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

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**Keywords**
Annual species; Fire-related cue; Fitness; Germination cue; Post-fire regeneration; Recruitment; Regeneration; Smoke chemicals; Soil seed bank

**Abbreviations**
MB = Mediterranean Basin; FDR = false discovery rate; GLM = generalized linear model; GLMM = generalized linear mixed model

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## Abstract

**Question:** In many plant species from fire-prone ecosystems germination is promoted by smoke. Mediterranean Basin (MB) flora is no exception. However, most information regarding germination response to smoke in the MB comes from a few experiments performed in laboratory conditions. This approach does not consider factors that occur in the field, such as species interactions, density-dependent processes or the fact that seeds spent time in the soil seed bank. In addition, species selection has been biased (e.g. there is a lack of information about annual species). Hence the importance of smoke relative to other fire cues is not clear, and we have a biased knowledge of post-fire community assembly in the MB. In this framework, we tested the following hypotheses: (1) smoke enhances seedling emergence and establishment from the soil seed bank of MB species, 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:** We performed a field experiment in which we applied a liquid smoke treatment and tracked seedling emergence and seedling establishment during 1 yr. Differences between smoke and control subplots with respect to seedling emergence and seedling establishment were analysed at different scales: community, growth form (annual or perennial), family and species level.

**Results:** At the community level, smoke played a clear role in seedling recruitment, increasing seedling emergence and seedling establishment. In addition, for most plots, families and species, establishment was higher in smoke subplots compared to the control. Annual species establishment was clearly stimulated by smoke but no effect was detected for perennials.

**Conclusions:** Smoke derived from wildfires has a key effect on plant recruitment and hence on community assembly in the MB vegetation.

## Introduction

Fire is one of the main drivers of vegetation dynamics in mediterranean climate ecosystems, where plant species have different mechanisms to persist under recurrent fires (Pausas & Keeley 2009; Keeley et al. 2012). One of these mechanisms is post-fire recruitment from soil or canopy seed banks (Pausas et al. 2004); plants having this mechanism take advantage of the reduced competition and the increased resources available after fire. The post-fire flush of seedling emergence has been associated with both the heat and the chemicals produced by the fire (Keeley & Fotheringham 2000). Moreover, different studies suggest that chemicals in smoke produce an improvement of seedling growth and resistance to stress (Blank & Young 1998; Daws et al. 2007; Jain et al. 2006; Moreira et al. 2010).

Since the seminal paper of De Lange & Boucher (1990) several laboratory experiments have demonstrated that

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certain chemical compounds of the smoke act as a germination cue in many species (Van Staden et al. 2000; Flematti et al. 2004, 2011), particularly in those from mediterranean climate ecosystems such as South Africa (Brown & Botha 2004), Australia (Dixon et al. 1995), California (Keeley & Fotheringham 1998) and the Mediterranean Basin (MB; Moreira et al. 2010). A positive smoke effect has also been demonstrated *ex situ* (Hill & French 2003; Måren et al. 2009; Ghebrehiwot et al. 2011; Anderson et al. 2012) and in field experiments (Rokich & Dixon 2007).

In the MB, data on seed germination in relation to smoke come from a limited number of laboratory experiments (Cruz et al. 2003; Pérez-Fernández & Rodríguez-Echeverría 2003; Buhk & Hensen 2006; Reyes & Trabaud 2009; Moreira et al. 2010). These laboratory experiments have used few species and, in some cases, the species selection criteria were subjective and biased. For example, while annual species are described as an important component of the smoke stimulated flora in other mediterranean climate ecosystems (e.g. California; Keeley et al. 1981), most of the experiments performed with MB species have inadvertently excluded this group (Paula et al. 2009; but see Daws et al. 2007). This biased species selection might have led to one-sided knowledge of vegetation response to fire. In addition, while these experiments provide valuable information on the potential reproduction of plants, they do not consider important factors such as species interactions, density-dependent processes or that the seeds have spent time in soil exposed to environmental factors (Baker et al. 2005). Thus, these experiments offer limited insight over the realized plant fitness (i.e. successful seedling establishment).

*In situ* field experiments allow monitoring of seedling recruitment in natural conditions up to the establishment phase (realized fitness) and thus are an important and reliable source of information in studying the role of smoke on plant recruitment. In these *in situ* experiments, species are framed in their community (i.e. in an unaltered environment and interacting with co-existing species), which allows to disentangle the effects of smoke on seedling emergence and on seedling establishment (i.e. effective recruitment).

In order to understand the actual effect of smoke on plant recruitment, we performed a field experiment in a fire-prone community of the western MB. We compared seedling emergence and establishment between smoke-treated and untreated subplots. This experiment allowed us to test the following hypotheses: (1) smoke enhances seedling emergence and establishment of MB species, and (2) annual species are an important component of smoke-stimulated flora of the MB.

## Methods

### Study area and sampling

The experiment was carried out in two adjacent areas in eastern Spain (Valencia region, western MB), Serra Calderona (39.728° N, 0.506° W) and Barranc de la Casella (39.090° N, 0.304° W). Selecting two different sites allowed us to maximize the range of species sampled. The vegetation in Serra Calderona study site is dominated by the shrubs *Anthyllis cytisoides, Cistus albidus, C. monspeliensis* and *Ulex parviflorus*, while the Barranc de la Casella study site is dominated by *Pistacia lentiscus, Quercus coccifera, Erica scoparia, E. multiflora, C. monspeliensis, Rosmarinus officinalis* and *U. parviflorus*. In both sites, scattered individuals of *Pinus halepensis* compose the tree layer, while *Brachypodium retusum* dominate the herbaceous layer. Climate in both areas is mediterranean, mean annual precipitation is 584 and 633 mm and mean annual temperature 17.4 and 17.7 °C in Serra Calderona and Barranc de la Casella study sites, respectively. Precipitation peaks in autumn, with a secondary peak in spring. Summer is the dry period, when fires are common (Pausas 2004).

In September 2007, 21 plots (2 × 1 m) were delimited, nine in Barranc de la Casella and 12 in Serra Calderona. In each plot, we cut all the standing vegetation and carefully removed the litter without disturbing the soil profile. Within each plot, we delimited eight subplots (30 × 30 cm), four of which received a smoke treatment and the remaining four were used as a 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 & Cohn 2000; Jäger et al. 1996; for details on the effectiveness of commercial liquid smoke). This concentration was chosen based on preliminary tests in the laboratory with representative species of the studied community (data not shown). In the smoke subplots we applied 0.5 L of liquid smoke and in the control subplots (interspersed design) 0.5 L of distilled water. This amount of liquid does not significantly change the natural pattern of soil water availability produced by rainfall. Although liquid smoke might leach through the soil profile (Roche et al. 1997a), the smoke effect remains for long periods on the seeds of the soil seed bank (Roche et al. 1997b).

Plots were surveyed ten times throughout 1 yr, starting 1 mo after the treatments (September, October, November and December 2007, and January, March, April, June, July and September 2008). In each survey, we recorded and tracked all seedlings within each subplot with the help of a grid (30 × 30 cm, divided in 5 × 5 cm cells) that allowed us to follow the fate of each seedling.

### Data analysis

Data on species growth form (perennial or annual) were obtained from Mateo & Crespo (2001) and Paula et al. (2009). A preliminary analysis showed that the effect of smoke was not significantly different between sites ($P = 0.95$) and thus, for all subsequent analyses, the data from the two sites were pooled.

We studied two main variables: seedling emergence and seedling establishment. Seedling emergence was studied by pooling all the emerged seedlings without distinction among species, families or growth forms because not all emerged seedlings could be properly identified. 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). All established seedlings were successfully identified (at least to genus level), which allowed us 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) levels. 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 subplot in smoke or in control subplots (i.e. at least four seedlings in one of the treatments).

We first evaluated whether the proportion of plots with more seedlings emerged in smoke-treated subplots, compared to the control, was different from the random expectation by means of a one-tailed binomial exact test. Then, for each plot, we evaluated the significance of the differences in total seedling emergence between smoke and control subplots (replicates) by means of generalized linear models (GLM). Finally, we performed the same comparison for the whole data set using generalized linear mixed models (GLMM), including plot as random effects. The same analyses were used for seedling establishment, considering the different levels: community, growth form (annuals and perennials), family and species. For GLM and GLMM analyses we 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 corrected using the false discovery rate method (FDR; Benjamini & Hochberg 1995).

All statistical analyses were carried out in the R language (R Foundation for Statistical Computing, Vienna, AT, US). Specifically, binomial tests, GLM analyses and FDR correction were performed using the 'stats' package and GLMM analyses were performed using the 'lme4' package.

## Results

### Seedling emergence

Seedling emergence occurred in all studied plots, ranging from two to 757 seedlings per subplot. Overall, we scored 14 203 seedlings. The number of plots in which seedling emergence was larger 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 12 plots (ranging from 27% to 350% increase due to smoke). In contrast, two plots showed significantly less seedling emergence in smoke than in control subplots (38% and 31% decrease due to smoke). No significant differences between smoke and control subplots were found in seven of the plots (Fig. 1). Analysing all the plots together, we detected a significant overall increase in seedling emergence produced by the smoke treatment (Table 1).

### Seedling establishment

Successful seedling establishment (i.e. seedlings that survived the first summer in the case of perennial species or reached maturity during the study period in the case of annual species) was recorded for only 1998 seedlings (14% of total seedlings emerged). These seedlings accounted for 59 species in 22 families, of which 33 species

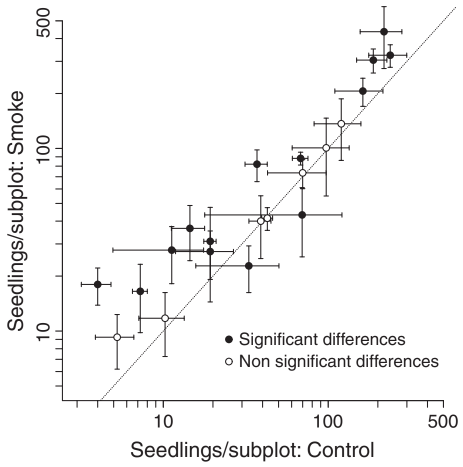

**Fig. 1.** Relation between mean seedling emergence in smoke and control subplots (30 × 30 cm) in the 21 studied plots. Horizontal and vertical bars represent SE in control and smoke subplots, respectively. Dashed line represents equal seedling emergence in smoke and control (1:1 line); plots above this line had higher emergence in smoke than in the control, and plots below this line had higher emergence in the control than with smoke. Differences are considered significant at $P < 0.05$ for FDR adjusted *P*-values. Axes are represented in logarithmic scale.

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

| Process | Subset | Total seedlings | Mean seedlings/ subplot | | *P*-Value |
|---|---|---|---|---|---|
| | | | Smoke | Control | |
| Emergence | Community | 14 203 | 98.9 | 70.2 | <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 | 0.098 |

in ten families reached the minimum seedling density to be studied (Appendix S1 and S2).

### Community level

The number of plots in which seedling establishment was higher 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, we found that the increase in seedling establishment due to smoke was significant in six plots (from 31% to 150% increase due to smoke). One plot showed significantly lower seedling establishment in smoke than in control subplots, while 13 plots showed no significant differences between treatments. In fact, the smoke effect was concentrated in plots with a 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).

### Annual species

Only 13 plots reached the minimum seedling density of annuals to be considered for this analysis. The proportion of plots with more seedlings established in smoke than in the control was significantly higher than the random expectation (12 out of 13, $P = 0.003$, binomial test; Fig. 3). This increase was significant in six plots (from 60% to 191% increase due to smoke). In the remaining seven plots there were no significant differences between treatments. Smoke effect was concentrated in plots with a higher density of seedlings (Fig. 3). Analysing all the plots together (including plot and species as a random effect in the model) allowed us to detect a significant increase in seedling establishment due to the smoke treatment (GLMM; Table 1).

### Perennial species

All but one plot reached the minimum density of seedlings to be considered for this analysis. The proportion of plots with more seedlings established in smoke than in the control was not significantly higher from the proportion expected by chance (14 out of 20, $P = 0.115$, binomial

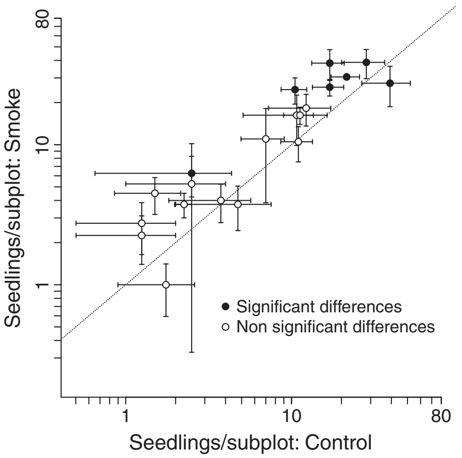

**Fig. 2.** Relation between mean seedling establishment in smoke and control subplots (30 × 30 cm) in the studied plots. Horizontal and vertical bars represent SE in control and smoke subplots, respectively. Dashed line represents equal seedling establishment in smoke and control (1:1 line); plots above this line had higher establishment in smoke than in the control, and plots below this line had higher seedling establishment in the control than in smoke. Differences are considered significant at $P < 0.05$ for FDR adjusted *P*-values. Axes are represented in logarithmic scale.

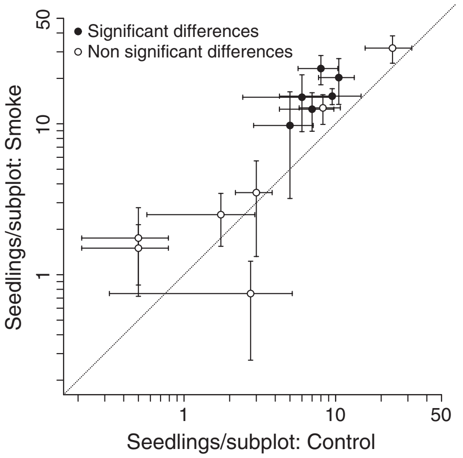

**Fig. 3.** Relation between mean seedling establishment of annual species in smoke and control subplots (30 × 30 cm) in plots with established annuals. Horizontal and vertical bars represent SE in control and smoke subplots, respectively. Dashed line represents equal seedling establishment in smoke and control (1:1 line); plots above this line had higher establishment in smoke than in the control and plots below this line had higher establishment in the control than in smoke. Differences are considered significant at $P < 0.05$ for FDR adjusted *P*-values. Axes are represented in logarithmic scale.

test). Seedling establishment of perennial species in smoke-treated subplots 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 non-significant effect of smoke on seedling establishment ($P = 0.098$; Table 1).

### *Family level*

Seedling establishment was higher in smoke than in control subplots in all studied families, and thus clearly higher than the proportion expected by chance. Lamiaceae showed significantly ($P < 0.05$) higher seedling establishment in smoke than in control subplots, and Scrophulariaceae showed a marginal ($P = 0.054$) positive effect (Appendix S1).

### *Species level*

From the 33 species that met the criteria for analysis, the proportion in which seedling establishment was higher in smoke compared to the control was significantly larger than expected by chance (26 out of 33, $P = 0.001$, binomial test). However, when analysing species individually, no single species showed a significant effect of smoke (Appendix S2).

## Discussion

The results of our field-based approach suggest that smoke not only enhances germination of MB species (Pérez-Fernández & Rodríguez-Echeverría 2003; Moreira et al. 2010), but also seedling emergence and establishment. The effect of smoke was also evident in annual species. This result coincides 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). This suggests that the importance of smoke chemicals for the regeneration of annual species might be a common feature in fire-prone mediterranean climate ecosystems (Keeley et al. 2012). Most of the emerged annual species were missing in the surrounding undisturbed vegetation, suggesting the presence of a persistent soil seed bank of ephemeral species, as described by other authors (Ferrandis et al. 1999; Ne'eman & Izhaki 1999). That is, our results suggest that in the MB flora, smoke-stimulated annuals might be more important in the post-fire environment 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 subplots, there were very few families and species in which the effect of smoke was statistically significant. Smoke effect was detected only in Lamiaceae, confirming the importance of smoke for regeneration of species in this family (Keeley & Fotheringham 1998; Moreira et al. 2010). This shortage of significant differences could be due to the inherent variability of field experiments – particularly those involving soil seed banks – that made the scale of our experiment and replication insufficient to properly test the hypotheses at family and species level. Controlled experiments, e.g. seed addition experiments, might help to overcome this limitation in future studies. In addition, the smoke effect might have been more conspicuous if we had used aerosol rather than liquid smoke because evidence exists that aerosol smoke might produce a higher stimulatory effect than liquid smoke (Roche et al. 1997b; van Staden et al. 2000); if so, our results are conservative in relation to our hypothesis. In any case, our results coincide with previous studies performed in Australian ecosystems, where a clear 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 eight- to ten-fold increase in seedling emergence, but in only two of the 18 studied species was the smoke effect significant (and it is worth mentioning that significance was not adjusted for multiple tests in Lloyd's study). Altogether, these results suggest that the community response to smoke cannot be inferred from individual species; it is the sum of small differences in each species towards the same direction that 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 the fact that our experimental approach did not emulate the conditions of a genuine wildfire (e.g. heat shock was not considered), our results support that in the MB flora, in addition to the effect on seed germination described in previous laboratory experiments, the chemicals produced during a fire have a positive effect on seedling emergence and establishment. Our results also suggest 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 MB.

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## Author contributions

BM and JGP conceived and designed the experiments. JT and BM performed the experiments. JT, BM and JGP analysed the data and wrote the manuscript.

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## Supporting Information

Additional supporting information may be found in the online version of this article:

**Appendix S1.** Total number of established seedlings and mean number of established seedlings per subplot (30 × 30 cm) in control and smoke-treated subplots for each of the studied families.

**Appendix S2.** Total established seedlings and mean number (seedling/subplot) of established seedlings in control and smoke-treated subplots (30 × 30 cm) for each of the studied species.