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# Germination patterns of the scrublands in response to smoke: The role of functional groups and the effect of smoke treatment method

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## ABSTRACT

Smoke is thought to be one of the most important fire cues stimulating seed germination of species from both fire-prone and fire-free ecosystems. The impact of smoke on seed germination at the community level of certain habitats in Western Asia has not been investigated yet. We aimed to analyze the effects of different smoke treatments on soil seed germination of different plant functional groups in a scrubland habitat. A soil seed bank was used to count seedling emergence following aerosol smoke (15 min or 30 min exposure) or smoke-water (1:1000 and 1:500, *v*/v) treatment. Smoke-induced germination was then separated into five functional groups (i.e. annual grasses, perennial grasses, annual herbs, perennial herbs, and legumes). The results showed that seeds of annuals were stimulated with smoke. In contrast, germination of perennial grasses was reduced by the smoke treatments, whereas perennial forbs and legumes showed no particular response to the smoke treatments. The longer exposure time to the aerosol smoke (30 min) and the higher concentration of smoke-water (1:500, *v*/v) were not at detrimental levels and resulted in maximum germination among the functional groups, other than perennial grasses. Our results indicate that smoke-stimulated germination of annuals plays an important role in species establishment after fire in the scrublands of Western Asia. Smoke treatments impacted differently on the different functional groups, and it is useful to determine the smoke response-classifications of these species to understand the effects of fires on the plant communities.

## 1. Introduction

Post-fire recruitment from soil seed banks is one of the most important mechanisms of species to persist under fire (Keeley and Fotheringham, 2000; Keeley et al., 2011). The long-term establishment of species regenerating from seeds may even depend strongly on soil seed survival and germination under specific habitat conditions (Poschlod et al., 2013; Abedi et al., 2014). Among different fire germination cues, smoke is known to be a major factor stimulating seed germination (Van Staden et al., 2000). One of the main chemicals responsible for this effect is a butenolide-type compound isolated from plant-derived smoke, referred to as karrikinolide (Flematti et al., 2009; Van Staden et al., 2004). Promotion of seed germination is the primary response to smoke, although plants establishing after exposure to smoke may also show improved post-germinative growth, often leading to a better establishment in disturbed soils (Roche et al., 1997; Read et al., 2000; Sparg et al., 2006; Daws et al., 2007; Kulkarni et al., 2008). Several studies since the early 1990s on the effects of smoke on germination have shown its usefulness as a germination promotor in both ex situ (Van Staden et al., 2000) and in situ conditions (Brown, 1993; Rokich and Dixon, 2007; Ghebrehiwot et al., 2012; Tormo et al., 2014). Therefore, smoke treatments are now widely accepted in fire simulation studies (Baskin and Baskin, 2014).

The profound and complex effects of smoke on vegetation may be related to several factors. Firstly, there are variable responses of species from both fire-prone and fire-free habitats (Brown et al., 2003; Keeley and Fotheringham, 1998; Van Staden et al., 2000). Different types of species including graminoids (Baxter et al., 1994; Read and Bellairs, 1999), forbs (Brown et al., 2003; Mojzes and Kalapos, 2014; Çatav et al., 2014), and woody species (Gómez-González et al., 2008) may positively response to smoke. Seemingly, a species response to smoke may be associated with their native or exotic conditions (Figueroa et al., 2009; Rawson et al., 2013), fire history (Bargmann et al., 2014) and resprouting ability (Luna et al., 2007). In addition, the effect of smoke may be related to fuel type (Razanamandranto et al., 2005), the smoke treatment method and exposure time (Keeley and Fotheringham, 1998) or the concentration of smoke-water (Måren et al., 2010), since smoke is also known to have an inhibitory effect on germination at high concentrations (Light et al., 2002).

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Research studies on the effects of smoke on germination ('smoke ecology') have mainly concentrated on South African ecosystems (Van Staden et al., 2000), Australian ecosystems (Dixon et al., 1995; Lloyd et al., 2000; Morris, 2000), Mediterranean ecosystems in Spain (Tormo et al., 2014; Moreira et al., 2010; Moreira and Pausas, 2016), Californian chaparral (Keeley and Fotheringham, 1998) and also fire-free ecosystems (Pierce et al., 1995; Bargmann et al., 2014), and information on species from other regions, especially Western Asia, is rare. Only a few selected plant species were recently studied with respect to their smoke response in Iran (Abdollahi et al., 2011; Naghipour et al., 2016; Zaki and Abedi, 2017; Zaki et al., 2017) and Turkey (Çatav et al., 2014; Çatav et al., 2015). However, to the best of our knowledge, a larger group of species from one habitat type in this region has not been examined.

Species-rich scrublands occurring in the eastern parts of the Hyrcanian forests are considered to be vulnerable and sensitive to fire due to the production of a remarkable amount of biomass from herbaceous species which dries out during July to September (Akhani, 1998). Furthermore, strong winds caused by the interaction of high air pressure from the Hyrcanian area in the west, and the low air pressure of Central Asia in the east, make this ecosystem particularly vulnerable to fire. Such scrublands occur throughout the north east of Iran, including the Golestan National Park, one of the most important National Parks and Biosphere Reserves in Iran. The vegetation type of the Golestan National Park phytogeographically belongs to the Irano-Turanian region, but some species from the Hyrcanian and Euro-Siberian region also contribute to the vegetation as well (Akhani, 1998). The habitat is characterized mainly by calcareous species-rich scrublands, which have been conserved for decades. Thus, wildlife grazing, drought, disturbance by wild boar and fire are the main drivers of vegetation change in this area.

In this present study, we aimed at evaluating the usefulness of aerosol smoke or smoke-water treatments to understand the effect of smoke (i.e. a fire cue) on different plant functional groups from the scrublands of Iran. The study was conducted in the Sharlegh watershed where, over the last 40 years, there has been no report of any fires (Golestan National Park Fire Report, personal communication, 2014). Whilst wildfire may not be considered an ecological driver in this particular habitat, fire events are increasing in most parts of Golestan National Park due to both drought and anthropogenic fires (Bhalkeh et al., 2017). Over the last few years there has been a 5-fold increase in fires, indicating its important role in future vegetation dynamics in Golestan National Park. Thus, it is important to understand how species from this vegetation respond to fire cues, including smoke. Using an approach based on evaluating germination from the soil seed bank, we attempted to answer the following questions: (i) How strongly is seed germination of species from this habitat influenced by smoke treatments; (ii) are the effects of smoke on seed germination of annuals more pronounced than perennials, and (iii) can the method of smoke treatment influence the germination responses observed?

## 2. Materials and methods

### 2.1. Description of the study area and vegetation

The study was conducted in Sharlegh watershed, located in the southern part of Golestan National Park, extending between latitudes 37°19′51.02″ N and 37°20′32.52″ N, and longitudes 56° 2′59.72″ E and 56° 0′34.96″ E. Altitude ranged between 960 and 1050 m. Mean annual precipitation and temperature are 149.9 mm and 9.87 °C, respectively. Two sites with similar topographical conditions were selected approximately 2 km apart. Both sites had well-developed scrub communities dominated by *Paliurus spina-christii* and associated with very rich herbaceous species, often covering up to 100% of the ground (Akhani, 1998). The *P. spina-christii* comprised the main element of these successional communities of the forest zone. The occurrence of young juniper shrubs (*Juniperus excelsa*) in our study site confirmed that there had been no recent fires in the area.

### 2.2. Soil seed bank collection and processing

Collection of soil seed bank samples was undertaken on 3 September 2014, when the fruit of most species had reached maturity and were ready to disperse their seeds. This allowed for recording of those species that form a transient soil seed bank. In order to test the germination responses of species to smoke, 10 soil seed bank samples were taken from two sites within the Sharlegh watershed ($n = 5$ for each site; Arzani and Abedi, 2015). Each soil sample had a volume of approximately 4 L and was collected to a depth of 5 cm. Each sample was mixed by hand and divided into five subsamples of about 800 mL. One of the subsamples was left untreated and served as a control. The remaining four subsamples were assigned one of the following treatments: (1) smoke-water 1:1000 (*v*/v), (2) smoke-water 1:500 (*v*/v), (3) aerosol smoke for 15 min, or (4) aerosol for 30 min. Each subsample was spread in a thin layer on a tray (with small drainage holes) filled with sterilized sand. All 50 trays were then placed randomly on shelves in the greenhouse with a natural day/night light regime and were kept moist by spraying regularly with tap water (Erfanzadeh et al., 2013). Five additional control trays, with sterilized soil only, were also prepared to monitor contaminations from any air-borne seeds in the greenhouse.

Smoke-water was prepared by burning dry leaf material, collected from the sampling sites (~5 kg), in a 20-L metal drum and passing it through a glass column containing 2 L of tap water for 45 min (Baxter et al., 1994). The two different concentrations of smoke-water used in this study were prepared by diluting 1 mL of the main smoke extract with 500 or 1000 mL distilled water (1:500 or:1000, *v*/v). Soil samples for the smoke-water treatments were moistened with 250 mL of the relevant solution every two weeks. In addition, aerosol smoke was produced by burning dry leaves collected from the sampling site in a 20-L metal drum (Dixon et al., 1995). The smoke was pumped through a 3-m metal tube covered with wet cotton, allowing the smoke to cool, before entering a closed plastic tent (2 m × 2 m × 1.5 m) wherein the trays with soil samples had been placed. The trays were removed from the tent after 15 and 30 min of exposure to the smoke and moved to the greenhouse for incubation.

For a period of six months, the germinating seedlings were counted and removed from each tray. In order to maximize potential germination from the soil seed bank samples, watering was stopped for two weeks at the end of the 6-month period, and the soil/sand was remixed and watered to allow for germination of any remaining seeds. Plants were identified down to species level and any unknown species were shifted to another tray to grow until flowering for identification. Total number of seedlings, and total richness (i.e. number of species germinating in each tray) was determined. To understand the effect of smoke on different functional groups, the following five groups were considered: annual grasses (AG), annual forbs (AH), perennial grasses (PG), perennial herbs (PH) and legumes (L).

### 2.3. Statistical analysis

All analyses were conducted using a generalized linear mixed effects model (GLMM) with a Poisson error distribution and a log-link function with sites and plots per sites as a random intercept. Analyses were run for seedlings of each functional group separately. All analyses were done using R version 3.1.3 (R Core Team, 2015), using the 'lme4' package (Bates et al., 2015). We assessed the significance of each effect by comparing the full model with a model in which a specific effect was withheld using likelihood-ratio-tests via the methods implemented in the 'afex' package (Singmann et al., 2015). Means estimates are based on the model obtained from the above model, and post hoc tests were applied using the 'lsmean' package (Lenth, 2016).

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