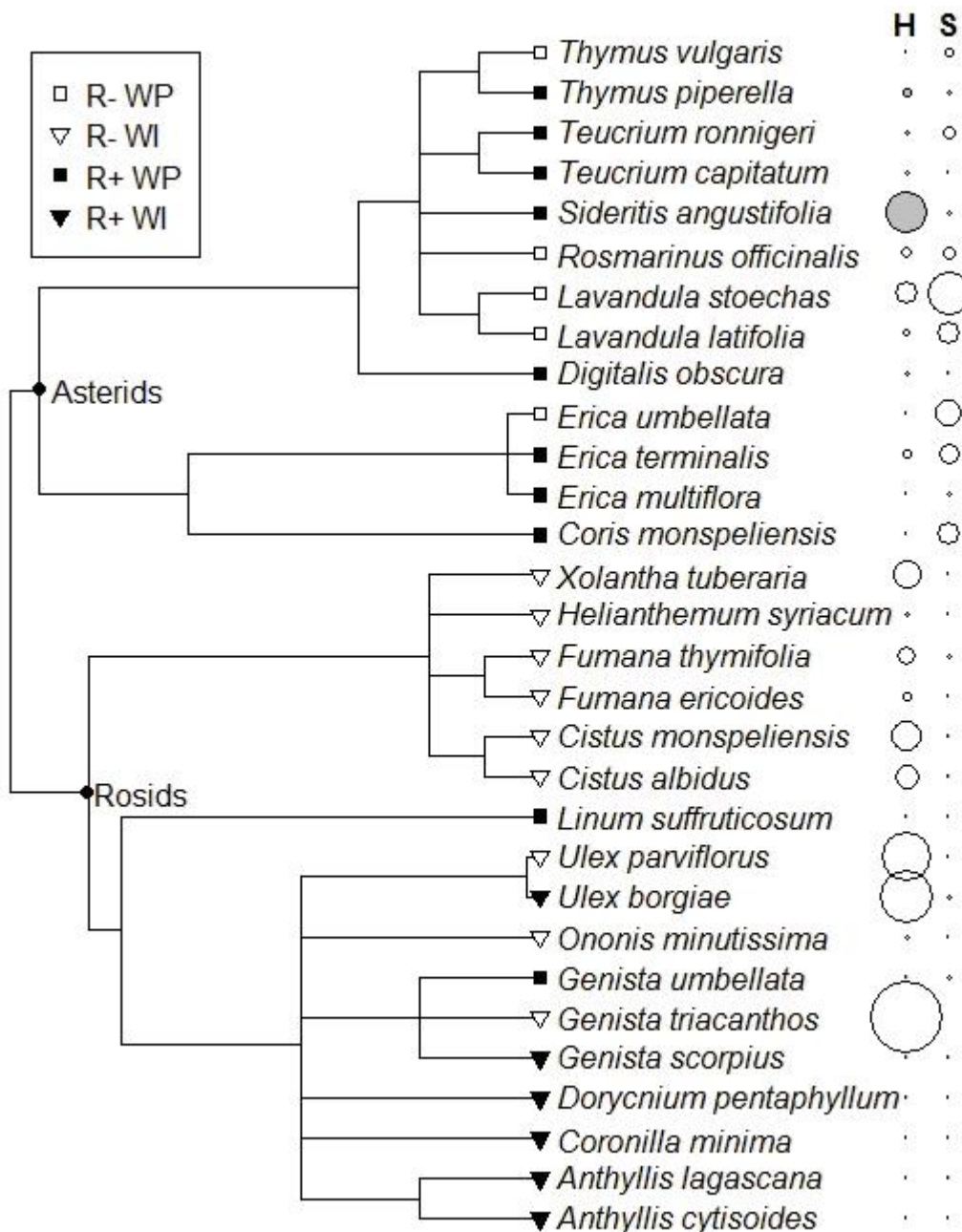


Figure 1. Phylogenetic relatedness between the species considered, indicating the two main clades (Asterids and Rosids), the species traits (symbols in the tips; R+: closed symbols, R-: open symbols; WP: squares, WI: triangles), and the mean magnitude of the heat (H) and smoke (S) treatments (size of the circles is proportional to the magnitude of the stimulation, with grey circles for negative effects).

Statistical analysis

We tested the role of resprouting ability (R+ vs R-), seedcoat type (WP vs WI), and treatment (heat vs smoke) on three processes associated with post-fire germination: seed dormancy, germination stimulation and seed thermal tolerance.

Seed dormancy was evaluated as the number of cases (seeds in the different species) with no germination in control conditions (probability of seed dormancy). Germination stimulation was assessed by three indicators: a) the number of cases (treatment levels in the different species) in which the germination was higher than in the control (probability of increased germination); b) the number of cases (treatment levels in the different species) in which the germination was faster than in the control (probability of faster germination); and c) the difference in germination percentage between treatments and control (magnitude of germination, %). Seed thermal tolerance was evaluated as the number of cases (treatment levels in the different species) in which seed mortality after heat treatments was higher than the control (probability of seed mortality). In all cases, the significance of the heat and smoke treatments compared with the respective control was based on Moreira et al. (2010, see their tables 1, 2 and 3).

The probability of seed dormancy (yes/no) was evaluated using a generalized linear mixed model (GLMM) with binomial error distribution, and including resprouting ability (R+ vs R-) and seedcoat type (WP vs WI) as fixed factors and species as random factor. The probability of increased germination (yes/no) and the probability of faster germination (yes/no) after treatments were also tested using the same GLMM but adding the treatment (heat vs smoke) as a fixed factor and the level of treatment as a random factor. The magnitude of germination (%) was tested using a linear mixed model (LMM) and including the same factors. The probability of seed mortality (yes/no) after heat shock was also tested with the GLMM but for heat treatments only. Although in Mediterranean flora the ability to resprout is correlated to seed characteristics (Pausas & Verdú 2005), in our data set, which considers P+ species only (i.e., seeders), there was no relation between seedcoat type and the ability to resprout ($\chi^2 = 0.57$, $p = 0.45$). This enabled us to include both resprouting ability and seedcoat type in the same statistical model.

Given that many traits, including regeneration traits, are evolutionary conserved (Blomberg et al. 2003, Bolmgren & Eriksson 2005, Pausas & Verdú 2005, 2008, Fig. 1), we expect that post-fire germination success and the response to different germination cues would depend on historical and phylogenetic constraints. In order to evaluate the role of phylogenetic affiliation in the post-fire germination, we tested the role of seedcoat type and resprouting ability on the germination stimulation using a phylogenetic-informed analysis. We first assembled a phylogenetic tree using the supertree of angiosperm families (Davies et al. 2004), grafted our species on it, and mapped the studied traits (seedcoat type and resprouting ability) and an

indicator of the response to heat and to smoke treatments. As indicator of stimulated germination for each species, we used the mean magnitude of stimulation (mean difference between treated seeds and control) by each treatment (heat and smoke). The differences in the magnitude of stimulation between different species traits (resprouting ability and seedcoat type) were tested using a Generalized Estimating Equation (GEE) procedure. GEE uses a GLM approach incorporating the phylogenetic relatedness among species as a correlation matrix in the model (Paradis & Claude, 2002).

Results

Seed dormancy

On average, seed dormancy (lack of germination under control conditions) was significantly higher for obligate seeders than for facultative seeders ($R\text{-}P^+ = 87.7\%$, $R^+P^+ = 60.0\%$; expressed as mean percentage of seeds that did not germinate in control conditions). Seed dormancy was also higher for species with water-impermeable seeds than for those with water-permeable seeds ($WI = 93.5\%$; $WP = 52.3\%$). The two traits explained a significant proportion of the variability (Table 1).

Table 1. Summary of the statistical analyses on seed dormancy (germination in control conditions), seed thermal tolerance (probability of seed mortality) and germination stimulation (probability of increased germination, magnitude of germination, and probability of faster germination) in relation to treatment (treat), resprouting ability (R), and seedcoat type (SC), (ns $p > 0.05$; * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$). Full details of the analyses are provided in the additional online supporting information (Table).

Process	Treat	R	SC	R x SC	Treat x SC	Treat x R	Table
Dormancy	-	**	**	ns	-	-	S1
Seed thermal tolerance	-	ns	*	ns	-	-	S2
Increased germination	ns	***	ns	ns	****	ns	S3
Magnitude of germination	ns	*	ns	ns	****	ns	S4
Faster germination	ns	**	ns	ns	****	ns	S5

Seed thermal tolerance

The differences in the probability of seed mortality in relation to resprouting ability and seedcoat type were in the limit of significance (Table 1) and only facultative seeders with water-impermeable seeds (R^+WI) had significantly higher probability of seed mortality (0.59) compared with all the other combinations of R and seedcoat type (R^+WP : 0.30; R^-WI : 0.30; R^-WP : 0.28; expressed as the proportion of heat levels with increased seed mortality, in relation to control).

Stimulated germination

Obligate seeders had higher germination stimulation for the three indicators considered (probability of increased germination, probability of faster germination and magnitude of the germination) than facultative seeders. In any of the cases, the interaction between resprouting and treatment was significant, suggesting that obligate seeders tend to germinate more and faster than facultative seeders in response to both heat and smoke (Table 1). Regarding seedcoat type, there were no differences between species with WI seeds and species with WP seeds in any of the three indicators of germination stimulation. However, the interaction of seedcoat type with treatment was highly significant for all the indicators, suggesting that species with WI seeds respond better to heat treatments while those with WP seeds respond better to smoke treatments (Table 1, Fig. 2 and 3).

Phylogenetic effects

Species with WI seeds were aggregated in the Rosids clade (e.g., Cistaceae and Fabaceae, Fig. 1), while none of the species in the Asterid group (Lamiaceae, Ericaceae, and Primulaceae) had WI seeds. Resprouting appeared in the two major clades. Heat-stimulated germination was dominant in Rosids, but also appeared in some Asterids (e.g., *Lavandula stoechas* and *Rosmarinus officinalis*; Fig. 1), while smoke-stimulated germination was exclusive of Asterids. The phylogenetic-informed analysis suggests that the magnitude of the germination stimulation by heat was significantly higher for R-P+ ($p=0.04$), and for species with WI seeds ($p=0.01$), while the stimulation by smoke was also higher for R-P+ ($p=0.006$) but lower for species with WI seeds ($p=0.005$). That is, the phylogenetic analysis supports both the differences in fire-stimulated germination between resprouting abilities and the interaction between treatment and seedcoat type found in the cross-species analyzes (Table 1, Fig. 2).

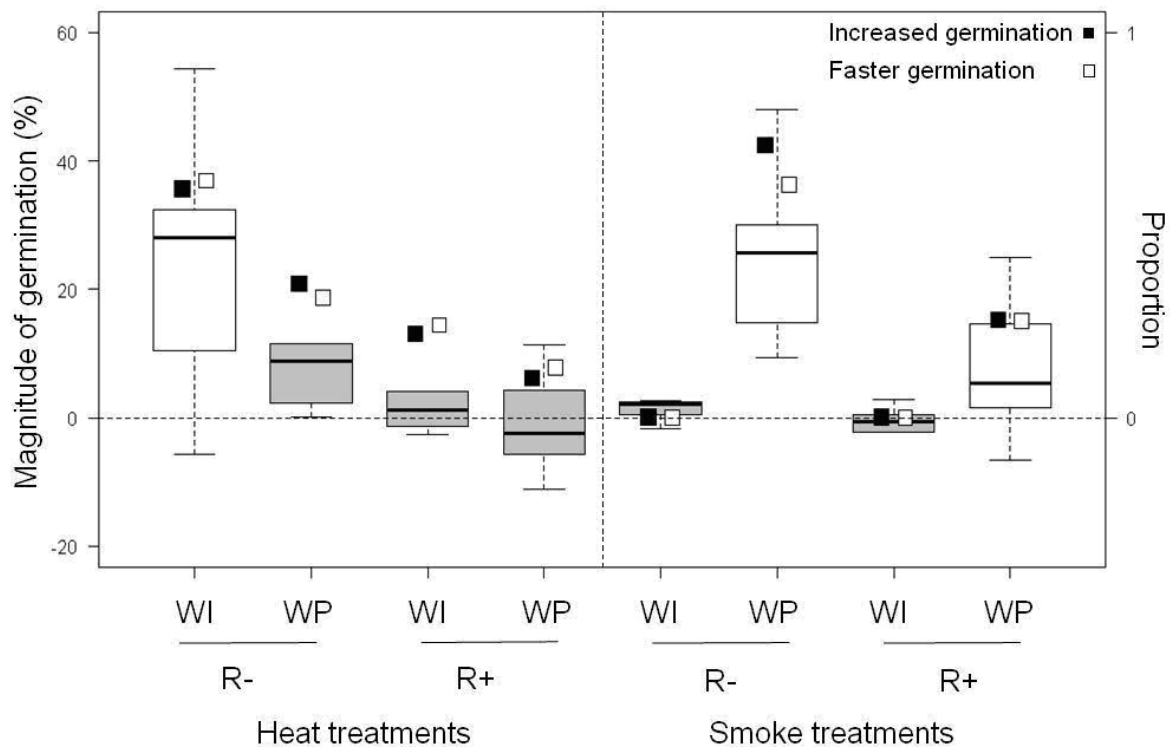


Figure 2. Germination response to the fire-related treatments (heat and smoke) for species with different resprouting ability (R- vs R+) and seedcoat type (WI and WP). Boxplots (median and percentiles) show the magnitude of the change in germination in relation to the control (%), left axis). Grey boxes are magnitude values that do not differ from 0 ($p > 0.05$). Squared symbols (and right axis) show the proportion of increased germination (filled symbols) and faster germination (open symbols) for each combination of resprouting ability and seedcoat type. These values are computed as the proportion of treatment levels that are higher than the control, for each species and then averaged for the given combination of traits. In the three variables, resprouting (R) and the interaction between seed coat and treatment (heat vs smoke) are significant (see table 1).

Discussion

Our results suggest that, in post-fire seeders, the processes behind post-fire germination (seed dormancy, seed thermal tolerance and germination stimulation) are linked to the ability to resprout, while the mechanisms of stimulated germination (heat vs smoke stimulation) are strongly linked to the seedcoat type (permeability). Obligate seeders (i.e., species with no resprouting ability) had higher seed dormancy and higher heat and smoke stimulated germination than facultative species (i.e. species with resprouting ability); but for each fire-related germination cue, the highest stimulation is dependent of the species' seedcoat type: obligate seeders with water-impermeable seeds are mostly stimulated by heat, while seeders with water-permeable seeds are stimulated by smoke (Figs 2 and 3). These results support the

importance of trait correlation in Mediterranean ecosystems (Herrera 1992; Pausas & Verdú 2008). However, because these correlations may not be universal, but associated to the evolutionary history of each flora, it is difficult to find general models based on a single trait (e.g., Bellingham & Sparrow 2000; Pausas 2001).

Obligate seeders (R-P+) had higher seed dormancy than facultative seeders (R+P+) and, since there is no clear evidence of differences in seed production when comparing these groups (Keeley 1977; Pausas et al. 2004), our results suggest that facultative seeders have a higher proportion of seeds that may germinate in the absence of fire and thus avoid its selective pressure. On the contrary, when a fire occurs, obligate seeders will have a greater proportion of seeds available in the seedbank, which will be subject to this selective pressure and will struggle for competitive advantages during and after a fire (seed thermal tolerance and stimulated germination). Accordingly, obligate seeders exhibited higher germination response than facultative seeders to the two fire-related germination cues considered (heat and smoke), suggesting that post-fire germination is more efficient in obligate than in facultative seeders (Keeley 1986; Lamont & Wiens 2003). This supports the hypothesis that the presence of one regeneration mechanism reduces the evolutionary pressure to acquire a second regeneration mechanism (Pausas & Verdú 2005; Paula & Pausas 2008). However, there were no differences between obligate and facultative seeders regarding seed thermal tolerance (Bell & Williams 1998). The lack of these differences together with the lower seed dormancy, suggests that post-fire germination of facultative seeders relies greatly on seed thermal tolerance. That is, facultative seeders successfully germinate and recruit after fire, despite the low levels of seed dormancy and fire-stimulated germination, due to their high seed thermal tolerance.

Species with WI seeds had higher seed dormancy than species with WP seeds but there were no differences regarding germination stimulation (Fig. 2). In addition, we did not find evidence of differences in seed thermal tolerance, suggesting that the presence of strongly lignified cell walls in the seedcoat of WI seeds has a limited role in protecting the embryo from high temperatures (Bell & Williams 1998). As predicted, there was a differential response to heat and smoke depending on the seedcoat permeability. Species with WI seeds had germination stimulated by the heat treatments, while species with WP seeds had germination stimulated by the smoke treatments (Fig. 3). These results support the lack of effect of heat over WP smoke-stimulated species (Keeley and Fotheringham 1998) and explain the observed coexistence of species with WI seeds and species with WP seeds in post-

fire regeneration (Keeley 1995). Nevertheless, for some species, smoke may also stimulate germination in species with WI seeds (Pérez-Fernández and Rodríguez-Echevarría, 2003; Crosti et al., 2006) and heat may be an important germination cue for some species with WP seeds (Moreira et al. 2010). For instance, the germination of *Lavandula stoechas* (Lamiaceae, with WP seeds) was stimulated by both heat and smoke (Figs 1 and 3).

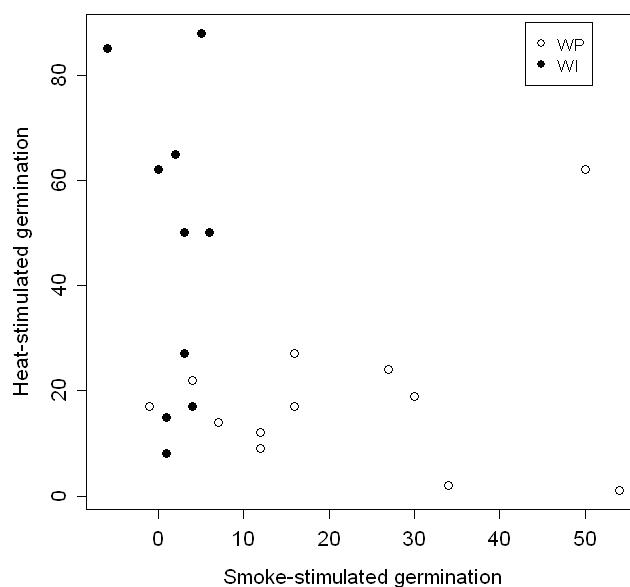


Figure 3. Relationship between heat- and smoke- stimulated germination for the species in which at least one of the two treatment types was significant (WP: open circles; WI: filled circles). Stimulated germination was measured as the maximum magnitude of the stimulation, that is, difference between the maximum germination (%) in treated seeds minus the germination in the corresponding control (%). The overall relationship was significant in both the cross-species analysis ($r = -0.497$, $p = 0.018$, log-transformed) and the phylogenetic-informed analysis ($p < 0.02$).

In conclusion, post-fire germination is more efficient in obligate seeders than in facultative seeders, supporting the hypothesis that having one trait that allows post-fire persistence (i.e. resprouting capacity) reduced the evolutionary pressure over a second post-fire persistence trait (i.e., post-fire germination). Moreover, different lineages have evolved post-fire germination ability, but this trait was acquired in response to different fire-related germination cues (smoke- vs heat-stimulation) depending on the seedcoat type of the lineage (Fig. 1). Although post-fire germination is a response to the evolutionary pressure exerted by recurrent fires, the specific strategies for acquiring this trait depend on historical effects (e.g., ancestral predisposition of the lineage). That is, different species have developed different solutions to the evolutionary pressure exerted by fire, allowing the coexistence in fire-prone ecosystems of species with different seedcoat type.

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Appendix

Table S1 - Analysis of the probability of germination in control conditions, in relation to resprouting ability (R) and seedcoat type (SC).

	df	AIC	BIC	logLik	Chisq	Chi	df	Pr(>Chisq)
Null	2	253.90	259.47	-124.95				
R	3	248.44	256.81	-121.22		7.45	1	0.006330
SC	4	240.00	251.15	-116.00		10.44	1	0.001232
R:SC	5	239.00	252.94	-114.50		3.00	1	0.083201

Table S2 - Analysis of the probability of heat-related seed mortality in relation to resprouting ability (R) and seedcoat type (SC).

	Df	AIC	BIC	logLik	Chisq	Chi	Df	Pr(>Chisq)
Null	3	164.72	174.76	-79.36				
R	4	165.45	178.84	-78.72		1.27	1	0.260150
SC	5	162.92	179.66	-76.46		4.53	1	0.033370
R:SC	6	162.14	182.22	-75.07		2.78	1	0.095230

Table S3 - Analysis of the probability of increased germination in relation to resprouting ability (R), seedcoat type (SC) and treatment (treat).

	df	AIC	BIC	logLik	Chisq	Chi	df	Pr(>Chisq)
Null	3	289.02	299.80	-141.51				
treat	4	290.06	304.42	-141.03		0.96	1	0.326698
R	5	279.96	297.92	-134.98		12.10	1	0.000506
SC	6	281.83	303.38	-134.92		0.13	1	0.716388
treat:SC	7	245.59	270.73	-115.80		38.24	1	<0.000001
treat:R	8	247.22	275.95	-115.61		0.37	1	0.543446
R:SC	9	249.02	281.34	-115.51		0.20	1	0.652420
treat:R:SC	10	251.02	286.93	-115.51		0.00	1	1.000000

Table S4 - Analysis of the magnitude of germination in relation to resprouting ability (R), seedcoat type (SC), and treatment (treat).

	df	AIC	BIC	logLik	Chisq	Chi	df	Pr(>Chisq)
Null	4	9371.50	9391.40	-4681.80				
treat	5	9373.60	9398.50	-4681.80	0.00		1	1.000000
R	6	9370.60	9400.40	-4679.30	5.04		1	0.024820
SC	7	9369.70	9404.60	-4677.90	2.84		1	0.092190
treat:SC	8	9231.90	9271.70	-4607.90	139.84		1	<0.000001
treat:R	9	9232.60	9277.40	-4607.30	1.30		1	0.254830
R:SC	10	9234.30	9284.10	-4607.10	0.30		1	0.586710
treat:R:SC	11	9229.80	9284.60	-4603.90	6.48		1	0.010890

Table S5 - Analysis of the probability of faster germination in relation resprouting ability (R), seedcoat type (SC) and treatment (treat).

	df	AIC	BIC	logLik	Chisq	Chi	df	Pr(>Chisq)
Null	3	249.60	259.99	-121.80				
treat	4	250.00	263.85	-121.00	1.60		1	0.205301
R	5	242.34	259.65	-116.17	9.66		1	0.001881
SC	6	244.16	264.94	-116.08	0.17		1	0.676008
treat:SC	7	213.90	238.15	-99.95	32.26		1	0.000000
treat:R	8	215.72	243.43	-99.86	0.18		1	0.672555
R:SC	9	217.48	248.65	-99.74	0.24		1	0.622033
treat:R:SC	10	219.48	254.12	-99.74	0.00		1	1.000000

Chapter 8

Local versus regional intraspecific variability in regeneration traits

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Abstract

Intraspecific trait variability has a fundamental contribution to the overall trait variability. However, little is known concerning the relative role of local (e.g. disturbances and species interactions) and regional (biogeographical) processes in generating this intraspecific trait variability. While biogeographical processes enhance plant trait variability between distant populations, in fire-prone ecosystems, recurrent fires may have a preponderant role in generating variability at a local scale. We hypothesize that plants respond to the local spatio-temporal heterogeneity generated by fire by having a relatively large local variability in regeneration traits in such a way that overrides the variability at a broader biogeographical scale. We test this hypothesis by assessing the intraspecific variability in fire-related regeneration traits of two species (*Cistus salviifolius* and *Lavandula stoechas*) growing in fire-prone ecosystems of the Mediterranean Basin. For each species, we selected six populations in two distant regions, three in the east (Anatolian Peninsula) and three in the west (Iberian Peninsula). For each species and population, we analysed the following regeneration traits: seed size, seed dormancy and stimulated germination by fire-related cues (heat and smoke). To evaluate the distribution of the variability in these traits, we decomposed the variability of trait values at each level, between regions (regional) and between population within region (local), using linear mixed-effect models. Despite the biogeographical and climatic differences between regions, for the two species, intraspecific variability in regeneration traits was higher at a local (within regions) than at a regional scale (between regions). Our results suggest that, in Mediterranean ecosystems, fire is an important source of intraspecific variability in regeneration traits. This supports the prominent role of fire as an ecological and evolutionary process, producing trait variability and shaping biodiversity in fire-prone ecosystems.

Keywords: Fire · Mediterranean ecosystems · Germination traits · Seed traits · Spatial scale

Introduction

Variability is a fundamental characteristic of life and the raw material for natural selection. Despite there being a large individual variability, species are commonly described by mean trait values, overlooking their intraspecific variability (Bolnick et al. 2011). Studying trait variability, and determining its patterns, causes and consequences, is essential for understanding evolutionary and diversification processes (Sandquist and Ehleringer 1997; Herrera 2009; Messier et al. 2010), as well as for improving predictions in the framework of global change (Garzón et al. 2011).

Biogeographical processes such as migration, glacial/ interglacial climatic fluctuations and isolation should cause distant plant populations to diverge, and thus enhance intraspecific trait variability at regional scales (Debussche and Thompson 2002; Hampe et al. 2003; Thompson 2005). However, local processes like disturbances, species interaction and heterogeneity in resource availability generate trait variability at a local scale. This local trait variability determines the species response to biotic and abiotic filters, which in turn has implications on the population dynamics and the community structure (Prinzing et al. 2008; Jung et al. 2010; Pausas and Verdú 2010; Bolnick et al. 2011; García Molinos and Donohue 2011). While there is an increasing bulk of information highlighting the important contribution of intraspecific variability to the overall trait variability (Boege and Dirzo 2004; Lecerf and Chauvet 2008; Cianciaruso et al. 2009; Albert et al. 2010), there is little information on the relative role of local versus regional processes in generating this variability.

The Mediterranean Basin provides an excellent framework to study the distribution of intraspecific plant trait variability at different spatial scales. This is because it has similar climatic conditions (Mediterranean climate) and common species. In addition, it is an extensive area (the distance from east to west is over 3,000 km, including a barrier in the middle, the sea), with many peninsulas, and thus provides opportunities for regional differentiation (Thompson 2005). However, wildfires are an important local process in this region, not only generating landscape dynamics and heterogeneity (Sousa 1984; Pausas 1999; Pausas and Lloret 2007) but also imposing a strong evolutionary pressure on plant traits (Pausas and Keeley 2009; Keeley et al. 2011). Indeed, in fire-prone ecosystems, evolutionary pathways (Pausas and Verdú 2005), genetic diversity (Dolan et al. 2008; Schrey et al. 2011) and community structure (Verdú and Pausas 2007) are strongly determined by fire history. These ecosystems can be viewed as a mosaic of patches with different fire histories and at different stages of succession (e.g. different recurrence, severity, time since fire; Vázquez and

Moreno 2001; Pausas and Lloret 2007). At the local scale, the heterogeneous burn patterns, together with other local factors such as species interaction and heterogeneity in resource availability, should generate variability in fire-related plant regeneration traits (such as seed size, seed dormancy and germination stimulated by fire-related cues). This should be especially evident in species that rely on post-fire germination as the only mechanism for regenerating after fires (i.e. species without the ability to resprout) which define the obligate seeding life history (Keeley 1995; Pausas et al. 2004). In addition, fires shorten the generation time of seeders (i.e. increase population turnover) and generate strong fluctuations in population dynamics. However, while there is evidence that fire favours genetic drift and population differentiation (Wade and McCauley 1988; Ellner and Hairston 1994; Segarra-Moragues and Ojeda 2010), little is known about its role generating intraspecific trait variability and phenotypic differentiation. Seeder species have seeds that are accumulated in the soil during the inter-fire periods. When a fire occurs and kills the adult plants, seeds resist the fire and the germination is stimulated by fire-related germination cues, i.e. by the heat or by the chemicals found in the smoke and charred wood (Keeley and Fotheringham 2000; Moreira et al. 2010). That is, fire selects for high levels of seed dormancy and germination stimulated by fire-related cues. In contrast, long inter-fire periods favour germination from seeds without a specific requirement for fire-related cues to germinate (Keeley 1991). Seed size also affects many regeneration processes such as persistence in the soil (Thompson et al. 1993; Moles et al. 2000; Peco et al. 2003; but see Leishman and Westoby 1998 for the lack of a relationship in Australian flora), seed dispersal (e.g. Ganeshiah and Shaanker 1991) and seedling size (e.g. Stock et al. 1990). In addition, it has been suggested that fire selects for large seeds, while small seeds are favoured during inter-fire periods (Delgado et al. 2001, 2008).

Previous studies suggested that seed size, seed dormancy (Cruz et al. 2003; Pérez-García et al. 2003) and germination stimulation in response to heat and smoke (Tieu et al. 2001; Cruz et al. 2003) are regeneration traits that exhibit intraspecific variability between populations at the local scale. However, no studies have been performed at a wider geographic scale (e.g. over the species distribution) that could allow the disentangling of the relative role of local (i.e. fire) versus regional (i.e. biogeographical) processes in generating this trait variability.

We hypothesize that plants with fire-dependent recruitment respond to the spatio-temporal heterogeneity of Mediterranean fire-prone ecosystems by having a relatively large local (within region) variability in regeneration traits in such a way that may override variability at a broader biogeographical scale. To test this hypothesis, we analysed the variability in seed

and germination traits in six different populations of two common species from two distant regions of the Mediterranean Basin. The two model species selected, *Cistus salviifolius* L. (Cistaceae) and *Lavandula stoechas* L. (Lamiaceae), are representative of two common families that exhibit contrasted mechanisms of fire-stimulated germination (see below). The two species were sampled from two peninsulas near the extremes of their distribution area, one in the east (Anatolia) and the other in the west (Iberia) (Fig. 1).

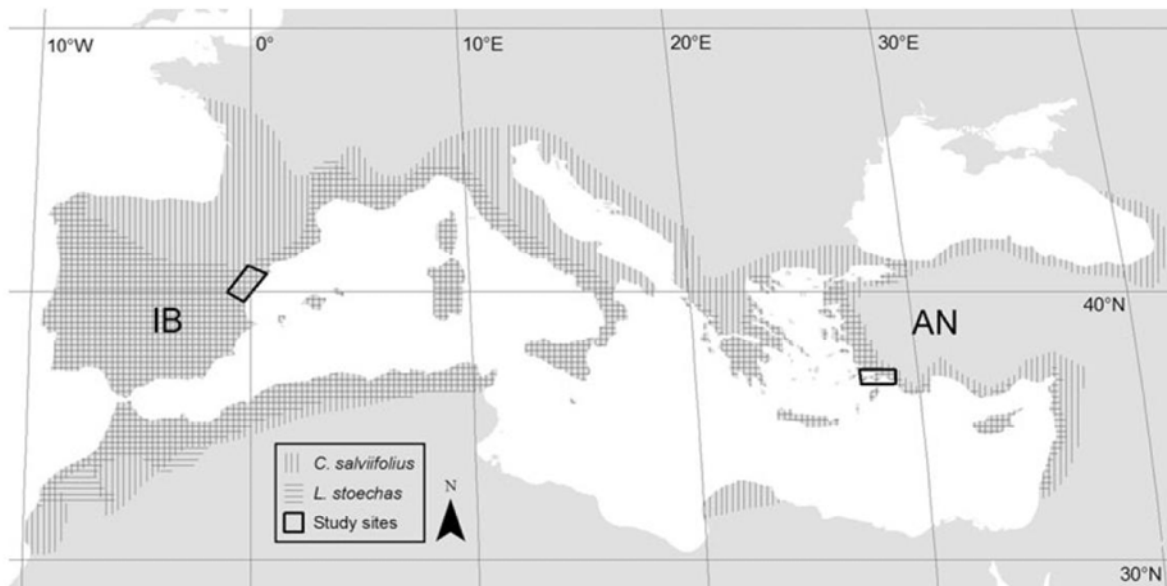


Figure 1. The Mediterranean basin with the location of the study areas (rectangle), in Iberia (IB) and Anatolia (AN). Distribution of the study species shown by shading: *Cistus salviifolius* (vertical lines) and *Lavandula stoechas* (horizontal lines). Areas where the species distributions overlap are indicated by crosshatching

Materials and methods

Species and study sites

We selected two shrub species growing in fire-prone communities throughout the Mediterranean basin: *Cistus salviifolius* (Cistaceae) and *Lavandula stoechas* (Lamiaceae). Both species are obligate seeders; that is, they lack the ability to resprout and rely on post-fire germination as the only mechanism for regenerating after fires (Pausas et al. 2004; Paula et al. 2009). The two species encompass the two main post-fire germination strategies in the Mediterranean Basin: Cistaceae are mainly stimulated by heat, while Lamiaceae are mainly stimulated by chemicals from the combustion (hereafter, “smoke-stimulated”; Moreira et al. 2010). The distribution area of the two species include most of the Iberian peninsula (Spain and Portugal), the southern rim of France, Italy, Greece, the Adriatic coast, the coastal regions of Anatolian peninsula (Turkey), the Near East (Lebanon, Syria, Cyprus, Palestine, Israel) and northern Africa (Morocco, Algeria, Tunisia) (Fig.1).

We selected six sites in two distant peninsulas of the Mediterranean Basin, three in the east (Mufla province, south-western Turkey, Anatolian Peninsula) and the other three in the west (Valencia province, eastern Spain, Iberian Peninsula) (Fig. 1; Table 1). Populations of the two species co-occurred at each site. The two sampling regions are separated by 2,600 km (straight-line), with the Mediterranean Sea inbetween them, while the distance between populations within regions ranged from 25 to 110 km. Consequently, gene flow has been certainly much higher within than between regions. In each region, each site had a different fire history and associated vegetation structure, but all were within the range of fire history currently found in the Mediterranean Basin. IB3 and AN3 had a recent very high frequency of crown-fires that precluded the occurrence of pine forests (shrubland with scattered regenerating pines). The most recent fires in IB3 occurred in 1985 and 1992, and in AN3 in 1996. IB1 and AN1 had the least fire activity of all sites; IB1 was surrounded by a managed cork oak forest and the last fire was a surface fire in 2003, and AN1 was an open maquis shrubland where no fires had occurred during at least the last 30 years. IB2 and AN2 were in an intermediate situation, where crown-fires were frequent but allowed the development of pines (co-dominated by regenerating pines and shrubs). The most recent fires in IB2 occurred in 1992, and in AN2 in 1993 (fire interval >20 years). Although the two regions have a clear Mediterranean climate, they also have some differences: average annual climatic data suggest that Anatolian populations grow under warmer ($T_{\text{mean}} > 18^{\circ}\text{C}$) and wetter ($P > 650$ mm) conditions than the Iberian populations ($T_{\text{mean}} < 17^{\circ}\text{C}$, $P < 600$ mm). In the two regions, there is a clear dry sea-son when fires are common; this dry season is longer and dryer in the Anatolian sites (Table 1).

Table 1. Location, elevation (m), aspect and average climatic conditions (T mean annual temperature, in $^{\circ}\text{C}$, $Prep$ mean annual precipitation, in mm, Dry duration of dry period, in months, $Prep_{dry}$ precipitation in the dry period as proportion of the annual precipitation, in %) of the Iberian and Anatolian studied sites (western and eastern Mediterranean Basin, respectively).

Site code	Location	Elevation (m)	Aspect	T ($^{\circ}\text{C}$)	Prep (mm)	Dry (months)	Prep _{dry} (%)
Iberian sites							
IB1	Serra Espadà	578	SE	13.3	586.9	3	9.4
IB2	Serra Calderona	741	NW	16.6	556.4	3	8.0
IB3	Desert de les Palmes	165	SE	15.7	546.2	3	14.2
Anatolian sites							
AN1	Datça	13	S	19.4	679.8	5	4.1
AN2	Fethiye	194	SW	18.0	970.5	5	4.1
AN3	Marmaris	265	NW	18.7	1211.7	5	4.7

The two species (*Cistus salviifolius* and *Lavandula stoechas*) occurred at all sites.

Sampling

During June–July 2009, for each population and species, we collected all seeds from 20–30 individuals separated ca. 10 m from each other (>100 seeds per individual). These seeds were pulled together and stored in paper bags until the germination experiment started at the end of July 2009. Seed weight was estimated with a digital weight from ten replicates of ten seeds.

Germination experiments

Seeds were placed in aluminium pockets and, for each population and species, four aluminium pockets (four replicates) were randomly allocated to each of the seven groups (three heat treatments, one smoke treatment, a combined treatment of heat plus smoke, and two controls; see below).

Heat treatments consisted of three different temperatures (100, 120 and 150°C) applied for 5 min using a temperature-controlled oven. For each heat treatment, the respective aluminium pockets were disposed randomly in the metal tray of the oven. This operation was repeated four times for each population and species to achieve four independent replicates of each treatment.

For the smoke treatment, we prepared four batches (replicates) of smoke solution by grinding dry leaves and thin twigs of *Quercus coccifera* (a common shrub of the study areas) and following the protocol described in Jäger et al. (1996). Seeds were incubated in this solution (four replicates; mean pH = 4.84) for 24 h before sowing in the Petri dishes. To test the effect of the combination of heat and smoke, this combined treatment was applied (100°C for 5 min plus smoke), consisting of seeds heated at 100°C for 5 min and then incubated in the liquid smoke solution for 24 h. Two controls were applied: a “dry control” (seeds without any treatment, sown directly into the Petri dishes) for comparing with the heat treatments, and a “watered control” (seeds immersed in distilled water for 24 h before sowing, pH of distilled water was 6.29) for comparing with the smoke treatment and the combined treatment of heat plus smoke.

For each replicate (of each treatment, species and population) we sowed 50 seeds in Petri dishes containing agar (0.9%) as substrate. All Petri dishes were then placed in a germination chamber and incubated at 20°C in darkness. This temperature is appropriate for the germination of many Mediterranean species (Thanos et al. 1991) including Cistaceae (e.g. Thanos and Georghiou 1988) and Lamiaceae (e.g. Thanos et al. 1995). The germination was

monitored every 2 days during the first 2 weeks and once a week after this period, and germinated seeds were scored and eliminated from the Petri dishes. The criterion used for scoring a seed as germinated was based on the observation of radicle emergence (0.5–1 mm) determined under a magnifying lens. During the recounts, rotten seeds and empty seeds (lacking embryo and storage tissue) were removed from Petri dishes. The initial number of sown seeds was corrected before any statistical analysis by excluding empty seeds. The experiment started at the end of July 2009 and was conducted during 90 days, when no germination was recorded during one week.

Data analyses

For each population and species, the final germination of each treatment was compared with their corresponding control using the analysis of deviance (GLM). Seed dormancy was measured as the proportion of non-germinated seeds in control conditions (“dry control”). To evaluate the relative distribution of intraspecific trait variability at the different spatial scales (regional vs. local) we used linear mixed-effects (LME) models with population (nested in region) and region as random factors. This allowed the decomposing of the variability of each trait (seed size, seed dormancy and germination stimulation) at each level (population and region) (see Messier et al. 2010 for a similar approach). To further evaluate the significance of population (local scale) and region (regional scale), we included in the model population as fixed factor (with region as random in the model) or region as fixed factor (with population as random in the model) respectively.

Results

The germination of *Cistus salviifolius* showed a strong increase after heat shock for all populations in the two regions while the effect of smoke was almost nil (Table S1 in the appendix). All populations of *Lavandula stoechas* had seed germination stimulated by heat treatments, by the combined treatment of heat plus smoke and by the smoke treatment alone, with the stimulation being highest for the treatments that included smoke. For both species, most intraspecific variability in seed size and seed dormancy was within regions (82% and 44% of the total variability for *Cistus*, respectively; 74% and 65% for *Lavandula*), and very little was left between regions (0% for both traits; Table 2). Consequently, for the two species and these two traits, population (within region) was a significant factor explaining the observed variability while region was not (Table 2).

Intraspecific variability in fire-stimulated germination (estimated as germination stimulation, in relation to control, after treatments) was also higher within than between regions. Most of the variability in germination stimulation by heat in *Cistus* and by smoke in *Lavandula* was at the local scale (Table 2). In *Cistus*, variability in germination stimulation by heat within regions was significant and accounted for 31–48% of the total variability. In *Lavandula*, variability in germination stimulation by treatments that included smoke was also significant within regions and accounted for 85–93% of the total variability. In both cases, the variability between regions was very low (~0%) and not significant. On the contrary, the variability in germination stimulation by smoke in *Cistus* was similar within and between regions (30–33%) and the variability in germination stimulation by heat in *Lavandula* depended on the treatment; for the extreme heat doses (100 and 150°C, respectively), variability was higher within regions, while for the intermediate heat treatment (120°C), variability was higher between regions (Table 2).

Table 2. Statistical analyses (LME), for each species (*Cistus salvifolius* and *Lavandula stoechas*), of trait variability in relation to region and population (nested in region)

Species	Trait	Variance (%)			Significance	
		Population	Region	Residuals	Population	Region
<i>Cistus</i>	Seed size	82.05	~0	17.95	<0.001	ns
	Seed dormancy	44.53	~0	55.47	0.007	ns
	Stim. H ₁₀₀	48.39	~0	51.61	0.003	ns
	Stim. H ₁₂₀	47.57	4.05	48.38	0.002	ns
	Stim. H ₁₅₀	31.09	~0	68.91	0.047	ns
	Stim. S	29.77	32.57	37.65	0.002	ns
	Stim. H _{100+S}	43.78	~0	56.22	0.007	ns
<i>Lavandula</i>	Seed size	73.51	~0	26.49	<0.001	ns
	Seed dormancy	65.01	~0	34.99	<0.001	ns
	Stim. H ₁₀₀	19.41	~0	80.59	ns	ns
	Stim. H ₁₂₀	~0	52.65	47.35	ns	0.001
	Stim. H ₁₅₀	27.89	22.59	49.51	0.009	ns
	Stim. S	92.66	~0	7.34	<0.001	ns
	Stim. H _{100+S}	85.14	~0	14.86	<0.001	ns

Traits are: seed size, seed dormancy and stimulation by heat treatments of 100, 120 and 150°C for 5 min (Stim. H₁₀₀, Stim. H₁₂₀, Stim. H₁₅₀), smoke treatment (Stim. S), and heat plus smoke treatment (Stim. H_{100+S}). Left columns show the variance components of population and region (nested design). Right columns show the significances of population as fixed factor (with region as random in the model) and of region as fixed factor (with population as random in the model). ~0 represent values lower than 1e⁻⁴
 ns non-significant

Discussion

The two studied species exhibited germination stimulated by fire-related cues; and independently of the region, the different populations of each species had a similar pattern of response. That is, *Cistus salviifolius* was stimulated by heat and *Lavandula stoechas* was mainly stimulated by smoke, although heat also exhibited a positive effect on the latter species.

The two studied regions are distant (Fig. 1), and consequently gene flow is much higher within than between regions. In addition, the two regions exhibit climatic differences (Table 1) and the species interact with different coexisting species in each of the regions. Despite all these factors acting towards more expected variability between than within regions (Farley and McNeilly 2000; Debussche and Thompson 2002; Hampe et al. 2003; Thompson 2005), our results unambiguously support the opposite pattern regarding traits relevant for persistence in fire-prone ecosystems. Variability in the studied fire-related traits was much higher within than between regions, and only in one case did we find significantly higher variability between regions (intermediate heat doses in *Lavandula stoechas*). Overall, these results suggest that intraspecific variability in regeneration traits allows species to recruit in different conditions (i.e. after fires with different intensity levels and during inter-fire periods). Consequently, the high intraspecific trait variability at the local scale increases the probability of persistence in fire-prone ecosystems and emphasizes the fundamental role of fire in the generation phenotypic variability. Recent molecular studies have also shown that local ecological processes are strongly relevant in generating genetic divergence in fire-prone ecosystems (Fernández-Mazuecos and Vargas 2010; Segarra-Moragues and Ojeda 2010; Schrey et al. 2011). Our results of fire-related regeneration traits contrast with previous observations of high variability at the regional scale in morphological traits, such as leaf size and internode length, between eastern and western Mediterranean populations of *Cistus salviifolius* (Farley and McNeilly 2000). This implies that variability in traits relevant for persistence in fire-prone ecosystems and in those unrelated to fire may respond to processes that are acting at different spatial scales.

Despite our results are based on only two species, these two species are representative of the woody species with fire-dependent recruitment. The two species are common and abundant in the whole Mediterranean Basin, they belong to different (and very common) plant families (Cistaceae and Lamiaceae) and they embrace the two main fire-stimulated germination mechanisms (i.e. heat-and smoke-stimulation). To what extent these results can be generalised

to species with different post-fire strategies (i.e. with no fire-dependent recruitment as obligate resprouters) remains to be explored.

Our results suggest that traits vary most at the same scale as the process that most affects them. In fire-prone ecosystems and for traits relevant for persistence in these ecosystems, fire is probably the main driver of the intraspecific variability observed in these traits, overriding any possible variability at the larger scale. This confirms the preponderant role of fire in shaping species and biodiversity in fire-prone ecosystems (Pausas and Keeley 2009; Keeley et al. 2011).

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Appendix

Table S1. Mean seed weight (SW, mg), seed dormancy (Dorm, non-germinated seeds in control conditions, %) and germination (%) after heat treatments (100°C, 120°C, and 150°C for 5 minutes), smoke treatments (S) and heat plus smoke treatment (H₁₀₀+S) with their corresponding control treatments (C), for each population (Pop) and species (*Cistus salviifolius* and *Lavandula stoechas*). Differences in germination between treatments and respective controls were evaluated using a GLM. ns: non-significant, * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001). For each species and region (Iberia and Anatolia) it is also shown the mean (and standard deviation, SD) of seed weight (mg) seed dormancy and of germination stimulation after each treatment (differences in germination between treatment and control, %).

Pop	SW (mg)	Dorm	C	Treatment					
				Heat 100°C	Heat 120°C	Heat 150°C	Smoke C	Smoke S	Heat + Smoke H ₁₀₀ +S
<i>Cistus salviifolius</i>									
IB1	1.0967	98	2	28****	84****	97****	7	6 ns	36****
IB2	1.1347	90	10	18 ns	90****	96****	22	11 **	19 ns
IB3	0.8011	96	4	56****	87****	100****	8	8 ns	51****
Mean (sd)	1.01 (0.18)	94.0 (4.4)		28.0 (22.3)	81.0 (2.0)	91.7 (5.9)		-4.0 (6.1)	23.0 (23.6)
AN1	0.8547	93	7	91****	86****	92****	6	8 ns	60****
AN2	1.2865	97	3	10 **	52****	99****	2	3 ns	4 ns
AN3	1.1524	90	10	32****	91****	99****	5	14 **	20****
Mean (sd)	1.09 (0.22)	93.3 (3.5)		37.7 (40.8)	69.7 (17.9)	90.0 (5.6)		4.0 (4.3)	23.7 (27.1)
<i>Lavandula stoechas</i>									
IB1	0.7045	46	54	50 ns	68 **	24****	51	99****	95****
IB2	0.8212	65	35	52***	59****	41 ns	33	95****	93****
IB3	0.6096	44	56	69 **	76****	26****	3	99****	91****
Mean (sd)	0.70 (0.11)	51.7 (11.6)		8.7 (11.1)	19.3 (5.0)	-18.0 (20.8)		48.7 (29.7)	44.0 (16.0)
AN1	0.7267	83	17	39****	69****	75****	19	100****	100****
AN2	0.8261	48	52	66 *	96****	54 ns	85	100****	100****
AN3	0.7068	52	50	50 ns	87****	97****	30	99****	99****
Mean (sd)	0.75 (0.06)	60.3 (19.7)		12.0 (11.1)	44.3 (7.5)	35.7 (29.7)		55.0 (35.2)	55.0 (35.2)

Chapter 9

Fires enhance flammability in *Ulex parviflorus*

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Introduction

Wildfires have been occurring in terrestrial ecosystems since the early evolution of plants (Bowman et al., 2009; Pausas and Keeley, 2009; Bond and Scott, 2010) and have shaped many species and ecosystems worldwide (Keeley et al., 2011). Plant flammability is an important driver of fire in terrestrial ecosystems and thus has a fundamental role in ecosystem dynamics and species evolution (Belcher et al., 2010; Bond and Scott, 2010; He et al., 2011). Many traits enhance plant flammability (Cornelissen et al., 2003), including structural traits such as high surface area to volume ratio and retention of standing dead biomass (Papió and Trabaud, 1990, 1991; van Wilgen et al., 1990; Schwilk, 2003), and chemical traits, such as high cellulose:lignin ratio and high levels of volatile compounds and waxes (Philpot, 1970; Rundel, 1981; Dimitrakopoulos and Panov, 2001; Alessio et al., 2008). Indeed, it has been suggested that flammability-enhancing traits have evolved to maintain fire-prone ecosystems. This hypothesis was first proposed at the community level, where it was stated that “fire-dependent plant communities burn more readily than non fire-dependent communities because natural selection has favoured development of characteristics that make them more flammable” (Mutch, 1970). However, this hypothesis was criticized, mainly for being group selectionist and for lacking an explanation on how increased flammability of an individual would increase its fitness (Snyder, 1984; Christensen, 1985; Troumbis and Trabaud, 1989). Later, the hypothesis on the evolution of flammability was reformulated at the individual level within the framework of inclusive fitness theory (Bond and Midgley, 1995). It suggested that flammability-enhancing traits could be favoured in individuals if the elevated flammability resulted in increased mortality of neighbours, and thus opened up space for recruitment opportunities of the flammable individual offspring (“kill thy neighbour” hypothesis, Bond and Midgley, 1995; also called ‘born-to-burn’ hypothesis, Zedler, 1995). More recent modelling studies have suggested different genetic mechanisms for the evolution of flammability (Kerr et al., 1999; Schwilk and Kerr, 2002). Indeed, the correlation between post-fire regeneration strategy and flammability across different species provides some evidence for the “kill thy neighbour” hypothesis (Keeley and Zedler, 1998; Schwilk and Ackerly, 2001; Cowan and Ackerly, 2010; Saura-Mas et al., 2010; He et al., 2011). However,

rigorous field evidence on whether fire enhances plant flammability within populations of a particular species is lacking.

If natural selection enhances flammability traits in fire-prone ecosystems through an increase in opportunities for post-fire recruitment (Bond and Midgley, 1995), the hypothesis should be better tested in obligate seeder species, that is, in species that lack resprouting ability and for whom post-fire regeneration relies only on germination from the seed bank (Bond and Van Wilgen, 1996; Pausas et al., 2004). This is because obligate seeders have shorter and non-overlapping generations and higher population turnover than resprouters (Verdú et al., 2007), and thus should be more sensitive to fire regime changes. Previous experimental studies on plant flammability in fire-prone ecosystems have focused on differences between species and, in particular, between regeneration strategies (e.g. Cowan and Ackerly, 2010; Saura-Mas et al., 2010), but none has studied the intraspecific variability and its relation to fire regime. In this study, we predict that individuals of seeder species growing in populations subject to recurrent fires will be more flammable than individuals of the same species growing in populations that rarely burn. In addition, because of this directional selection and the different population dynamics in contrasted fire regimes, we also predict that seeder species living in different fire regimes should differ in the variance structure of flammability enhancing traits (Endler, 1986). We tested these predictions in *Ulex parviflorus* (Mediterranean gorse, Fabaceae), a shrub species of the Mediterranean Basin lacking the ability to resprout and whose germination is triggered by fire. We quantified, at the individual level, flammability-enhancing traits in populations with contrasted fire regimes.

Materials and Methods

Species description

Ulex parviflorus Pourr. (Fabaceae) is a thorny perennial shrub that, in mature stages, retains a high proportion of dead biomass in the plant, giving it a high flammability (Baeza et al., 2011). Post-fire regeneration is exclusively by seedling recruitment (i.e. it lacks the ability to resprout; Paula et al., 2009). Seeds have physical dormancy and form a persistent soil seed bank. The heat produced during a fire breaks seed dormancy and stimulates germination in post-fire conditions (Baeza and Vallejo, 2006; Paula et al., 2009; Moreira et al., 2010). However, *U. parviflorus* also recruits in the absence of fire, in open spaces such as old fields (Baeza et al., 2011), making this species an ideal model to test the consequences of living in different selective environments.

Study area

The study populations were located in the Valencia region (Spain, eastern Iberian Peninsula). Using the local government forest fire database (spatially explicit data from 1978), we selected areas with high fire recurrence (two or more fires since 1978) and areas with no fire incidences for a long time. The identification of the latter areas was based on the absence of fire records (since 1978), and the presumably much longer history without fires according to the information from local managers. After a careful field survey, we selected two sites within the high fire recurrence area and two sites within the unburned area in which *U. parviflorus* was abundant (hereafter HiFi and NoFi populations, respectively; Table 1). The four sites were shrublands. NoFi populations grew in old fields, and the recruitment of recent generations was independent of fire (old-field colonization); indeed, *Juniperus phoenicea*, a slow-growing fire-sensitive tree, was present in the two NoFi sites, suggesting a long time since fire and agricultural abandonment. By contrast, HiFi populations were the product of recurrent fires (Table 1), and the recruitment of most individuals was assumed to have been mediated by fire (post-fire regeneration).

All four populations grew on calcareous bedrock, and the slightly different climatic conditions were not related to fire regime; indeed, the climatic conditions of HiFi populations were within the range of the conditions of NoFi populations (Table 1). The mean distance between populations was 66 km; three of the populations were between 12 and 26 km apart, and the fourth (a NoFi population) was between 110 and 115 km from the other three; that is, there was no geographical segregation between HiFi and NoFi populations. The populations might have differed in age. HiFi plants were 10 and 16 yr old (time since last fire; Table 1); we do not know the exact age of the NoFi populations, but, most probably, they were of a similar age to, or older than, HiFi populations (but certainly not younger). Changes in fuel structure with age in *U. parviflorus* are observed mainly at the early stages of plant development; for instance, Baeza et al. (2006) found differences in fuel structure between 3- and 9-yr-old stands, but not between 9- and 17-yr-old stands. That is, in our sites, age is unlikely to have driven differences in whole-plant flammability; moreover, if there was an age effect, it would be expected to be towards higher flammability in NoFi populations (i.e. against our hypothesis). In addition, flammability was tested at the twig level, in the last growing season twigs located at the tips of the branches (see the next section).

Flammability traits

In August (summer) 2010, we selected 40–46 mature individuals in each population (a total of 170 individuals), separated by *c.* 5 m, and, in each individual, we measured plant structural traits and performed flammability experiments in live twigs using an epiradiator.

Plant structure

We estimated plant size in the field by measuring the maximum height and crown diameters (maximum and perpendicular diameters). We also measured the basal stem diameter; in multiple-stemmed individuals, we measured the diameter of each basal stem. We then clipped all the aboveground biomass of each individual and took it to the laboratory, where we fractionated the live and dead plant biomass into fine (< 6 mm in diameter) and coarse (≥6 mm in diameter) portions (fuel classes). All of these fractions were oven dried at 80°C for a minimum of 48 h and immediately weighed. From these data, we estimated, for each individual, the proportion of dry biomass of the different fuel classes (%) and the plant bulk density (i.e. plant dry biomass per volume, g cm).

Twig flammability

Flammability tests were performed for each individual on 13 twigs selected in the laboratory immediately after sampling. All selected twigs were live green twigs of a similar size (ranging from 4.5 to 6.0 cm in length) and corresponded to the last growing season (tips of the branches). Plant moisture content is known to influence flammability (e.g. Gill et al., 1978; Alessio et al., 2008; Plucinski and Anderson, 2008; Saura-Mas et al., 2010); to eliminate differences in plant moisture that could have been induced by environmental variability between sites during sampling, twigs were hydrated for 24 h at 5°C in dark conditions and then allowed to balance to controlled conditions for 24h (24°C in dark conditions and 60% humidity in a growth chamber). Immediately before each test, three of the 13 twigs were weighed and then oven dried (24 h at 70°C) to obtain the dry mass and to calculate twig moisture content (%) at the time of the flammability assay. The remaining 10 twigs were individually weighed (overall mean, 0.40 g; standard deviation (SD), 0.17 g; fresh mass) and used for flammability assays. Some replicates were discarded because of methodological issues (e.g. the twig was not placed correctly in the centre of the epiradiator), and thus the final number of replicates per individual ranged from eight to ten. Flammability assays were carried out in a fume hood using an epiradiator of 500 W (Helios Italquartz, Milan, Italy) with a thermocouple connected to a data-logger (temperature range from -50 to 1100°C; time resolution of 2 s) and placed 8 cm above the heating plate. At this point, the background

temperature (i.e. without any fuel) ranged between 130 and 150°C, and on the surface of the epiradiator between 640 and 660°C. The flammability tests involved placing each twig in the centre of the epiradiator and registering the time to ignition (i.e. time to initiate a flame) and time to flame extinction. The heat released during combustion was then computed as the area under the temperature–time curve during the flame duration divided by the sample fresh biomass ($^{\circ}\text{C s g}^{-1}$), and the mass loss rate as the sample fresh biomass consumed divided by the flame duration (mg s^{-1}). The maximum temperature ($^{\circ}\text{C}$) reached by the flame in each twig was also recorded. A few twigs (2%) failed to ignite; in these cases, the time to ignition was set to the maximum observed value (60 s), which is a conservative estimate.

Table 1. Location, altitude, average climatic conditions (P_{ann} , annual precipitation (mm); P_{sum} , summer precipitation (mm); T , mean annual temperature ($^{\circ}\text{C}$)), fire years during the period 1978–2010 and fire regime considered (high fire recurrence area (HiFi) and unburned area (NoFi)) for the four studied populations of *Ulex parviflorus*.

<i>Location</i> (<i>province</i>)	<i>Altitude</i> (<i>m asl</i>)	P_{ann} (<i>mm</i>)	P_{sum} (<i>mm</i>)	T ($^{\circ}\text{C}$)	<i>Fire</i> <i>years</i>	<i>Fire regime</i>
Ares del Maestrat (Castelló)	820	760	116	14.4	None	NoFi
Cheste (Valencia)	170	422	65	17.7	None	NoFi
Sot de Chera (Valencia)	775	600	78	14.2	1978, 1986, 1994	HiFi
Chiva (Valencia)	800	553	60	15.0	1990, 1994, 2000	HiFi

Statistical analysis

The time to ignition (s), heat released ($^{\circ}\text{C s g}^{-1}$), mass loss rate (mg g^{-1}), maximum temperature ($^{\circ}\text{C}$) and moisture (%) were averaged at the individual plant level. We first compared these four twig flammability variables, plus plant bulk density and biomass fractions, between plants living in populations under different fire regimes (HiFi vs NoFi). Because the measured parameters related to twig flammability were significantly related to twig moisture at the time of the flammability test, this variable was included as a covariate in the model when testing the effect of fire regime. Statistical comparisons were performed with a linear mixed-effects model using population as a random factor, and the significance of twig moisture content and fire regime was obtained using a likelihood ratio test (LR; $\text{df} = 1$). For the traits that were significant, we also compared the variance between fire regimes (HiFi vs NoFi). To do this, we compared the observed variance in each fire regime with the distribution of variances generated by resampling the data and estimating their variance 1000

times (bootstrap replications). Because twig flammability depends on twig moisture, variance values for each trait were computed from the residuals against moisture. If the observed variance falls in the lower tail ($P < 0.05$), the variance is considered to be lower than the null expectation; if it falls in the upper tail ($P > 0.95$), it suggests that the variance is greater than expected; otherwise ($0.05 < P < 0.95$), variance values are not significantly different from the null (random) expectation.

Results

The above-ground plant biomass (dry weight) of the studied individuals varied considerably (5% quantile, 232.8 g; 95% quantile, 1877 g; mean, 855 g; SD, 535 g), but was not significantly different between HiFi and NoFi populations (LR = 0.26, $P = 0.61$). Similarly, the area of basal stems did not differ between fire regimes (LR = 0.107, $P = 0.744$). On average, individual plant biomass was quite evenly distributed in the following three fractions: coarse live (33.6%; SD, 11%), fine live (29.8%; SD, 13%) and fine dead (35.6%; SD, 10%); however, these fractions were very variable among individuals (ranging from $< 15\%$ to $> 60\%$); dead coarse biomass always represented a small fraction of the total biomass (mean, $< 1\%$). None of these fractions were significantly different between fire regimes (LR < 2 , $P > 0.10$). By contrast, bulk density (g cm^{-3}) was significantly higher in HiFi than in NoFi (LR = 5.5, $P = 0.019$; Fig. 1).

For most individuals, all twigs tested ignited correctly; however, for 15 individuals (9%), some of the twigs (1–5; mean, 2) did not ignite. Most of these individuals (14 of 15) were from NoFi populations ($\chi^2 = 12.25$, $P < 0.001$). The mean twig moisture of each individual at the time of the flammability tests varied greatly (mean, 56.8%; SD, 21.8%; 5% quantile, 20.9%; 95% quantile, 84.1%), but was not different between the fire regimes (LR = 0.04, $P = 0.83$). Moisture content was positively related to time to ignition (LR = 110.6, $P < 0.0001$; Fig. 2a) and mass loss rate (LR = 29.18, $P < 0.0001$; Fig. 2b); that is, samples with high moisture content took longer to dry out and ignite, and, because the mass remaining was lower (a high percentage of the total weight was water), they burnt more quickly. By contrast, the moisture content was negatively related to the heat released (LR = 118.42, $P < 0.0001$; Fig. 2c) and maximum temperature (LR = 58.82, $P < 0.0001$; Fig. 2d); that is, the higher the moisture content of the samples, the lower the heat released and maximum temperature attained. Considering moisture content as a covariate, plants from HiFi populations ignited earlier (LR = 8.72, $P = 0.0032$; Fig. 2a), burned more slowly (LR = 7.10, $P = 0.0079$; Fig. 2b) and released more heat (LR = 5.76, $P = 0.0164$; Fig. 2c) than plants from NoFi populations.

The maximum temperature reached during the flammability tests was marginally higher in plants from HiFi populations than in those from NoFi populations (LR = 2.8, P = 0.09; Fig. 2d).

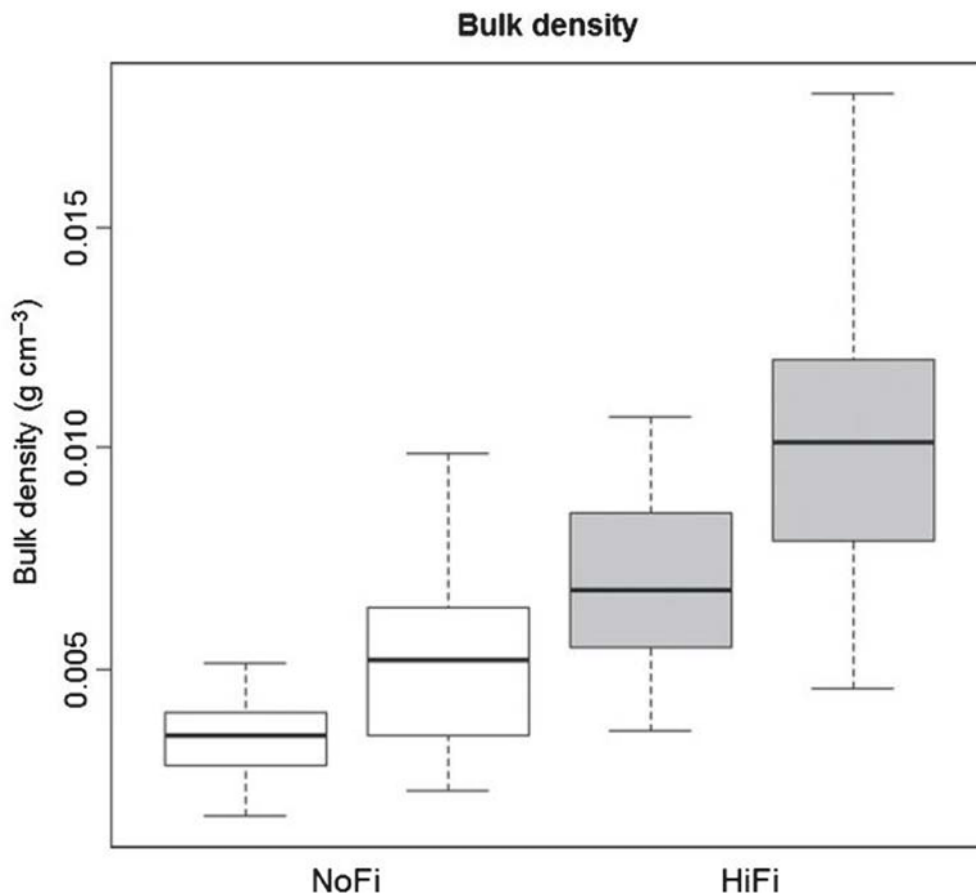


Figure 1. Bulk density in the four studied plots located in the two fire regimes (high fire recurrence area (HiFi) and unburned area (NoFi)); differences between the fire regimes are significant (likelihood ratio (LR) = 5.5, P = 0.019).

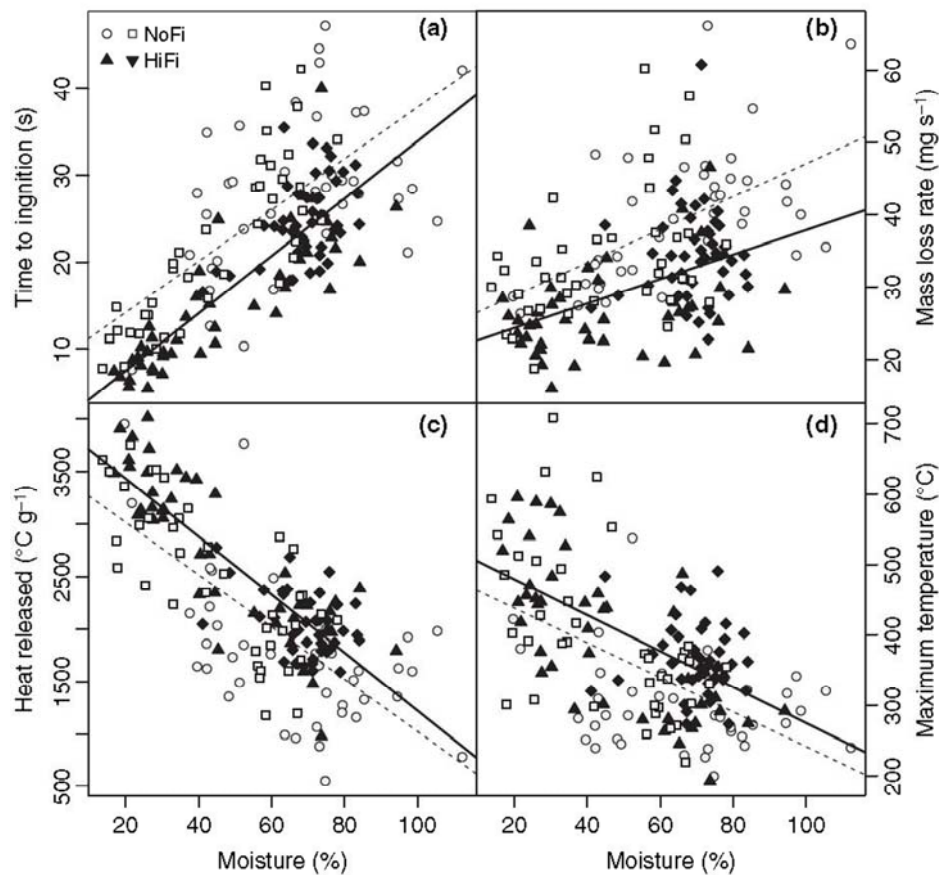


Figure 2. Time to ignition (a), mass loss rate (b), heat released (c) and maximum temperature (d) in relation to twig moisture content for plants growing in different fire regimes. Different symbols refer to different populations, and different colours refer to different fire regimes (high fire recurrence area (HiFi), black symbols and continuous lines; unburned area (NoFi), white symbols and dashed lines). In all cases, the moisture content was significant ($P < 0.001$); once accounting for the differences in moisture, the differences between fire regimes were also significant (a–c: $P = 0.0032$, $P = 0.0079$, $P = 0.0164$) or marginally significant (d, $P = 0.09$); interactions were not significant in any of the cases.

Flammability-enhancing traits also showed different variance structure between fire regimes. In the four twig flammability traits, the variances were lower in HiFi than in NoFi populations (Table 2). Moreover, for these traits, HiFi populations showed significantly lower variance than the null expectation, whereas trait variances in the NoFi populations were no different or were greater than the null expectation (Table 2). Variance in bulk density did not show any trend with fire regime. The same results were obtained when using the raw data instead of the residuals against moisture (not shown).

Table 2. Variance of the five studied flammability-enhancing traits in the two fire regime scenarios (high fire recurrence area (HiFi) and unburned area (NoFi)).

Trait	HiFi		NoFi	
	Variance	P	Variance	P
Time to ignition	19.26	<0.001	50.09	0.969
Mass loss rate	44.37	0.024	62.85	0.469
Heat released	127048	<0.001	306330	0.996
Maximum temperature	4575.8	0.005	7747.9	0.972
Bulk density	0.000021	0.336	0.000015	0.166

For the twig traits (i.e. time to ignition, mass loss rate, heat release and maximum temperature), the values represent the variance of the residuals in the regression against moisture (Fig. 2). $P < 0.05$ represents significantly lower variance than expected by chance; $P > 0.95$ represents significantly greater variance than expected by chance; otherwise ($0.05 < P < 0.95$), variance values are not significantly different from the null (random) expectation.

Discussion

Plants of *U. parviflorus* growing in recurrently burnt populations (HiFi) are more flammable than those growing in populations that have not suffered any fire (NoFi, i.e. populations established in old fields by colonization). Specifically, plants from HiFi populations ignite more quickly, burn more slowly and release more heat than NoFi plants. In addition, these plants have higher bulk density which, in *U. parviflorus* (Santana et al., 2011), as well as in other shrub species (Bradstock and Auld, 1995), is associated with higher temperatures in the soil during a fire. All of these observations support the hypothesis that recurrent fires enhance plant flammability. Enhanced flammability is probably selected to ensure the elimination of neighbours, allowing the success of offspring recruited from the seed bank (Bond and Midgley, 1995; Midgley and Bond, 2011). Indeed, in this species, the heat released during fires breaks seed dormancy and stimulates germination (Moreira et al., 2010). These results support the hypothesis proposed by Mutch (1970) and Bond and Midgley (1995) on the evolutionary role of flammability. To what extent the variability in flammability (and fire intensity) is associated with enhanced post-fire recruitment, as predicted by the ‘kill thy neighbour’ hypothesis (Bond and Midgley, 1995), remains to be explored. However, there is evidence of higher seedling recruitment under increased fire intensity for a range of Mediterranean ecosystems, including South Africa (Bond et al., 1990), California (Moreno and Oechel, 1991; Schwilk, 2003; Keeley et al., 2005) and Australia (Knox and Clarke, 2006). In addition, future genetic analysis evaluating the heritability of flammability enhancing traits would be needed to fully test the ‘kill thy neighbour’ hypothesis.

The high proportion of dead standing biomass observed in these populations of *U. parviflorus* (ranging from 16% to 65% of the total biomass; mean, 36%) is within the range observed in previous studies for mature populations in this species (Baeza et al., 2006, 2011). This trait was highly variable among individuals and populations, and thus was not significantly different between fire regimes. That is, although dead standing biomass confers high flammability to plants (Schwilk, 2003), we did not find a consistent variability to indicate that this trait is under selection by fire in *U. parviflorus*. Our results suggest that the increased flammability in *U. parviflorus* populations subjected to recurrent fires is caused by changes in plant structure (e.g. plant bulk density and twig structure) or chemical composition, rather than by changes in dead standing biomass. These results also have management implications as they challenge the efficiency of recurrent prescribed burns for fuel reduction in *U. parviflorus* shrublands.

Previous studies have suggested that recurrent fires act as a community filter, precluding some species from entering the community, depending on their traits (phenotypic clustering; Verdú and Pausas, 2007; Pausas and Verdú, 2008; Ojeda et al., 2010). Here, we demonstrate that recurrent fires also act at the population level, structuring intraspecific variability of flammability-enhancing traits. The lower trait variability in recurrently burned populations may be a result of historical effects (in situ post-fire regeneration vs colonization) or of a directional selection (Falconer, 1981; Endler, 1986); with our data, we cannot disentangle these two possibilities. Our results support the idea that fire is an evolutionary pressure shaping plant traits (Keeley et al., 2011), and provide field evidence suggesting that natural selection acts on flammability traits in such a way that recurrent fires enhance plant flammability.

It could be argued that the phenotypic differences observed between populations may be related to environmental conditions, plasticity or biogeographical patterns. However, this is quite improbable because differences in environmental conditions do not follow the observed differences in flammability (Table 1). For instance, populations at the highest and lowest altitudes have both been regenerated by old-field colonization (NoFi; Table 1) and are the populations with the lowest flammability. Furthermore, the four populations do not exhibit geographical aggregation following the different fire regimes; indeed, one of the NoFi populations is c. 110–115 km from the other three populations, and the other three populations are 12–26 km apart; thus, biogeographical differences between NoFi and HiFi should be ruled out. Although some flammability parameters are expected to be correlated

(e.g. maximum temperature and heat released), most of the studied flammability-enhancing traits are not directly linked to each other (e.g. time to ignition, heat released, bulk density), and thus we found different and independent lines of evidence towards more flammability in HiFi populations. Plant age can also be discarded as a driver of our results because: (1) in this species, age-related changes in flammability traits are observed at younger age classes than those considered here (Baeza et al., 2006; see also the Materials and Methods section); (2) the basal area is probably a good indicator of plant age, at least in species that do not resprout, and we found no differences in basal area among our populations; and (3) flammability differences between fire regimes were observed not only at the whole-plant scale (bulk density), but also at the scale of the twigs (last growing season twigs). Therefore, the most parsimonious explanation for the higher flammability and lower trait variability in HiFi populations is the origin of the populations (post-fire vs colonization) and the associated fire history. Flammability-enhancing traits have also been linked to different fire regimes in *Pinus* species (Keeley and Zedler, 1998). Recent studies have demonstrated that changes in flammability are associated with changes in the fire regime during the Triassic–Jurassic boundary (Belcher et al., 2010), with the spread of angiosperms in the Cretaceous (Bond and Scott, 2010) and with the diversification of some species (He et al., 2011). All of these studies place flammability as a fundamental trait in plant evolution.

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Chapter 10

Flammability as a biological concept

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'No description of the variability and predictability of the environment makes sense without reference to the particular range of scales that are relevant to the organism or processes being examined.'

Levin (1992)

Recurrent fires enhance flammability

Studies examining plant flammability descriptors as fire-adaptive traits (e.g. Schwilk, 2003; Scarff and Westoby, 2006; Cowan and Ackerly, 2010; Saura-Mas et al., 2010; Pausas et al., 2012) are normally formulated within the framework of inclusive fitness theory. In such a framework, flammability-enhancing traits are considered to favour individuals if the elevated flammability confers fitness benefits. In post-fire seeder species (i.e. those with fire-stimulated germination from a persistent seed bank), higher flammability could increase the recruitment opportunities for the offspring of the individual with enhanced flammability by increasing the chance of opening spaces and by producing the necessary cues for triggering germination from the seedbank (“kill thy neighbour” hypothesis; Bond and Midgley, 1995). This might be particularly relevant in non-resprouting (obligate) seeders with strong spatial population structure (e.g., with short-distance dispersal). Because there is some evidence of heritability for both seed dormancy (e.g., Baskin et al., 2000; Huang et al., 2010) and flammability-enhancing traits (e.g., Sampedro et al., 2010), we would expect a selection for higher flammability with repeated fires.

In a recent paper, we provided evidence that individuals of an obligate seeder species (*Ulex parviflorus*, Fabaceae; a shrub from the Mediterranean Basin) growing in populations recurrently burnt (HiFi populations) were more flammable than individuals of the same species in populations arising from old-field colonization that did not suffer any fire (NoFi populations, i.e. with fire-independent recruitment; Pausas et al., 2012). Specifically, twigs of plants from HiFi populations ignited quickly, burnt slowly and released more heat than twigs of NoFi plants. In addition, HiFi plants had higher bulk density than NoFi plants. Previous

studies have showed that bulk density in *U. parviflorus* (Fig. 1), as well as in other shrub species (e.g. Bradstock and Auld, 1995; Tachajapong et al., 2008), is associated with higher temperatures and longer residence time of high temperatures in the soil during a fire. Thus, the results at the twig and the whole-plant scale were in agreement and suggested that HiFi plants should ignite easily and reach higher temperatures and produce higher heat doses in the soil than NoFi plants (Fig. 1). This higher probability of ignition and higher heat in the soil would increase the chance of recruitment of *U. parviflorus* from the soil seed bank by opening spaces and by enhancing seedling emergence (the heat shock from fire breaks seed dormancy and stimulates germination in this species; Baeza and Vallejo, 2006; Moreira et al., 2010). Thus, these results are in agreement with the “kill thy neighbour” hypothesis.

By including our data on *U. parviflorus* bulk density in a fire behaviour model, Fernandes and Cruz (2012) predict lower fire spread rates in HiFi populations, which implies higher fire residence time and thus higher heat dose in the soil and in the seed bank (Bradstock and Auld, 1995; Gagnon et al., 2010). Their simple modelling approach inadvertently provides further support for our results, although a modelling framework accounting for variability and uncertainty would have been much more appropriate. That is, the conclusions by Pausas et al. (2012) remain firm: in *U. parviflorus* shrublands there is a divergence on flammability traits between populations living in different selective environments, and the mechanism by which plant fitness would be enhanced is driven by the increase in both the probability of ignition and the heat released to the soil.

Diversity of paradigms, metrics and scales

Fernandes and Cruz (2012) criticize flammability experiments performed in laboratory conditions because they are not “adequate surrogates for real-world, full-scale fire behaviour and dynamics”, neglecting that predicting real-world broad-scale fire behaviour is not necessarily the objective of all flammability studies. Fire has effects at a diverse array of scales and the appropriate metric at which flammability should be measured depends on the scale and on the objective of the study (Levin, 1992). For instance, the durations of soil heating as well as maximum temperatures (Fig. 1) are closely tied to biological processes such as post-fire resprouting and seed regeneration (Beadle, 1940; Auld and O’Connell, 1991; Schwilk, 2003; Vesik et al., 2004; Paula and Pausas, 2008). However, even though fireline intensity might be a useful metric for modelling fire behaviour, it is often not predictive of soil heating because heat is mainly transferred upwards (Hartford and Frandsen, 1992; Bradstock and Auld, 1995). This is why the use of fire line intensity has been discouraged for

describing fire effects (Keeley, 2009). On the contrary, the rate of fire spread is related to soil heating, and the relationship is negative because faster fires tend to burn both with shorter residence times and higher above the ground than slower-moving fires (Bradstock and Auld, 1995). In fact, enhancing fire spread rates has been suggested as a mechanism for fire protection in plants (Gagnon et al., 2010).

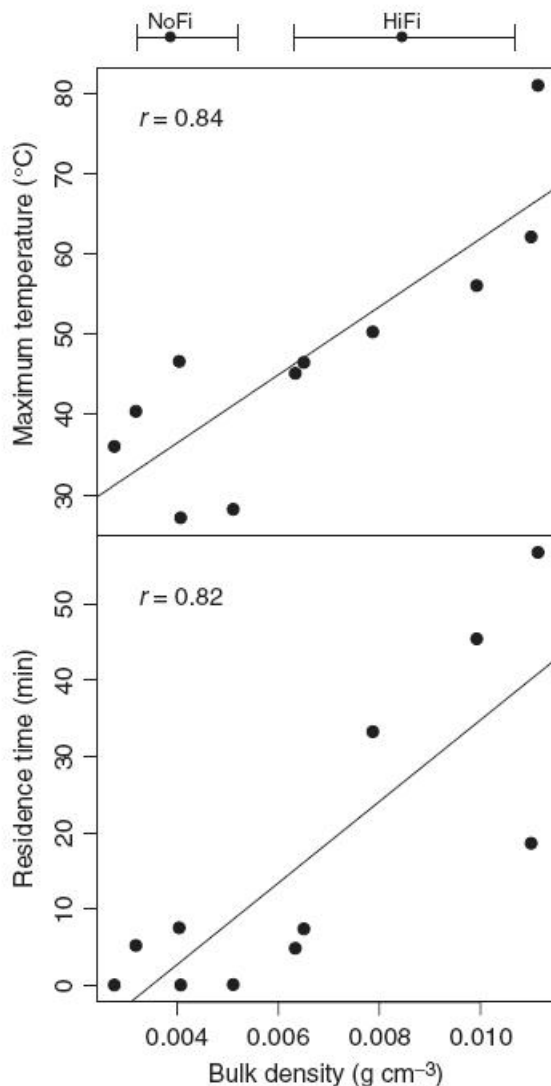


Figure 1. Relation between bulk density (in g cm^{-3}) of different individuals of *Ulex parviflorus* and maximum temperature ($^{\circ}\text{C}$) and residence time above 40°C (in min) in the soil profile (1 cm depth) during experimental fires in the Valencia region, eastern Spain (Santana et al., 2011). Regressions are significant at $P < 0.002$. The segments on the top represent the median and the 25th and 75th percentiles of the bulk density values observed for NoFi (old-field colonization that did not suffer any fire) and HiFi (recurrently burnt) populations in Pausas et al. (2012).

Modelling fire behaviour requires detailed information of the spatial structure of the fuel bed in the landscape at a given time. Unfortunately, the simulation by Fernandes and Cruz (2012) does not consider parameters related to the structure of the ecosystem (e.g. flammability of the coexisting species, the size, cover density and spatial distribution of dead and live individuals, etc.) and thus their fire spread estimates may be unrealistic at the community and landscape scales. In contrast to fire behaviour modelling, ecological and evolutionary studies of flammability are mainly performed at the individual level and considering long-term processes (fire regimes), because natural selection (and the genetic control) acts on individual trait variation. Indeed, most of these studies perform standardized flammability measurements

in leaves or small branches in controlled laboratory conditions (e.g. size or mass and moisture of the sample are standardized). Although burning full individuals in field conditions could conceptually be a better approach than working at a small scale in the laboratory, the dimensions and logistics of such experiments and the difficulties of making standardized measurements limit the use of this approach. In addition, variability between whole individuals in the field might have various causes that are not genetically controlled, rendering them less informative for an evolutionary analysis. An extreme case may be illustrative: the number and cover of dead individuals in a community are important for modelling fire behaviour; however, these values might be of limited relevance for understanding evolutionary processes because they are not genetically controlled (mortality may be distributed among different species and depends on many other factors such as droughts, pests, age, competition, etc.).

Some species enhance their flammability by retaining dead biomass (Keeley and Zedler, 1998; Schwilk, 2003). For instance, the retention of dead biomass is an omnipresent trait in *U. parviflorus* (Pausas et al., 2012). While the presence of this trait is probably genetically controlled (e.g., see references on architectural traits in many horticultural plants), the amount of dead biomass retained is unlikely to have a genetic basis, as many other local factors (e.g. water availability, micro topography, light incidence) are involved. Consistently, when analysing this trait in *U. parviflorus* populations with contrasted fire regime, we found high individual variability in standing dead biomass and no differences between fire regimes (Pausas et al., 2012). Flammability is a complex trait that can be defined in different ways (e.g., probability of ignition, heat released, temperature reached, velocity of combustion, fuel structure, chemical composition, etc.) and measured at different scales (from leaves to landscapes). Because all these indicators are not necessarily correlated, the simple use of the term flammability can generate some confusion. However, the results become clear when the flammability indicators are viewed in detail and at the scale that is relevant to the organism or process being examined (Levin, 1992).

Research directions

Fire science is multidisciplinary and researchers need to recognize the diversity of approaches and the importance of evolutionary biology. We definitely need experimentation in laboratories, even if they are (necessarily) reductionist, as is most experimental biology. Understanding the evolution of flammability is extraordinarily complicated and laboratory experiments can be considered a first step in sorting out this biological phenomenon. As our

methods and technology advance, we will perhaps be able to account for the whole phenotypic variability, but currently we are forced to work at smaller scales, and risk being criticized for being reductionist. Experimentation cannot be replaced by computer models in which assumptions are embedded in the code; it is a basic tool in science and useful for the understanding of many biological processes. Laboratory experiments are also useful for calibrating models that are later used for fire modelling to test alternative management scenarios. Models can be a useful tool for scaling up laboratory experiments, because these experiments are performed at the scale of selection (individual level), but fire also spreads through communities and landscapes. Fire research and landscape management have historically been based only on the physical paradigm of fire (i.e. fuel management and fire behaviour modelling). This physical framework has yielded remarkable results, yet it has failed to provide an integrative view of how fire shapes nature and how fire-prone ecosystems should be managed for a sustainable world. Only by considering the complementarity of the different disciplines (physics, forestry, ecology, evolution, genetics, etc.) can fire science make a significant advance in land management (Pyne, 2007).

Current trait-based flammability studies might have limitations for predicting broad-scale fire behaviour (but see Schwilk and Caprio, 2011). However, they contribute to understanding the role of fire in generating trait divergence, fire adaptations and, ultimately, species persistence in fire-prone ecosystems (Keeley et al., 2011; Pausas and Schwilk, 2012). Fire modelling studies also have limitations, but contribute to understanding fire behaviour and might be valuable for fire management. Although the flammability concept is useful to foresters and engineers for predicting fire risk and modelling fire behaviour, it is a biological trait and its origin and variability are determined by biological processes. In fact, flammability is now becoming a key factor for understanding plant evolution (Keeley and Rundel, 2005; Bond and Scott, 2010; He et al., 2011, 2012). With the recent advent of new generation sequencing and genotyping methods, we are closer to the possibility of genotyping whole populations with highly polymorphic markers to infer relatedness among individuals in the field, and thus to fully test the “*kill thy neighbour*” hypothesis. In addition, the emerging trait-based discipline of community genetics and the concept of the extended phenotype (Whitham et al., 2003) may provide an appropriate evolutionary framework for linking leaf traits and community flammability. Indeed, community genetics can potentially make a significant contribution to fire ecology (Wymore et al., 2011). Current studies linking traits and flammability at different scales (Schwilk and Caprio, 2011; Pausas et al., 2012) are contributing to build this promising

approach. This is a very exciting time for fire ecology; we hope that fire modelling and landscape management will also benefit from these new emerging ideas.

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Chapter 11

Discussion

Traditionally, wildfires have been considered a recent perturbation (from the Quaternary), tied to human presence, and an ecological disaster. However, wildfires have been occurring in terrestrial ecosystems since the early evolution of plants (Bowman et al. 2009, 2011; Pausas and Keeley 2009; Bond and Scott 2010) and have shaped many species and ecosystems worldwide (Keeley et al. 2011). They are important not only generating landscape dynamics and heterogeneity (Sousa 1984; Pausas 1999; Pausas and Lloret 2007) but also imposing a strong evolutionary pressure on plant traits (Pausas and Keeley 2009; Keeley et al. 2011). Indeed, in fire-prone ecosystems, evolutionary pathways (Pausas and Verdú 2005), genetic diversity (Dolan et al. 2008; Schrey et al. 2011) and community structure (Verdú and Pausas 2007) are strongly determined by fire history. Altogether, we have now strong evidence that many of the traits with adaptive value for persistence in fire-prone ecosystems might have evolved by natural selection driven by fire (Keeley et al. 2012). In addition, intraspecific variability is also now considered of paramount importance for predicting species dynamics (Bolnick et al. 2011) and the response to environmental changes (Ramírez-Valiente et al. 2010), as well as to understand evolutionary processes (Pausas et al. 2012, Pausas and Moreira 2012). This is especially evident in fire-prone environments, where the regime of a strong disturbance such as fire is very sensitive to global change drivers (Dubinin et al. 2011, Pausas and Fernández- Muñoz 2012).

This thesis has focused on traits that plants living in fire-prone ecosystems have acquired throughout evolution, trying to incorporate the importance of intraspecific variability in these traits. Specifically, it has given particular attention to traits that allow plants to persist in these fire-prone ecosystems and thus have an adaptive value. These traits are the capacity to resprout, the post fire seedling recruitment (seed resistance and fire-stimulated germination) and flammability.

The ability to resprout has often been considered as a binary trait (yes/no). Despite the fact that this dichotomous classification of species into resprouters and non-resprouters accounts for a high proportion of the interspecific variability in resprouting, it overrides the great intraspecific variability in this trait. Different studies have related resprouting variability to factors such as disturbance regime, and spatial, environmental or ecotypic variability (e.g., Keith 2002, Cruz et al. 2003). However, for a given disturbance and environmental condition,

intraspecific variability in resprouting is determined by both the stored (pre-disturbance) resources, and the initial (post-disturbance) resource acquisition capacity. Stored reserves are important for initiating resprout and for the initial growth (first for maintaining root and buds alive and subsequently for producing new leaves and shoots). However, once plants have resprouted (using the stored reserves), they rely on the initial post-disturbance capacity to acquire resources (i.e. resprouting vigour) for surviving. Intraspecific variability in resprouting might play a key role in determining the fate of resprouting plant species in a world with continuous changes in the disturbance regime (**Chapter 2**).

It is also likely that obligate resprouters (R+P-) and facultative species (R+P+) exhibit differences in their resprouting capacity, following the hypothesis that having one trait that allows post-fire persistence reduces the evolutionary pressure over a second post-fire persistence trait (see below). Thus, it would be expectable that obligate resprouters are more efficient in the different steps of the resprouting process that we have proposed (Chapter 2); the initial resprouting ability, resprouting vigour and post-resprout survival. These differences might be associated with differential levels of stored reserves and initial post-fire growth and will result in differences in the overall resprouting success of species, depending on their strategy and are likely to be more important the stronger is the disturbance pressure (e.g., severity, frequency).

From an evolutionary perspective, there is evidence that resprouting ability is an ancient trait in land plants and, in some lineages, it is likely that it has been maintained by fire. However, despite the importance of this mechanism for persistence in fire prone ecosystems, changes in fire regime (associated with increase in aridity and predictability of high intensity fires) created greater opportunities for the evolution towards post-fire seedling recruitment (either facultative or obligate). In most cases these species have changed their reproductive strategy, capitalizing recruitment into a single post-fire pulse. Seeder species have several traits that allow the success of this mechanism. Annual seed production is mostly accumulated in a seedbank (in the canopy or in the soil) because seeds are dormant. In addition, seeds resist the heat produced by fire and, frequently, germination is stimulated by fire-related germination cues (e.g., heat and smoke).

Some species might display a gradual loss of seed dormancy over time to take advantage of occasional recruitment opportunities but maintain a considerable seed bank as a bet-hedging mechanism. This results in a large commitment of seeds to take advantage of the favourable

post-fire conditions and a smaller commitment for recruitment over multiple seasons, between fires. Indeed, physical seed dormancy might have evolved in such a way that the temperature threshold associated with the dormancy release is above the maximum summer temperatures (Ooi et al. 2012). This ensures the maintenance of a persistent seed bank until a fire occurs. Despite the fact that some species with physical seed dormancy in fire-prone ecosystems may have a fraction of seeds with fire-independent germination, temperature thresholds that break physical seed dormancy are better explained as a response to fire than as a response to the summer temperatures (**Chapter 3**).

Fire may stimulate germination through different mechanisms, depending on the species or even populations of the same species. Indeed, both heat and smoke are important germination cues for many Mediterranean Basin woody species and the successful post-fire recruitment of these species is due not only to seed resistance to heat shock, but also to the enhanced germination by heat and/or smoke (in both germination percentage and germination rate). Furthermore, seedlings of some species develop faster and thus with greater fitness under post-fire conditions than after other disturbances. Indeed, the chemicals in smoke enhance not only seed germination but also both seedling emergence and establishment. Our results also suggested that the effect of smoke in annual species might be more relevant than previously thought, encouraging further work on the role of annual species for post-fire regeneration in the Mediterranean Basin (**Chapter 4 and 5**). These results point towards that, as in the other Mediterranean ecosystems, fire-cued germination in the Mediterranean Basin is an evolutionary response to the frequent fires. This needs to be considered when addressing evolutionary questions on plant traits at global scale.

In the Mediterranean Basin the pressure exerted by fire did not select plants to have germination in response to the obligatory combination of both fire-related germination cues (**Chapter 6**). This is because the ecological significance of a species having exclusively seeds that germinate when exposed simultaneously to both factors would only be understandable to 1) avoid germination related to very low intensity fires in which conditions for seedling establishment are not improved (e.g., fire does not produce gaps) and/or to 2) avoid dormancy release by summer temperatures. However, 1) it is likely that these fires are not very common in Mediterranean shrublands and 2) Mediterranean species have other mechanisms that minimize the loss of seed dormancy by summer temperatures (chapter 3).

Fire has played a different role shaping germination in different species. Post-fire germination is more efficient in obligate seeders than in facultative species, supporting the hypothesis that having one mechanism that allows post-fire persistence (i.e. resprouting capacity) reduced the evolutionary pressure over a second post-fire persistence mechanism (i.e., post-fire seedling recruitment). Moreover, different lineages have evolved post-fire germination ability, but this mechanism was acquired in response to different fire-related germination cues (smoke- vs heat-stimulated germination) depending on the seedcoat type of the lineage. Although post-fire germination is a response to the evolutionary pressure exerted by recurrent fires, the specific strategies for acquiring this trait depend on historical effects (e.g., ancestral predisposition of the lineage). That is, different species have developed different solutions to the evolutionary pressure exerted by fire, allowing the coexistence in fire-prone ecosystems of species with different seedcoat type (**Chapter 7**).

For a same species, populations living under different fire regimes, present variability in traits that improve their fitness under the particular disturbance regime where they have evolved. Indeed, at a local scale, the heterogeneous burn patterns, together with other local factors such as species interaction and heterogeneity in resource availability, generate variability in fire-related plant regeneration traits (such as seed size, seed dormancy and germination stimulated by fire-related cues). This intraspecific variability in regeneration traits allows species to recruit in different conditions (e.g., under different fire regimes) and thus increases the probability of persistence in fire-prone ecosystems (**Chapter 8**).

It is likely that flammability-enhancing traits have played a key role in the evolution of seeder species. Positive selection for flammability-enhancing traits is only expected if it is associated with other characteristics that confer higher fitness to the individuals possessing those traits. For instance, traits resulting in a higher probability of ignition and higher heat in the soil might increase the chance of recruitment of seeder species from the soil seed bank by opening spaces and by enhancing seedling emergence (the heat shock from fire breaks seed dormancy and stimulates germination in many seeder species, particularly in species presenting physical seed dormancy). Thus, it is not surprising that individuals of obligate seeder species growing in populations recurrently burnt (HiFi populations) were more flammable than individuals of the same species in populations arising from old-field colonization that did not suffer any fire (NoFi populations, i.e. with fire-independent recruitment; **Chapter 9 and Chapter 10**). This presumes a marked individual variability (within each population) of the traits associated with flammability and post-fire seedling recruitment, and a positively correlation between both. As

with flammability, it is likely that seeder species present variability in traits related to post-fire seedling recruitment, between populations under different fire regimes. The most obvious traits which might vary between populations under different fire regimes are seed resistance and stimulated germination by fire-related germination cues (i.e., heat and smoke).

At a genetic level, it is also likely that fire produces a selection pressure that favours individuals with certain genetic variants (that might be associated to increased flammability and post fire seedling recruitment). In addition, fire might also contribute to changes in gene frequencies (a reduction of intra-population genetic diversity and increase in population genetic differentiation). These hypothesis can be validated by comparing phenotypic characteristics related to the fire (traits related to flammability and post-fire seedling recruitment) and the genetic structure, in populations subjected to different fire regime (high vs. low frequency).

With the recent advent of new generation sequencing and genotyping methods, we are closer to the possibility of genotyping whole populations with highly polymorphic makers to infer relatedness among individuals in the field, and thus to fully test multiple hypothesis of fire-driven evolution. In addition, the emerging trait-based discipline of community genetics and the concept of the extended phenotype (Whitham et al. 2003) may provide an appropriate evolutionary framework in which this extended phenotype can be traced from the individuals possessing the trait, to the community, and to ecosystem processes. Indeed, community genetics can potentially make a significant contribution to fire ecology (Wymore et al. 2011).

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Chapter 12

Conclusions

1. For a given disturbance and environmental condition, intraspecific variability in resprouting is determined by both the stored (pre-disturbance) resources, and the initial (post-disturbance) resource acquisition capacity. Stored reserves are important for initiating resprout and for the initial growth (first for maintaining root and buds alive and subsequently for producing new leaves and shoots). However, once plants have resprouted (using the stored reserves), they rely on the initial post-disturbance capacity to acquire resources (i.e. resprouting vigour) for surviving.
2. Some species might display a gradual loss of seed dormancy over time to take advantage of occasional recruitment opportunities but maintain a considerable seed bank as a bet-hedging mechanism. Despite the fact that some species with physical seed dormancy in fire-prone ecosystems may have a fraction of seeds with fire-independent germination, temperature thresholds that break physical seed dormancy are better explained as a response to fire than as a response to the summer temperatures.
3. Both heat and smoke are important germination cues for many Mediterranean Basin woody species and the successful post-fire recruitment of these species is due not only to seed resistance to heat shock, but also to the enhanced germination by heat and/or smoke (in both germination percentage and germination rate).
4. The chemicals in smoke enhance not only seed germination but also both seedling emergence, growth and establishment. The effect of smoke in annual species is also relevant in the Mediterranean Basin.
5. In the Mediterranean Basin the pressure exerted by fire did not select for germination in response to the obligatory combination of both fire-related germination cues (heat and smoke).
6. Post-fire germination is more efficient in obligate seeders than in facultative species. Moreover, different lineages have evolved post-fire germination ability, but this

mechanism was acquired in response to different fire-related germination cues (smoke- vs heat-stimulated germination) depending on the seedcoat type of the lineage.

7. For a same species, populations living under different fire regimes, present variability in traits that improve their fitness under the particular disturbance regime where they have evolved. Indeed, at a local scale, the heterogeneous burn patterns, together with other local factors such as species interaction and heterogeneity in resource availability, generate variability in fire-related plant regeneration traits (such as seed size, seed dormancy and germination stimulated by fire-related cues). This intraspecific variability in regeneration traits allows species to recruit in different conditions (e.g., under different fire regimes) and increases the probability of persistence in fire-prone ecosystems.
8. In *U. parviflorus* shrublands there is a divergence on flammability traits between populations living in different selective environments. Individuals growing in populations recurrently burnt (HiFi populations) are more flammable than individuals of the same species in populations arising from old-field colonization that did not suffer any fire (NoFi populations, i.e. with fire-independent recruitment).
9. The mechanism, by which plant fitness would be enhanced, in plants with higher flammability, is driven by the increase in both the probability of ignition and the heat released to the soil.

Resumen

Introducción general

Los ecosistemas mediterráneos, incluyendo la cuenca Mediterránea, han sufrido una larga historia de incendios (Pausas y Keeley 2009, Keeley et al. 2012). Esta presión recurrente ejercida por el fuego es un factor clave en la evolución de las plantas y en la estructura de las comunidades vegetales. Muchas especies tienen rasgos y mecanismos que les permiten persistir y regenerarse después de un incendio. Por lo tanto, estas características tienen un valor adaptativo y son esenciales para comprender la evolución y la diversidad de especies en las zonas propensas a incendios (Bond y van Wilgen 1996, Bond y Midgley 2001, Keeley et al. 2011, 2012).

En los matorrales y bosques Mediterráneos, que son típicamente compuestos por una vegetación relativamente densa, los incendios son frecuentemente incendios de copa; es decir, el fuego afecta toda la parte aérea de la vegetación (de los distintos estratos; herbáceo, arbustivo y arbóreo), incluyendo las copas del estrato de vegetación dominante. La intensidad y, en general, la severidad de estos incendios es elevada y la gran parte de la vegetación se ve muy afectada por el fuego. Los dos principales mecanismos que permiten la regeneración de las plantas, tras estos incendios, son la capacidad de rebrotar (R) y la capacidad de reclutamiento de plántulas (P) tras el fuego. Estos dos mecanismos de persistencia al fuego se dan a dos niveles jerárquicos diferentes, al nivel individual y al nivel de población, respectivamente (Pausas y Lavorel 2003, Pausas et al. 2004) y permiten la recuperación de los ecosistemas afectados por el fuego. Los individuos de una especie persisten tras el fuego si tienen la capacidad de rebrotar (R+). Si los individuos no persisten (R-), pueden persistir las poblaciones, si estas han formado un banco de semillas que resista al fuego, lo que permite el reclutamiento de nuevos individuos tras el incendio (P+). Estos dos mecanismos de regeneración tras el fuego no son excluyentes, pudiéndose dar los dos a la vez, de manera que las especies se pueden clasificar en cuatro estrategias: especies rebrotadoras obligadas (R+P-), especies germinadoras obligadas (R-P+), especies facultativas (R+P+) y especies sin ningún mecanismo de regeneración post-incendio (R-P-).

La inflamabilidad de la planta también es un mecanismo importante para la persistencia en zonas propensas a incendios. Se considera que los rasgos que incrementan la inflamabilidad favorecen a los individuos si la elevada inflamabilidad les confiere una mayor aptitud o eficacia biológica. En las especies germinadoras, una mayor inflamabilidad puede aumentar

las oportunidades de reclutamiento para la descendencia del individuo con mayor inflamabilidad mediante el aumento de la posibilidad de abrir espacios y por la producción de las señales necesarias para activar la germinación del banco de semillas (hipótesis "kill thy neighbour"; Bond y Midgley, 1995). Además, una mayor inflamabilidad (por ejemplo, medida como un incremento en la velocidad de propagación del fuego) ha sido sugerido como un mecanismo de protección contra el fuego (Gagnon et al. 2010) y podría ser importante, particularmente para especies rebrotadoras.

A pesar de la importancia de estos mecanismos de persistencia al fuego (capacidad de rebrote y reclutamiento de plántulas tras el fuego, y inflamabilidad) en zonas propensas a incendios, tanto para la comprensión de la evolución de las especies como para predecir futuros cambios en el ecosistema, el actual nivel de conocimiento todavía presenta algunas lagunas.

Rebrote

La capacidad de rebrotar tras un incendio, tras la eliminación de toda su biomasa aérea, caracteriza las especies rebrotadoras y es una característica importante para la persistencia en zonas propensas al fuego. El rebrote consiste en la producción de nuevos tallos a partir de yemas protegidas (por ejemplo, por el suelo o por la corteza). Este mecanismo confiere persistencia a nivel individual (y por lo tanto a nivel de la población), ya que parte del individuo (al menos la parte subterránea) resiste el fuego y no muere. Estas especies acumulan reservas de carbohidratos y nutrientes en los órganos subterráneos (que están protegidos por el suelo) y la presencia de estas reservas se ha asociado con la capacidad de rebrote (Bell et al. 1996, Canadell y López Soria-1998, Bell y Ojeda 1999).

Las especies han sido, a menudo, clasificados como rebrotadoras (R+) o no rebrotadoras (R-, Pausas et al. 2004, Veski et al. 2004). Sin embargo, hay evidencias de una cierta variabilidad intraespecífica en este mecanismo (Paula et al. 2009). Además, la información disponible sobre los factores que otorgan la capacidad de rebrote, el vigor de rebrote, o el éxito general del rebrote es muy escasa; dicha información es prácticamente inexistente en especies que, aunque frecuentes, no dominan el paisaje.

Reclutamiento de plántulas

La capacidad de reclutar nuevos individuos (plántulas) después de un fuego caracteriza a las especies germinadoras y es también un mecanismo importante para la persistencia en ambientes con incendios recurrentes. Las especies germinadoras tienen varios rasgos que

permiten el éxito de este mecanismo. La producción anual de semillas es en su mayoría acumulada en bancos de semillas (en la copa o en el suelo) ya que las semillas presentan dormición (ej., física, fisiológica, etc). Además, las semillas resisten al calor producido por el fuego y, con frecuencia, la germinación es estimulada por señales asociados al fuego (por ejemplo, el calor y los compuestos químicos, que resultan de la combustión de la vegetación durante el fuego y presentes en el humo y carbón vegetal).

Las especies germinadoras son a menudo clasificadas como tales por observaciones de emergencia de las plántulas en el campo después del paso del fuego. En muchos casos hay una emergencia masiva de plántulas tras el incendio (con el primer período de lluvias) y el soporte para la clasificación de una especie como germinadora es obvio. Sin embargo, el reclutamiento de plántulas después del fuego es un mecanismo complejo y puede depender de varios factores abióticos (precipitación en otoño/invierno después del fuego, la intensidad del fuego, etc) así como de los rasgos de las plantas. En la Cuenca Mediterránea, las especies germinadoras (P+) han sido casi exclusivamente vinculadas a especies con semillas que presentan una cubierta dura y impermeable al agua (como en la mayoría de las especies pertenecientes a las familias Cistaceae y Fabaceae). Se asume comúnmente que es este tipo de cubierta lo que otorga resistencia al fuego y la estimulación de la germinación (por calor), permitiendo la persistencia a nivel de población. También se ha propuesto que el efecto acumulativo de las temperaturas de verano en el suelo desnudo (por ejemplo, claros en la vegetación) podría tener un efecto similar al fuego (es decir, la dosis total de calor podría ser similar y por lo tanto, tener un efecto análogo sobre la dormición de las semillas). Esto ha llevado a la sugerencia que los umbrales de temperatura asociados a la liberación de la dormición de las semillas (principalmente la dormición física) podrían haber evolucionado independientemente de fuego (Bradshaw et al. 2011).

El efecto de los compuestos químicos, que resultan de la combustión de la vegetación durante el fuego, y están presentes en el humo y carbón vegetal, que es muy relevante en otros ecosistemas mediterráneos (por ejemplo, Sudáfrica, California, Keeley y Bond 1997; Keeley y Fotheringham 1998), solo se ha estudiado muy superficialmente en la Cuenca Mediterránea. De la grande cantidad de compuestos químicos producidos durante un incendio, diferentes compuestos ya han sido aislados y demostrado que estimulan la germinación (por ejemplo, Karrikinas: Flematti et al. 2004, 2005; o Cianohidrininas: Flematti et al. 2011). Esta señal relacionada con el fuego puede tener un efecto significativo en algunas especies, especialmente en especies que tienen semillas con cubierta permeable al agua. Esto es, la

germinación estimulada por el fuego y el reclutamiento de plántulas después de los incendios está relacionada no sólo con el debilitamiento (permeabilización) de la cubierta de la semilla (dormición física), sino también con la inducción de procesos fisiológicos (Keeley 1987, Baskin y Baskin, 1998). De hecho, hay evidencia de emergencia de las plántulas tras el fuego en especies que carecen de semillas con cubierta impermeable al agua y muchas de estas especies son abundantes en zonas propensas a incendios. Sin embargo, la germinación estimulada por los compuestos químicos presentes en el humo sólo puede tener un valor adaptativo en ecosistemas con incendios recurrentes si las semillas también resisten al calor producido por el fuego.

Para entender los mecanismos asociados a la dormición de las semillas y a la germinación y reclutamiento de plántulas tras el fuego, es necesario hacer experimentos (es decir, someter las semillas a diferentes tratamientos; tratamientos que simulen las temperaturas de verano, las temperaturas asociadas al fuego y tratamientos con humo) para cuantificar el grado de resistencia de las semillas al calor y averiguar que señales estimulan la germinación.

Inflamabilidad

Existe evidencia, tanto en la Cuenca Mediterránea como en otros ecosistemas mediterráneos, que las especies germinadoras están más presentes en las zonas con incendios recurrentes que en áreas con baja frecuencia de incendios (Pausas et al. 2004, 2006), específicamente en zonas con alta recurrencia de incendios estas especies están más presentes que lo esperado a partir del acervo de especies regional (Verdú y Pausas 2007). De hecho, se ha sugerido que, en estas especies que se ven favorecidas por el fuego (el fuego incrementa el tamaño de la población), no sólo se han seleccionado los mecanismos de regeneración tras el fuego, pero también rasgos que favorecen la ocurrencia de incendios, tales como rasgos que incrementan la inflamabilidad. Esta hipótesis fue inicialmente formulada a nivel de comunidad (Mutch 1970) y reformulada a un nivel individual (según la teoría de la aptitud inclusiva, hipótesis "*kill thy neighbour*", Bond y Midgley, 1995).

El **objetivo genérico** de esta tesis es profundizar y ampliar nuestro conocimiento en los mecanismos de persistencia al fuego de plantas mediterráneas (es decir rebrote y reclutamiento de plántulas tras el fuego, y inflamabilidad), y entender las consecuencias ecológicas y evolutivas de estos mecanismos, en particular en las especies de la Cuenca Mediterránea.

Los **objetivos específicos** son: 1) identificar los factores que determinan el éxito del rebrote en plantas mediterráneas (por ejemplo, reservas de hidratos de carbono, nutrientes) y comprender los distintos procesos que componen este mecanismo; 2) identificar los factores que determinan la capacidad de germinación en plantas mediterráneas (por ejemplo, el grado de resistencia y / o la estimulación de las semillas por el calor y el humo); 3) determinar la importancia del tipo de cubierta de la semilla (cubierta permeable al agua vs cubierta impermeable al agua) en la resistencia de las semillas y en la estimulación de la germinación por las señales asociadas al fuego (calor y humo); 4) evaluar la variabilidad intra-específica en rasgos asociados a la germinación tras el fuego (por ejemplo, grado de resistencia y/o estimulación de las semillas por el efecto del fuego (calor y humo) y los factores responsables por esta variabilidad; 5) evaluar la variabilidad intra-específica en la inflamabilidad y su correlación con la presión ejercida por el fuego (historial de incendios).

Independientemente de la relevancia del fuego en el origen de los mecanismos de persistencia al fuego, **la hipótesis general** de partida de esta tesis es que el fuego ha contribuido a moldear las especies y ecosistemas mediterráneos mediante procesos de selección natural y filtraje ecológico, y que la adquisición y/o mantenimiento de estos mecanismos de persistencia ha conducido a variaciones en los rasgos de las especies.

La información disponible sobre la magnitud y la variabilidad (intra- e inter-específica) de la capacidad de rebrote y reclutamiento de plántulas tras el fuego, y de la inflamabilidad de las plantas es muy escasa. Entender la variabilidad en los rasgos asociados a los mecanismos que confieren, a las plantas, persistencia al fuego es fundamental para comprender los procesos evolutivos que determinan la estructura y diversidad de las comunidades mediterráneas, y las respuestas a variaciones del régimen de incendios (incluyendo el cambio global).

La capacidad de rebrotar, tras un incendio que ha eliminado toda la biomasa aérea, está relacionada con la presencia de órganos subterráneos que contienen reservas y yemas (Bond y Midgley 2001). Se han encontrado evidencias de diferencias en la presencia de reservas de carbohidratos en órganos subterráneos entre especies rebrotadoras y no rebrotadoras y que este es un factor importante para que se produzca el rebrote (Bell et al. 1996, Bell y Ojeda 1999). Existen también evidencias de otros factores que pueden influir en la capacidad de rebrote de las plantas, como la cantidad de reservas subterráneas de macronutrientes minerales (Canadell y López-Soria 1998). Como consecuencia, es posible que la cantidad de estas reservas (carbohidratos y nutrientes) sea también responsable por la variabilidad intra-

específica en la capacidad de rebrote de plantas adultas de especies de rebrotadoras (Hipótesis 1, Capítulo 2).

Se asume comúnmente que, en la Cuenca Mediterránea, el calor estimula la germinación post-incendio, especialmente en especies con semillas de cubierta dura e impermeable al agua (es decir, Cistaceae y Fabaceae). En cambio, se supone que el humo tiene un papel menos relevante, en la estimulación de la germinación y reclutamiento de plántulas tras el fuego, en comparación con otros ecosistemas mediterráneos, lo que podría sugerir que la estimulación de la germinación por el fuego es menos relevante en la Cuenca Mediterránea, si comparamos con otros ecosistemas mediterráneos (por ejemplo, California). De hecho, se ha sugerido que incluso la dormición física (en las semillas con cubierta impermeable al agua) exhibida por muchas especies mediterráneas puede ser una adaptación a las condiciones de verano de los ecosistemas mediterráneos y no al fuego. Sin embargo, estudios recientes muestran que la estructura de las comunidades vegetales Mediterráneas está fuertemente condicionada por la capacidad de germinar tras el fuego (P). Por consiguiente, es probable que la dormición física presentada por las semillas de especies mediterráneas se explique mejor como una adaptación al fuego que a las condiciones (temperaturas) de verano asociadas al clima mediterráneo (Hipótesis 2, Capítulo 3) y que tanto el calor como el humo asociados al fuego tengan un efecto significativo en el proceso de reclutamiento tras el fuego de las especies de la Cuenca Mediterránea (por ejemplo, en la estimulación de la germinación y en el crecimiento inicial y establecimiento de las plántulas) (Hipótesis 3, Capítulo 4 y Capítulo 5). Además, la respuesta germinativa, de las diferentes especies, al calor y al humo puede depender del tipo de cubierta de la semilla; es decir, semillas con cubierta permeable al agua responden al humo, mientras que las semillas con cubierta impermeable al agua responden al calor (Hipótesis 4; Capítulo 7).

Aunque la germinación tras el fuego, en las plantas de la Cuenca Mediterránea, sea estimulada tanto por el calor como por los compuestos químicos presentes en el humo (hipótesis 3) y que el fuego pueda haber jugado un papel distinto en la germinación de diferentes especies e incluso de diferentes poblaciones dentro de una misma especie (hipótesis 4), es probable que la presión ejercida por el fuego en la Cuenca Mediterránea no haya seleccionado las plantas para tener germinación en respuesta a la combinación obligatoria de ambas señales. Esto es porque el significado ecológico de tener exclusivamente semillas que germinan cuando son expuestas simultáneamente a ambos factores sólo sería comprensible para evitar la germinación relacionada con incendios de muy baja intensidad en que las

condiciones para el establecimiento de plántulas no se ven mejoradas; y estos incendios no son muy frecuentes en matorrales Mediterráneos (Hipótesis 5, Capítulo 6).

En la Cuenca Mediterránea, Pausas y Verdú (2005) han encontrado una correlación evolutiva negativa entre la capacidad de rebrotar (R) y la capacidad de reclutar nuevos individuos después de un incendio (P), lo que sugiere que ha habido una tendencia evolutiva a seleccionar especies con uno de los dos mecanismos de persistencia. La posesión de ambos mecanismos (R+P+, especies facultativas) no parece haber sido favorecida a lo largo de la evolución en la Cuenca Mediterránea (aunque sí bajo otros regímenes de incendios; Pausas et al. 2004, 2006.). Esto puede ser debido a que por el hecho de presentar dos mecanismos de persistencia, la presión de selección sobre cada uno de ellos disminuye. Como consecuencia, la persistencia de las semillas y la estimulación de la germinación por el fuego en especies facultativas (R+P+) debe ser menos eficiente que en especies germinadoras obligadas (R-P+) (Paula y Pausas 2008; Hipótesis 6, Capítulo 7).

Los incendios producen una elevada dinámica y heterogeneidad en el paisaje y los ecosistemas mediterráneos pueden ser vistos como un mosaico con distinta historia de incendios y en distintos estadios de la sucesión (ej., con distinta recurrencia, severidad y tiempo desde el último incendio). A una escala local, la heterogeneidad producida por el fuego, en conjunto con otros factores de importancia local como la interacción entre especies y la heterogeneidad en la disponibilidad de recursos, debe generar variabilidad en los rasgos asociados a la regeneración (ej., tamaño de las semillas, grado de dormición, y magnitud de estimulación de la germinación por factores asociados al fuego). Esta variabilidad debe ser especialmente marcada en especies que tienen el reclutamiento post-incendio como el único mecanismo de persistencia post-incendio (R-P+, especies germinadoras obligadas). Como consecuencia, las especies germinadoras obligadas deben responder a la heterogeneidad espaciotemporal de los ecosistemas mediterráneos propensos al fuego, poseyendo una variabilidad en estos rasgos de regeneración relativamente grande a escala local (dentro de regiones), de tal forma que supera la variabilidad a escalas mayores (biogeográficas). Es decir, en zonas con elevada frecuencia de incendios, los incendios y otros procesos locales que generan variabilidad deben ser más importantes que los procesos biogeográficos en la estructuración de la biodiversidad (Hipótesis 7, Capítulo 8).

Como las especies germinadoras se ven favorecidas por el fuego (el fuego incrementa el tamaño de la población), se ha sugerido que estas especies tienden a ser más inflamables

(Schwilk y Ackerly 2001, Saura-Mas et al. 2010). Esta relación entre la germinación y la inflamabilidad se ha formulado tanto entre especies dentro de la comunidad (Mutch 1970) como entre los individuos a nivel de población (Bond y Midgley 1995), aunque en ambos casos muy pocos estudios han demostrado claramente esta relación. Si la selección natural favorece los rasgos relacionados con la inflamabilidad, los individuos de especies germinadoras, en poblaciones quemadas de forma recurrente, deben tener una mayor inflamabilidad que los individuos de la misma especie en poblaciones con baja presión del fuego (Hipótesis 8; Capítulo 9 y Capítulo 10).

Estas hipótesis se han validado mediante experimentos de rebrote, experimentos de germinación y experimentos de inflamabilidad. El objetivo es estudiar la variabilidad en tres distintos mecanismos de persistencia al fuego; la capacidad de rebrote, la germinación y reclutamiento post-incendio y la inflamabilidad. La tesis se centra mayoritariamente en especies leñosas, dada su importancia y dominancia en los ecosistemas mediterráneos, y porque permite un estudio homogéneo desde el punto de vista de técnicas de muestreo. Los datos actualmente disponibles tienen muchas limitaciones; de este modo, es necesario realizar experimentos con especies y localidades concretas para obtener datos rigurosos, cuantitativos y comparables.

Resultados principales

Esta tesis se ha centrado en rasgos que las plantas que viven en ecosistemas propensos al fuego han adquirido a lo largo de la evolución, intentando incorporar la importancia de la variabilidad intra-específica en estos rasgos. Se ha prestado especial atención a los rasgos que permiten a las plantas persistir en estos ecosistemas con incendios recurrentes y por lo tanto que tienen un valor adaptativo. Estos rasgos son la capacidad de rebrotar, el reclutamiento de plántulas tras el fuego (resistencia de las semillas y estimulación de la germinación por el fuego) y la inflamabilidad.

Rebrote

La capacidad de rebrotar se ha considerado a menudo como un rasgo binario (sí/no). A pesar de que esta clasificación dicotómica de las especies en rebrotadoras y no rebrotadoras incorpora una alta proporción de la variabilidad inter-específica en el rebrote, deroga la gran variabilidad intra-específica en este rasgo. Diversos estudios han relacionado la variabilidad en el rebrote con factores como el régimen de perturbación, y la variabilidad espacial,

ambiental o ecotípica (ej., Keith 2002, Cruz et al. 2003). Sin embargo, para una misma perturbación y condiciones ambientales, la variabilidad intra-específica en el rebrote está determinada tanto por los recursos almacenados (previamente a la perturbación), como por la capacidad inicial (tras la perturbación) de adquisición de recursos. Las reservas almacenadas son importantes para iniciar el rebrote y para el crecimiento inicial (primero para mantener la raíz y brotes vivos y posteriormente para la producción de nuevas hojas y tallos). No obstante, una vez las plantas hayan rebrotado (utilizando las reservas almacenadas), dependen de la capacidad inicial, tras la perturbación, de adquirir recursos (es decir, del vigor de rebrote) para sobrevivir. La variabilidad intra-específica en el rebrote puede desempeñar un papel clave en determinar el destino de las plantas rebrotadoras en un mundo de continuos cambios en el régimen de perturbaciones (Capítulo 2).

Reclutamiento de plántulas

Las semillas de algunas especies pueden mostrar una pérdida progresiva de dormición con el tiempo para lograr obtener ventaja de las oportunidades ocasionales de reclutamiento pero manteniendo un considerable banco de semillas. Esto resulta en un elevado compromiso de las semillas para aprovechar las condiciones favorables tras el fuego y un menor compromiso para el reclutamiento al largo del periodo entre incendios. En efecto, la dormición física exhibida por las semillas de algunas especies, abundantes en el Mediterráneo, puede haber evolucionado de tal manera que el umbral de temperatura asociado con la rotura de la dormición está por encima de las temperaturas máximas de verano (Ooi et al. 2012). Esto asegura el mantenimiento de un banco de semillas persistente hasta que se produzca un incendio. A pesar del hecho que algunas especies con semillas con dormición física, en ecosistemas propensos al fuego, puedan tener una fracción de las semillas con germinación independiente del fuego, los umbrales de temperatura que rompen la dormición física de las semilla se explican mejor como una respuesta al fuego que como una respuesta a las temperaturas de verano en climas mediterráneos (Capítulo 3).

El fuego puede estimular la germinación mediante diferentes mecanismos, dependiendo de la especie o incluso de la población (para una misma especie). En efecto, tanto el calor como el humo son estímulos importantes para la germinación de muchas especies leñosas de la Cuenca Mediterránea y el éxito del reclutamiento de estas especies tras el fuego es debido no sólo a la resistencia de las semillas al calor, sino también a la germinación estimulada por el calor y/o humo (tanto en el porcentaje como en la velocidad de germinación). Además, las plántulas de algunas especies se desarrollan más rápidamente y por lo tanto con una mayor

aptitud bajo las condiciones post-incendio que después de otras perturbaciones. De hecho, los productos químicos presentes en el humo no sólo estimulan la germinación, pero también potencian tanto la emergencia como el establecimiento de las plántulas. Los resultados obtenidos en la presente tesis también sugieren que el efecto del humo en las especies anuales puede ser más relevante que lo que se suponía anteriormente, alentando a nuevos trabajos sobre el papel de las especies anuales en la regeneración tras el fuego en la Cuenca Mediterránea (capítulos 4 y 5). Estos resultados apuntan hacia que, así como en otros ecosistemas mediterráneos, la germinación estimulada por el fuego en la Cuenca Mediterránea es una respuesta evolutiva a los incendios recurrentes. Esto necesita ser considerado al abordar cuestiones evolutivas sobre los rasgos de las plantas a escala global.

En la Cuenca Mediterránea la presión ejercida por el fuego no ha seleccionado las plantas para tener germinación en respuesta a la combinación obligatoria de los dos estímulos asociados al fuego (capítulo 6). Esto es porque el significado ecológico de tener exclusivamente semillas que germinan cuando son expuestas simultáneamente a ambos factores (calor y humo) sólo sería comprensible para 1) evitar la germinación relacionada con incendios de muy baja intensidad en que las condiciones para el establecimiento de plántulas no se ven mejoradas, o 2) evitar una pérdida de la dormición por las temperaturas de verano, lo que podría ser especialmente importante para las especies que presentan semillas con dormición física. Sin embargo 1) es probable que estos incendios no sean muy frecuentes en matorrales Mediterráneos y 2) como se puede ver en el capítulo 3 de la presente tesis, las especies Mediterráneas presentan otros mecanismos que minimizan la pérdida de dormición de las semillas por las temperaturas de verano.

El fuego ha tenido un papel distinto en la germinación de las diferentes especies. La germinación tras el fuego es más eficiente en especies germinadoras obligadas que en especies facultativas, apoyando la hipótesis de que tener un mecanismo que permite la persistencia tras el fuego (es decir, la capacidad de rebrote) redujo la presión evolutiva sobre un segundo mecanismo de persistencia tras el fuego (es decir, reclutamiento tras el fuego). Además, diferentes linajes han desarrollado y adquirido la capacidad de germinación tras el fuego, pero este mecanismo fue adquirido en respuesta a diferentes señales de germinación relacionadas con el fuego (germinación estimulada por humo *vs* germinación estimulada por calor), dependiendo del tipo de cubierta de la semilla del linaje. Aunque la germinación tras el fuego sea una respuesta a la presión evolutiva ejercida por los incendios recurrentes, las estrategias específicas para la adquisición de estos rasgos dependen de efectos históricos (ej.,

la predisposición ancestral del linaje). Es decir, diferentes especies han desarrollado diferentes soluciones frente a la presión evolutiva ejercida por el fuego, permitiendo la coexistencia en zonas con incendios recurrentes de especies con semillas con diferente tipo de cubierta (Capítulo 7).

Para una misma especie, poblaciones que viven bajo diferentes regímenes de fuego, presentan variabilidad en los rasgos que mejoran su aptitud en el régimen de perturbación en que han evolucionado. De hecho, a escala local, los patrones heterogéneos de fuego, junto con otros factores locales como la interacción entre especies y la heterogeneidad en la disponibilidad de recursos, generan variabilidad en los rasgos de regeneración de las plantas, asociados al fuego (ej., tamaño de semillas, grado de dormición de las semillas y germinación estimulada por señales asociadas al fuego). Esta variabilidad intra-específica en los rasgos de regeneración permite a las especies el reclutamiento en distintas condiciones (ej., bajo distintos regímenes de incendios) y por lo tanto aumenta la probabilidad de persistencia en ecosistemas propensos a incendios (capítulo 8).

Inflamabilidad

Es probable que rasgos asociados a un aumento de la inflamabilidad hayan desempeñado un papel clave en la evolución de las especies germinadoras. Una selección positiva de estos rasgos asociados a un aumento de la inflamabilidad sólo es esperable si van asociados con otras características que confieran una mayor aptitud a los individuos que poseen esos rasgos. Por ejemplo, rasgos que resultan en una mayor probabilidad de ignición y mayor calor en el suelo pueden aumentar las posibilidades de reclutamiento de especies germinadoras, a partir del banco de semillas del suelo, por la apertura de espacios y por la estimulación de la emergencia de plántulas (el calor de los incendios rompe la dormición y estimula la germinación en muchas especies germinadoras, principalmente especies con semillas que presentan dormición física). Por lo tanto, no es de extrañar que los individuos de especies germinadoras obligadas, en poblaciones recurrentemente quemadas (poblaciones HiFi) sean más inflamables que los individuos de la misma especie en poblaciones derivadas de antigua colonización de campos abandonados que no sufrieron ningún incendio (poblaciones NoFi; es decir, con reclutamiento independiente del fuego; Capítulo 9 y Capítulo 10).

Discusión general

Tradicionalmente, los incendios han sido considerados una perturbación reciente (del Cuaternario), ligados a la presencia humana, y un desastre ecológico. Sin embargo, los incendios se han producido en los ecosistemas terrestres desde el inicio de la evolución de las plantas (Bowman et al 2009, 2011; Pausas y Keeley 2009; Bond y Scott 2010) y han moldeado muchas especies y ecosistemas a nivel global (Keeley et al. 2011).

Los incendios son importantes no sólo al generar dinamismo y heterogeneidad en el paisaje (Sousa, 1984; Pausas 1999; Pausas y Lloret 2007), sino también al imponer una fuerte presión evolutiva sobre los rasgos de las plantas (Pausas y Keeley 2009; Keeley et al. 2011). De hecho, en los ecosistemas propensos al fuego, las rutas evolutivas (Pausas y Verdú 2005), la diversidad genética (Dolan et al. 2008, Schrey et al. 2011) y la estructura de las comunidades (Verdú y Pausas 2007) están fuertemente determinadas por la historia de incendios. Hay ahora fuertes evidencias de que muchos de los rasgos con valor adaptativo para la persistencia en ecosistemas con incendios recurrentes pueden haber evolucionado por selección natural conducida por el fuego (Keeley et al. 2012).

Además, la variabilidad intra-específica también es ahora considerada de vital importancia para la predicción de dinámica de especies (Bolnick et al. 2011) y respuesta a cambios ambientales (Ramírez-Valiente et al. 2010), así como para entender procesos evolutivos (Pausas et al. 2012, Pausas y Moreira 2012). Esto es especialmente evidente en zonas propensas a incendios, en que el régimen de una perturbación fuerte como el fuego es muy sensible a los factores responsables por el cambio global (Dubinin et al. 2011, Pausas y Fernández-Muñoz 2012).

En esta tesis se han aportado evidencias que apoyan la relevancia del fuego en la evolución de los mecanismos que permiten a las plantas persistir en zonas con incendios recurrentes. También se ha profundizado el conocimiento existente sobre distintos rasgos que están asociados a estos mecanismos y la importancia de la variabilidad exhibida en estos rasgos.

Con el reciente advenimiento de la nueva generación de métodos de secuenciación y genotipado, estamos ahora más cerca de la posibilidad de genotipar poblaciones enteras con marcadores altamente polimórficos para inferir relaciones entre individuos en el campo, y por lo tanto testar integralmente múltiples hipótesis de evolución asociada al fuego. Además, la disciplina emergente basada en rasgos, de genética de comunidades, y el concepto de fenotipo

extendido (Whitham et al. 2003) pueden proporcionar un marco evolutivo adecuado en el que este fenotipo extendido se puede trazar desde los individuos que poseen el rasgo, a la comunidad y procesos de los ecosistemas. Efectivamente, la genética de comunidades puede potencialmente hacer una contribución significativa a la ecología del fuego (Wymore et al. 2011).

Conclusiones finales

1. Para una perturbación dada y condición ambiental determinada, la variabilidad intra-específica en el rebrote está determinada tanto por los recursos almacenados (antes de la perturbación), como por la capacidad inicial (después de la perturbación) la adquirir recursos. Las reservas almacenadas son importantes para la iniciación del rebrote y para el crecimiento inicial (primero para mantener la raíz y yemas vivas y posteriormente para la producción de nuevas hojas y tallos). Sin embargo, una vez que las plantas han rebrotado (con las reservas almacenadas), dependen de la capacidad inicial, posterior a la perturbación, de adquirir recursos (es decir, del vigor de rebrote) para sobrevivir.
2. Algunas especies pueden revelar una pérdida gradual de dormición de las semillas al largo del tiempo para beneficiar de las oportunidades ocasionales de reclutamiento sin fuego, pero mantienen la mayoría del banco de semillas para reclutamiento tras el fuego. De hecho, a pesar de que, en ecosistemas propensos al fuego, algunas especies con semillas con dormición física puedan tener una fracción de las semillas con germinación independiente del fuego, los umbrales de temperatura que rompen la dormición física de la semilla se explican mejor como una respuesta al fuego que como respuesta a las temperaturas del verano .
3. Tanto el calor como el humo son señales importantes de germinación para muchas especies leñosas de la Cuenca Mediterránea y el éxito del reclutamiento tras el fuego de estas especies se debe no sólo a la resistencia de las semillas al choque térmico, sino también a la germinación estimulada por el calor y/o humo (tanto en el porcentaje como en la velocidad de germinación).
4. Los productos químicos presentes en el humo, no solo estimulan la germinación, pero también la emergencia, crecimiento y establecimiento de las plántulas. El efecto del humo en las especies anuales es también relevante en la Cuenca Mediterránea.

5. En la Cuenca Mediterránea, la presión ejercida por el fuego no ha seleccionado las plantas para que la germinación sea en respuesta a la combinación obligatoria de las dos señales de germinación relacionadas con el fuego (calor y humo).
6. La germinación estimulada por el fuego es más eficiente en especies germinadoras obligadas que en especies facultativas. Además, la capacidad de germinación tras el fuego ha evolucionado en diferentes linajes, pero este mecanismo fue adquirido en respuesta a diferentes señales de germinación relacionadas con el fuego (germinación estimulada por humo vs germinación estimulada por calor), dependiendo del tipo de cubierta de semilla del linaje (cubierta permeable al agua vs cubierta impermeable al agua).
7. Para una misma especie, poblaciones que viven bajo diferentes regímenes de incendios, presentan variabilidad en rasgos asociados a la regeneración que mejoran su aptitud en el régimen de perturbaciones en que han evolucionado. De hecho, a escala local, el patrón heterogéneo de incendios, junto con otros factores locales, como la interacción de especies y la heterogeneidad en la disponibilidad de recursos, generan variabilidad en rasgos de las plantas, relacionados con la regeneración tras el fuego (como el tamaño de las semillas, la dormición y la germinación estimulada por señales asociadas al fuego). Esta variabilidad intra-específica en los rasgos de regeneración permite a las especies reclutar en diferentes condiciones (por ejemplo, bajo diferentes regímenes de incendios) y aumenta la probabilidad de persistencia en ecosistemas propensos al fuego.
8. En matorrales de *U. parviflorus* hay una divergencia en los rasgos de inflamabilidad entre las poblaciones que viven en diferentes ambientes selectivos. Los individuos que crecen en poblaciones recurrentemente quemadas (poblaciones HiFi) son más inflamables que los individuos de la misma especie en poblaciones resultantes de la colonización de antiguos campos de cultivo y que no sufrieron ningún fuego (poblaciones NoFi, es decir, con reclutamiento independiente del fuego).
9. El mecanismo, mediante el cual la aptitud de las plantas se puede ver incrementada, en plantas con mayor inflamabilidad, está asociado al aumento tanto de la probabilidad de ignición como del calor liberado hacia el suelo.

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