CrossMark

Fire-related germination and early seedling growth in 21 herbaceous species in Central Anatolian steppe

Çağatay Tavşanoğlu [a, *], Şükrü Serter Çatav [a, b], Barış Özüdoğru [c]

[a] *Fire Ecology and Seed Research Lab., Division of Ecology, Department of Biology, Hacettepe University, Beytepe 06800, Ankara, Turkey*
[b] *Department of Biology, Muğla Sıtkı Koçman University, Kötekli, 48000, Muğla, Turkey*
[c] *Division of Botany, Department of Biology, Hacettepe University, Beytepe 06800, Ankara, Turkey*

ARTICLE INFO

ABSTRACT

The role of fire in shaping plant traits in crown fire ecosystems is well understood, but little attention has been given to surface fire ecosystems. We studied the effect of fire-related cues (heat shock and smoke) on the germination and early seedling growth of 21 herbaceous species in Central Anatolian steppe, Turkey. We made an experiment including one smoke and five heat-shock treatments (60, 80, 100, 120 and 140 °C for 5 min) to test whether germination percentage, mean germination time, seedling growth, and seedling vigor enhance or reduce in the treatments in comparison to the controls. Seeds of all species were able to tolerate low heat shocks, but moderate and high heat shocks had a negative impact on germination and seedling growth. In *Stachys byzantina*, germination was stimulated by the smoke treatment. Smoke and low heat shocks positively affected the seedling vigor index of six species. The results suggest that the seeds of plant species in Central Anatolian steppes are resistant to low-intensity surface fires, but not high-intensity crown fires and that some species take advantage from surface fires. Our results contribute to understanding the role of fire in temperate grassland ecosystems.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

Fire is a global phenomenon affecting many ecosystems in the world including forests, shrublands and grasslands in temperate and tropical regions (Archibald et al., 2013). Although the natural role of fire has been acknowledged for many years (Naveh, 1975), the importance of fire in the evolution of plants has recently been understood (Keeley et al., 2012). Many studies conducted in recent years showed how fire shapes plant traits such as flammability, serotiny, germination and seed size (Keeley et al., 2011; Moreira et al., 2012; Pausas et al., 2012; Tavşanoğlu and Çatav, 2012). However, most of the studies related to fire-adapted plant traits come from frequently burned Mediterranean-type ecosystems. One of the interesting questions in relation to natural role of fires, therefore, is how fire affects plant regeneration traits (e.g. germination) in fire-prone vegetation types out of the Mediterranean ecosystems (Çatav et al., 2014). In such regions, there is still a shortage of information on fire-related plant traits, and therefore relatively less is known about fire's role in the ecosystem. Three of

those understudied regions are the grasslands of temperate, subtropical and tropical regions, which are all fire-prone ecosystems. Recently, subtropical and tropical grasslands/savannas have received some attention in relation to fire-related plant traits (Gashaw and Michelsen, 2002; Williams et al., 2003; Overbeck et al., 2006; Dayamba et al., 2008; Dantas et al., 2013; among others). However, such studies have relatively less conducted in temperate grasslands (but see; Clarke and French, 2005; Gonzalez and Ghermandi, 2012; Schwilk and Zavala, 2012). Studying fire-related regeneration traits in plant species in surface-fire ecosystems will improve our understanding of pyrogeography, the study of the distribution of fire on Earth (Archibald et al., 2013). Moreover, data from such regions will be useful to be compared to the Mediterranean ecosystems, and to learn about the relevance of fire regime (crown vs. surface) in shaping plant regeneration traits.

Heat shock and smoke are the most commonly studied cues for fire-related germination and seedling growth. Heat shock causes the release of physical seed dormancy in fire-adapted species with impermeable seed coat, while the chemicals found in smoke break physiological seed dormancy in fire-adapted species with permeable seed coat (Moreira et al., 2010). These traits allow the stimulation of germination after fire by breaking dormancy of seeds in

the soil seed bank (Keeley and Fotheringham, 1998). Moreover, smoke is known to stimulate seedling growth in several species (Daws et al., 2007; Moreira et al., 2010). It is clear that the germination response of plant species to smoke is a fire adaptation at least in Mediterranean ecosystems (Keeley et al., 2011). On the other hand, there is still a large gap on the role of smoke as fire-related germination and seedling growth in different ecosystems in the world.

In temperate, sub-tropical and tropical grasslands, plant species have positive, negative or neutral germination response to fire-related cues. Most of the studies tested germination response of grassland species to various heat shock doses failed to show any stimulative effect of heat on germination (Gashaw and Michelsen, 2002; Overbeck et al., 2006; Dayamba et al., 2008; Gonzalez and Ghermandi, 2012; Schwilk and Zavala, 2012). However, at the same time, many species can resist low to moderate heat shock treatments while intense heat shock has a lethal effect on seeds of some species. Among grassland species, germination stimulation by heat shock has been shown in some Australian Poaceae (Clarke and French, 2005) and a *Rumex* species from Japan (Tsuyuzaki and Miyoshi, 2009). In most cases, smoke does not affect the germination of species found in grasslands either (Dayamba et al., 2008; Gonzalez and Ghermandi, 2012; Schwilk and Zavala, 2012). But there are records of smoke-stimulated germination (Tsuyuzaki and Miyoshi, 2009; Ghebrehiwot et al., 2012; Gonzalez and Ghermandi, 2012; Schwilk and Zavala, 2012; Le Stradic et al., 2015) and seedling growth (Blank and Young, 1998) in some grassland species.

Temperate grasslands cover extensive areas in Anatolian peninsula. Anatolia hosted the first human settlements in the world (dated back to 10,000 years before present), and the first domestications of wild plants and animals also occurred around this region. At the same time, Anatolia has an extraordinary biodiversity created by its variable topography and microclimates and enhanced by the existence of three biogeographic regions inside (Şekercioğlu et al., 2011). Due to the long history of human civilization in this region, Anatolian people have converted forests into grasslands and grasslands into crop fields, and have intensely used grasslands as rangelands for their livestock (Fırıncıoğlu et al., 2009). Therefore, Central and Eastern Anatolian grasslands (i.e., steppes) have been under intense human pressure for millennia (Asouti and Fairbairn, 2002). Agricultural burning has also been a common practice in Central Anatolia, to clean crop residues in agricultural areas after harvesting period and to grow grasses more in rangelands for goat and sheep grazing. Satellite data indicate a high burning activity in Central Anatolia in recent years especially concentrated on September (Giglio et al., 2003; NASA, 2012), but the short durations of those fires are a sign of agricultural ones (Amraoui et al., 2013). The anthropogenic origin fire regimes seem to be present for a few millennia in Central Anatolia after agriculture became intensified (Kaniewski et al., 2008), as the lake sedimentary charcoal data suggest (Turner et al., 2008). However, during the early and mid-Holocene periods (before agricultural fires become widespread), natural grassland fires were also common in Central Anatolia and the grasslands covered this region were maintained by dry-season fires (Turner et al., 2010; Vanniére et al., 2011). Since Central Anatolian steppes have a fire history dated back to the end of the last glacial period, plant species found in this ecosystem should have been under the selective pressure of surface fires for millennia. It can be expected, therefore, that regeneration characteristics of plant species (such as seed germination and seedling growth) in Central Anatolian steppes are shaped by fire. To date, however, no study has been performed on fire-related germination and seedling growth in the plant species in the Central Anatolian steppe ecosystem.

In this study, considering the observations and assumptions above, we hypothesized that germination and seedling vigor will be enhanced or not be affected by fire in plant species in the Central Anatolian steppe. To test this hypothesis, we studied the effect of fire-related cues (heat shock and smoke) on the germination and early seedling growth of 21 herbaceous species growing in steppe vegetation near Ankara, Central Anatolia, Turkey. We made a laboratory experiment including one smoke and five heat-shock treatments to test if germination percentage, mean germination time, seedling growth, and seedling vigor index of the studied species enhance or reduce in the treatments in comparison to the controls.

2. Materials and methods

2.1. Study area, study species and seed collection

We collected the fruits of twenty-one plant species (Table 1) from Hacettepe University Beytepe Campus area, Ankara, Turkey (39° 52′ N, 32° 43′ E, 1025 m) between July and September 2010. This period corresponds to the time just before the seed dispersal starts for each particular species. All the studied species have hemicryptophyte or therophyte life form and are herbaceous except the suffruticose *Fumana aciphylla*. The distribution range varies among species, extending from Anatolian endemics to widespread throughout the world (Table 1). Between these two extremes, some species have regional distribution range (only in the Mediterranean Basin or Eastern Europe or the Middle East) whereas some distributed more locally (only in Anatolia or around). Although the vegetation of the study area is originally characterized by Central Anatolian steppe, *Pinus nigra* (black pine) plantations have substituted natural flora in many parts of the landscape due to afforestation and landscape planning activities for nearly 40 years. Fortunately, plant species from steppe vegetation remained in the open patches that are not covered by planted trees.

Flora of the study area consists of 504 species, 264 genera, and 57 families (Mutlu et al., 2008). Although the selected 21 species for the study constitutes a small sample of the local flora, they are among the most common ones, and they more-or-less represent the major phylogenetic lineages of Angiosperms growing in Anatolia. Many of the selected species are also present in other localities throughout the Central Anatolian steppe (Davis, 1965—1985). Therefore, we assumed that our selection is a representative one of the local flora.

The climate of the study area is semi-arid continental one according to Köppen's climate classification, with cold winters and hot summers. Annual total precipitation is 407.3 mm, and the mean annual temperature is 12.1 °C (data from Turkish State Meteorological Service, 1980—2011). During the cold period (November to March) mean temperature is 3.6 °C while total precipitation is 47.1 mm during the dry period (July to September).

We collected fruits from the field into paper bags from at least five individuals for each species, however in some cases more individuals were needed to be collected to obtain enough number of fruits to conduct an experiment. Seeds were removed from fruits by hand in the laboratory and then stored in paper envelopes under room conditions until the start of the experiment in October 2010.

Nomenclature follows Davis (1965—1985), but also Angiosperm Phylogeny Website (Version 12.07.2012; http://www.mobot.org/MOBOT/research/APweb/) for the current family names.

2.2. The experiments

We performed five heat shock treatments and an aqueous smoke treatment in the study. We also used untreated seeds in the experiment serving as a control for heat shock treatments, whereas

Table 1
Taxonomic status and the ecological characteristics of the study species. GF is growth form (a: annual herb, b: biennial herb, p: perennial herb, sf: suffruticose herb), LF is Raunkier's life form (t: therophyte, H: hemicryptophyte), Range is the distribution range of species (W: widespread, R: regional, and L: local; Anatolian endemics indicated with asterisks), and SM is mean seed mass in mg. Taxa codes given here are used in the tables throughout the study.

Code	Family	Taxon	GF	LF	Range	SM
BRA	Apiaceae	*Bifora radians*	a	t	R	9.12
DCA	Apiaceae	*Daucus carota*	b	H	W	1.05
CDE	Asteraceae	*Carthamus dentatus*	a	t	R	25.3
CFO	Asteraceae	*Crepis foetida* ssp. *rhoedafolia*	a	t	W	0.51
CCR	Asteraceae	*Crupina crupinastrum*	a	t	R	6.87
TPO	Asteraceae	*Tragopogon porrifolius* ssp. *longirostris*	b	H	R	1.30
ALE	Boraginaceae	*Anchusa leptophylla* ssp. *leptophylla*	p	H	R	6.33
DTE	Brassicaceae	*Diplotaxis tenuifolia*	p	H	W	0.25
GPI	Caryophyllaceae	*Gypsophila pilosa*	a	t	R	2.48
FAC	Cistaceae	*Fumana aciphylla*	sf	H	R	2.82
SAR	Caprifoliaceae	*Scabiosa argentea*	p	H	R	1.55
OTO	Fabaceae	*Onobrychis tournefortii**	p	H	L	7.35
PPU	Lamiaceae	*Phlomis pungens*	p	H	R	8.13
SSY	Lamiaceae	*Salvia syriaca*	p	H	R	1.92
SAN	Lamiaceae	*Stachys annua* ssp. *annua*	p	H	W	2.22
SBY	Lamiaceae	*Stachys byzantina*	p	H	R	3.15
LHI	Linaceae	*Linum hirsutum* ssp. *pseudoanatolicum**	p	H	L	1.14
RCR	Polygonaceae	*Rumex crispus*	p	H	W	1.35
RLU	Resedaceae	*Reseda lutea*	p	H	W	0.71
SMI	Rosaceae	*Sanguisorba minor* ssp. *minor*	p	H	W	2.58
GFL	Rubiaceae	*Galium floribundum* ssp. *floribundum*	a	t	L	0.24

a watered control was used to serve as a control for aqueous smoke treatment (see below).

Heat shock treatments applied at several temperatures (60 °C, 80 °C, 100 °C, 120 °C and 140 °C) for 5 min. These treatments correspond to the temporal pattern of the upper zone soil temperatures during surface and crown fires (Trabaud, 1979). By selecting a range of heat shock intensities, we simulated low-intensity surface fire (60 and 80 °C), moderate intensity fire (100 °C), and high-intensity crown fire (120 and 140 °C). Before we apply the heat shock in a temperature-controlled oven, seeds were put into aluminum pockets and placed in the center of the oven. Each time, the seed pockets of one replicate of all species were heated at the corresponding heat shock temperature. This procedure was repeated four times for each treatment to obtain four independent replicates.

For smoke treatment, an aqueous smoke solution was prepared and then applied to the seeds. To achieve that, first, dry leaves of *Phillyrea latifolia* were separated into small pieces. Four replicates of 5 g of this plant material were heated separately in metallic containers in the oven for 30 min at 195 °C. The mouth of each container was tightly covered by an aluminum foil to capture the smoke generated from the burnt plant material. After the treatment, 50 mL of distilled water was added to the container and let stand for 10 min. By this procedure, the active chemicals found in smoke dissolved in water. The solution was filtrated into a bottle to obtain smoke in liquid form. Seeds of each species were incubated in this solution (pH = 5.94) for 24 h before sowing in the Petri dishes (Moreira et al., 2010; Çatav et al., 2014). For further explanation of the preparation and application of the smoke solutions, see Jäger et al. (1996).

For each species, four replicates of seed lots sown directly in the Petri dishes without subjecting any treatment to serve as the control for the heat shock treatments (hereafter, dry control). Additionally, for each species, four replicates of seed lots incubated in distilled water (pH = 7.74) for 24 h before sowing in the Petri dishes to serve as the control for the smoke treatments (hereafter, watered control).

After the treatments had been applied, seeds were sown in Petri dishes containing agar (0.7%) as a substrate. Each treatment consisted of four replicates of 25 seeds. We placed Petri dishes in a germination cabinet set at 20.0 ± 1.0 °C in darkness. Seed germination was checked every day during the first eight days, every two days until the end of the second week, and then once a week until the end of the experiment. Radicle emergence was the criterion to keep an account of germination. After germination had recorded, the seeds removed from Petri dishes and recorded in data tables. We also remove empty (seeds without an embryo) and rotten seeds and recorded. We finalized the experiment on the 63rd day of the incubation period when no more germination occurred in any of the treatments for one week. The viability of non-germinated seeds was assessed by a cutting test, and we considered the seeds with intact internal content as viable. To investigate the early seedling growth of the species in the controls and after the treatments, germinated seeds were transferred into Petri dishes covered with two layers of filter paper moistened initially with 5 ml distilled water. This procedure was applied only during the first eight days of the experiment when germinations checked daily to be sure of the exact germination day of the seedlings. Only the seedlings belonging to the same species were put into the same dish, and the maximum number of seedlings within each dish was delimitated by six (ten for species with very small seeds) to avoid the crowd effect. Petri dishes containing newly germinated seedlings were placed in the germination cabinet and incubated at 20.0 ± 1.0 °C in darkness for seven days. We preferred dark conditions to perform seedling growth experiment for practical reasons, and based on the information that aqueous smoke solutions can improve seedling growth under light (Moreira et al., 2010) or dark (Kulkarni et al., 2006, 2007) conditions either. The filter papers were regularly moistened with distilled water to protect seedlings to get water stress. At the end of the incubation period, the total length of seedlings (root + shoot length) was measured by a ruler with 1 mm precision to determine their growth within seven days.

In the case of lack of seeds to perform all the treatments in a species, we preferred to include smoke treatment first due to the scarcity of studies on smoke-stimulated germination, especially in the study region. Then, we preferred heat shock treatment of 100 °C and the other treatments in turn. Through this procedure, we tested germination response of 21 species to smoke, 17 species to 100 °C heat shock, 15 species to 80 °C, 120 °C, and 140 °C heat shocks and 13 species to 60 °C heat shock treatment. As a consequence, the

seedling length could not be measured for some species in the treatments in which we did not perform germination experiment. Moreover, in some treatment × species combinations mean seedling length and seedling vigor index could not be estimated because of a few number of germinations in the corresponding treatments.

2.3. Data analysis

Before the analysis, germination data of each species were corrected by removing empty seeds from the data. Therefore, the total number of seeds used in each Petri dish (each replicate) varied to how many empty seeds were found in each. Most of the species had >20 seeds in each replicate in each treatment at the end, except *Salvia syriaca* (~18 seeds per Petri dish), *Phlomis pungens* (~16 #), *Sanguisorba major* (~15 #), *Onobrychis tournefortii* (~14 #), *Fumana aciphylla* (~11 #), and *Scabiosa argentea* (~10 #). The germination percentage was estimated as the mean of four replicates of each treatment for each species. For each species, seeds were classified as germinated or non-germinated, and then the final germination of each treatment was compared with the corresponding control using the analysis of deviance (GLM) and assuming a binomial error distribution (Moreira et al., 2010).

Mean germination time (hereafter, MGT) was calculated using the equation $\sum(nD)/\sum n$, where n is the number of seeds germinated on day D and D is the number of days from the beginning of the incubation period (Tompsett and Pritchard, 1998). Seedling vigor was evaluated as the combination of early seedling growth and germination traits, and calculated with equation $(s + r)G$ (hereafter, seedling vigor index), where s and r are the shoot length (in mm) and root length (in mm), respectively, at the end of the incubation period for seedlings and G is the final percentage germination (Kulkarni et al., 2007). Both indexes were calculated for each replicate of the each treatment × species combination. Therefore, MGT and seedling vigor index values were estimated as the mean of four replicates of each treatment for each species.

The number of measured seedlings varied to species and treatments since it depends heavily on the amount of seeds germinated in each treatment × species combination. In the case of fewer than four seedlings were obtained, we excluded this treatment × species combination from the analysis. To increase the power of the analysis, we tried to equalize the number of seedlings measured in each treatment in the same species by excluding surplus measures randomly. In summary, minimum and maximum numbers of seedlings measured were four (in *Carthamus dentatus*) and 19 (in *Gypsophila pilosa*), while each treatment included between 8 and 12 seedlings in the most of the species. For each species, the difference of seedling length, seedling vigor index values and germination time between the treatments and corresponding controls were tested by one-way analysis of variance (ANOVA). Multiple comparisons were held by Dunnett test, a post-hoc test used when a set of comparisons are made to one group designated as control (Zar, 1996). Data normality and homoscedasticity were tested using the Shapiro-Wilk and Bartlett's tests before each analysis, respectively. If needed, logarithmic and square root transformations were done. In the case of assumptions of parametric tests were not met, the Kruskal-Wallis test and the non-parametric version of the Dunnett test (Zar, 1996) were used. Because of the large number of pairwise comparisons, the significance level was assumed as P < 0.01 in all analyzes. This approach is less conservative than the Bonferroni correction (Moran, 2003), and has been effectively used in many researches in recent years (e.g. Moreira et al., 2010; Tavşanoğlu and Gürkan, 2014).

3. Results

Dormancy level was quite variable among the studied species. Four species had very low dormancy with >90% germination in the controls while six species had high-degree dormancy with <3% germination. Dormancy degree or germination percentage in the control of the remaining species ranged between these two extremes (Table 2).

All of the study species resisted to low (60 °C 5′ and 80 °C 5′) heat shock treatments; while the moderate heat shock treatment (100 °C 5′) significantly decreased germination percentage in more than half of the species studied. Intense heat shock treatments (120 and 140 °C 5′) resulted in zero germination and total mortality of seeds (Table 2). Germination percentage increased ca. two-fold (from 19% to 36%) in *Reseda lutea* in the 60 °C 5′ heat shock treatment in comparison to the control, but this increase was not significant (P > 0.01). Heat shock treatments did not affect early seedling growth in most of the species studied (Fig. 1). However, the moderate heat shock treatment lengthened the MGT in most of the species tested (Table 3). Seedling vigor index values enhanced in two species (*R. lutea* and *Diplotaxis tenuifolia*) in low heat shock treatments, but decreased in four species (*Daucus carota*, *Galium floribundum*, *Rumex crispus* and *Tragopogon porrifolius* ssp. *longirostris*) in low heat shock treatments, and decreased in most of the species tested in the moderate heat shock treatment (Fig. 2).

In *Stachys byzantina*, germination was stimulated by the smoke treatment (Table 2). Germination of *Sanguisorba minor* enhanced after the smoke treatment but this improvement was not statistically significant (P > 0.01). Most of the remaining species were not affected by the smoke application, except two species (*C. dentatus* and *R. lutea*) in which inhibition of germination was observed. Apart from the negative effect on *D. carota*, the smoke treatment did not affect MGT (Table 3). Early seedling growth improved in two species (*Crepis foetida* and *D. carota*) in the smoke treatment in comparison to the control (Fig. 1). Moreover, four species (*C. foetida*, *Crupina crupinastrum*, *D. carota* and *S. minor*) had significantly higher values of the seedling vigor index in the smoke treatment in comparison to the control (Fig. 2). Seedling growth and seedling vigor index negatively affected from the smoke treatment only in *G. pilosa*.

Fire-related cues significantly enhanced seedling vigor index values in six species (*D. tenuifolia* and *R. lutea* in low heat shock treatments; *C. foetida*, *C. crupinastrum*, *D. carota* and *S. minor* in the smoke treatment) out of the 13 species that seedling vigor index values could be obtained (P < 0.01; Fig. 2). On the other hand, moderate and high heat shock treatments had no positive effect on the seedling vigor index.

4. Discussion

The results show that the seeds of the studied species can tolerate the heat effect of low-intensity fire, but that of moderate and high-intensity fires have a negative impact on the germination and seedling growth. This result suggests that the seeds of plant species found in Central Anatolian steppes are resistant to low-intensity surface fires, but not to high-intensity crown fires. The results also indicate that low heat shocks and smoke enhance seedling vigor in many species, suggesting that some of the species in Central Anatolian steppes take advantage of the presence of low-intensity fires over the others. These results are in accordance with those of many studies conducted in grassland ecosystems worldwide.

In temperate grasslands (Clarke and French, 2005; Gonzalez and Ghermandi, 2012; Schwilk and Zavala, 2012) and tropical grasslands/savannas (Gashaw and Michelsen, 2002; Overbeck et al.,

Table 2
Mean (±SE) germination percentage in the heat shock treatments, after smoke treatment and in the corresponding controls. Taxa codes are given in Table 1. WC and DC are watered control and dry control, respectively. Statistically significant values (P < 0.01) were given in bold. The probability of obtaining a result equal to the corresponding control for each treatment is also given (ns, P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001).

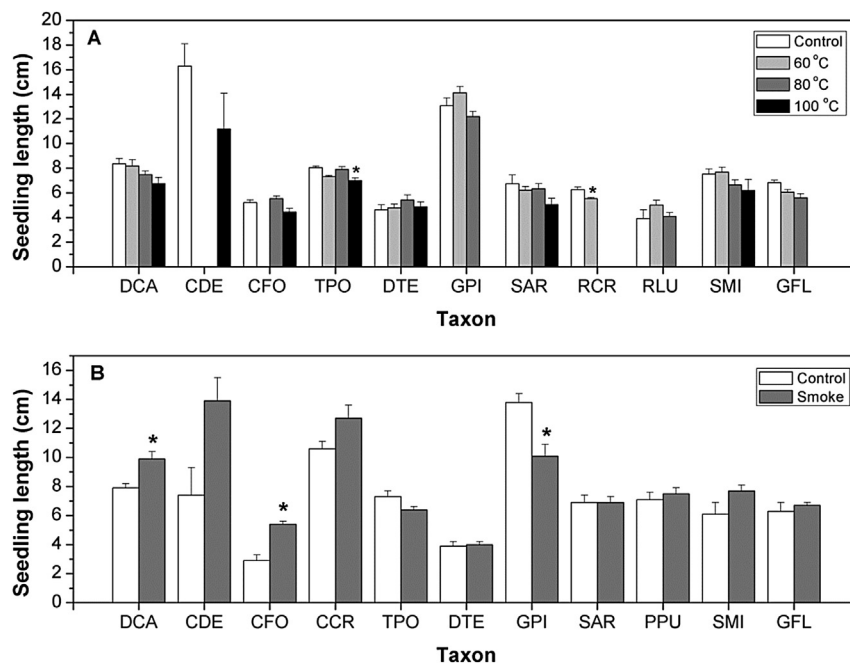| Taxon	WC	Smoke	DC	Heat-shock treatments				
				60 °C	80 °C	100 °C	120 °C	140 °C
BRA	0 ± 0	0 ± 0^{ns}	1 ± 1	2 ± 1^{ns}	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}
DCA	61 ± 9	68 ± 3^{ns}	69 ± 6	52 ± 3*	73 ± 5^{ns}	55 ± 9*	**0 ± 0******	**0 ± 0******
CDE	79 ± 2	**58 ± 6****	43 ± 4	–	–	**10 ± 6******	–	–
CFO	99 ± 1	96 ± 0^{ns}	92 ± 3	–	94 ± 0^{ns}	**58 ± 7******	**0 ± 0******	**0 ± 0******
CCR	51 ± 14	62 ± 4^{ns}	46 ± 12	–	–	**10 ± 4*****	–	–
TPO	99 ± 1	100 ± 0^{ns}	98 ± 1	95 ± 2^{ns}	98 ± 1^{ns}	91 ± 3*	**0 ± 0******	**0 ± 0******
ALE	15 ± 5	12 ± 3^{ns}	–	–	–	–	–	–
DTE	74 ± 5	83 ± 5^{ns}	28 ± 7	26 ± 3^{ns}	42 ± 13^{ns}	25 ± 4^{ns}	**0 ± 0******	**0 ± 0******
GPI	69 ± 8	52 ± 3*	76 ± 2	71 ± 5^{ns}	80 ± 6^{ns}	**43 ± 15******	**0 ± 0******	**0 ± 0******
FAC	21 ± 2	15 ± 6^{ns}	–	–	–	–	–	–
SAR	91 ± 4	81 ± 4^{ns}	88 ± 6	82 ± 6^{ns}	82 ± 9^{ns}	**49 ± 24****	**0 ± 0******	**0 ± 0******
OTO	54 ± 14	57 ± 7^{ns}	–	–	–	–	–	–
PPU	70 ± 8	77 ± 9^{ns}	–	–	–	–	–	–
SSY	0 ± 0	0 ± 0^{ns}	0 ± 0	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}
SAN	2 ± 1	5 ± 3^{ns}	0 ± 0	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}
SBY	1 ± 1	**28 ± 1******	2 ± 1	–	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}	0 ± 0^{ns}
LHI	1 ± 1	1 ± 1^{ns}	2 ± 1	2 ± 1^{ns}	2 ± 2^{ns}	2 ± 1^{ns}	0 ± 0^{ns}	0 ± 0^{ns}
RCR	1 ± 1	3 ± 2^{ns}	13 ± 2	12 ± 4^{ns}	2 ± 2*	2 ± 2*	**0 ± 0******	**0 ± 0******
RLU	24 ± 4	**3 ± 2******	19 ± 5	36 ± 6*	24 ± 5^{ns}	9 ± 4^{ns}	**0 ± 0******	**0 ± 0******
SMI	55 ± 5	79 ± 3*	70 ± 8	60 ± 3^{ns}	68 ± 15^{ns}	**39 ± 11****	**0 ± 0******	**0 ± 0******
GFL	95 ± 3	93 ± 4^{ns}	97 ± 2	99 ± 1^{ns}	95 ± 3^{ns}	**3 ± 3******	**0 ± 0******	**0 ± 0******

Fig. 1. Mean (±SE) seedling lengths (in cm) in heat shock treatments (A), in the smoke treatment (B), and in corresponding controls (Dry control in A, watered control in B) in each species. Asterisk indicates statistically significant (P < 0.01) increase or decrease in treatment in comparison to the corresponding control. Taxa codes are given in Table 1.

2006; Dayamba et al., 2008; Ribeiro and Borghetti, 2014; Le Stradic et al., 2015), the seeds of plant species are not affected by low heat shocks (60 °C and 80 °C for 5′ in our study), but are negatively affected by high heat shocks (120 °C and 140 °C for 5′ in our study). Our study confirms that this general pattern is also valid for the plant species in Central Anatolian steppe. In the case of moderate heat shocks (100 °C for 5′ in our study), however, there is not a consistent germination response pattern among grasslands/savannas of different biogeographic regions. For example, moderate heat shocks are lethal for seeds in Northern American temperate grasslands (Schwilk and Zavala, 2012), while the seeds of many Poaceae species in Australian temperate grasslands can resist moderate heat shocks (Clarke and French, 2005). Moreover, moderate heat shocks do not affect the seeds of grassland species of tropical zones. The seeds of many species in Afrotropical savannas (Gashaw and Michelsen, 2002; Dayamba et al., 2008), Neotropical grasslands (Overbeck et al., 2006; Le Stradic et al., 2015) and savannas (Ribeiro and Borghetti, 2014), and Australian savannas (Williams et al., 2003) can also resist to moderate heat shocks. In our study, the seeds of the most of the species were killed by moderate heat shocks. Based on our results of heat shock treatments, therefore, we conclude that post-fire germination response

Table 3
Mean (±SE) MGT (days) in the heat shock treatments, after smoke treatment and in the corresponding controls. Taxa codes are given in Table 1. WC and DC are watered control and dry control, respectively. Statistically significant values (P < 0.01) were given in bold. The probability of obtaining a result equal to the corresponding control for each treatment is also given (ns, P > 0.05; *P < 0.05; **P < 0.01; ***P < 0.001; ****P < 0.0001).

Taxon	WC	Smoke	DC	Heat-shock treatments		
				60 °C	80 °C	100 °C
DCA	5.5 ± 1.1	**10.8 ± 1.6****	3.8 ± 0.3	8.0 ± 1.5^{ns}	6.2 ± 2.1^{ns}	**26.6 ± 4.0******
TPO[b]	1.8 ± 0.1	2.2 ± 0.1*	1.1 ± 0.1	2.1 ± 0.0^{ns}	2.3 ± 0.2^{ns}	**4.7 ± 0.4*****
CDE	2.0 ± 0.0	2.1 ± 0.0^{ns}	2.2 ± 0.1	–	–	–
CFO[a]	1.5 ± 0.0	3.0 ± 0.3*	1.8 ± 0.2	–	3.2 ± 0.6*	**6.4 ± 0.2******
CCR	2.9 ± 0.1	4.1 ± 0.4*	3.2 ± 0.4	–	–	–
ALE[a]	2.2 ± 0.2	2.5 ± 0.5^{ns}	–	–	–	–
DTE[a]	2.2 ± 0.1	2.3 ± 0.2^{ns}	13.1 ± 5.0	18.4 ± 2.0^{ns}	5.6 ± 0.6^{ns}	16.9 ± 4.2^{ns}
GPI[a,b]	3.2 ± 0.2	6.9 ± 1.0*	4.9 ± 0.2	3.8 ± 0.1^{ns}	7.3 ± 2.8^{ns}	**14.1 ± 0.5****
FAC	34.8 ± 9.3	25.6 ± 8.0^{ns}	–	–	–	–
SAR[b]	4.2 ± 0.5	3.8 ± 0.3^{ns}	6.0 ± 0.3	5.3 ± 0.2^{ns}	5.5 ± 1.0^{ns}	8.9 ± 0.5^{ns}
OTO	5.9 ± 0.7	8.9 ± 1.2^{ns}	–	–	–	–
PPU	5.6 ± 0.8	5.1 ± 0.2^{ns}	–	–	–	–
SBY	–	5.9 ± 0.5	–	–	–	–
RCR	–	–	5.5 ± 0.3	5.5 ± 0.2^{ns}	–	–
RLU[b]	4.8 ± 0.3	–	5.7 ± 0.3	6.0 ± 0.3^{ns}	8.4 ± 2.6^{ns}	–
SMI[b]	3.2 ± 0.2	3.4 ± 0.3^{ns}	4.0 ± 0.3	3.8 ± 0.1^{ns}	6.7 ± 0.9^{ns}	**13.7 ± 1.4******
GFL	8.1 ± 1.5	7.8 ± 0.6^{ns}	3.8 ± 0.1	3.0 ± 0.2^{ns}	5.1 ± 0.4*	–

[a] Non-parametric Kruskal-Wallis test was used to compare smoke treatment with WC.
[b] Non-parametric Kruskal-Wallis test was used to compare heat-shock treatments with DC.
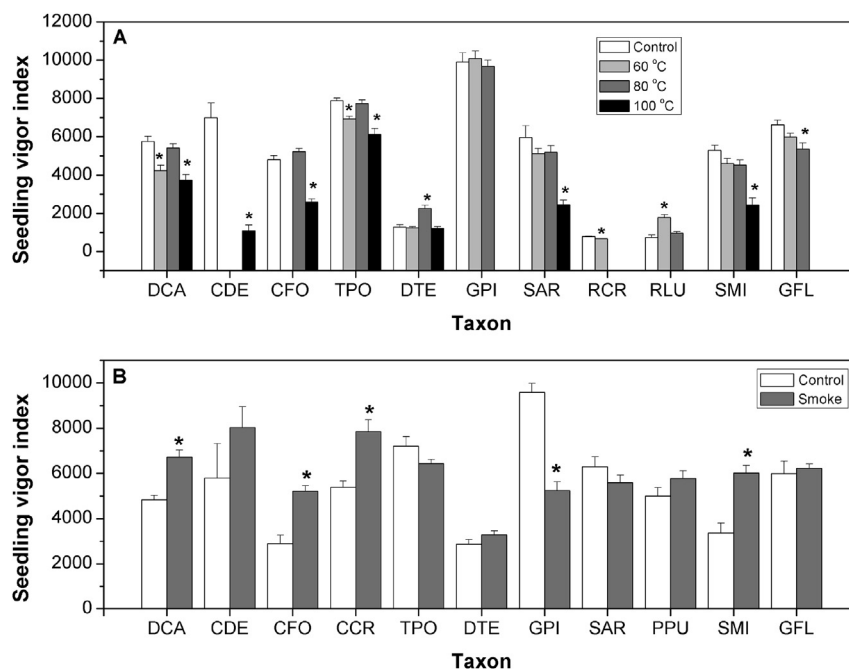

Fig. 2. Mean (±SE) seedling vigor index values in heat shock treatments (A), in the smoke treatment (B), and in corresponding controls (Dry control in A, watered control in B) in each species. Asterisk indicates statistically significant (P < 0.01) increase or decrease in treatment in comparison to the corresponding control. Taxa codes are given in Table 1.

of plant species in Central Anatolian steppe is clearly different from the ones in tropical grassland ecosystems, and follows the pattern observed in temperate grassland ecosystems.

Evidence of smoke-stimulated germination is scarce among plant species in grasslands of temperate (Tsuyuzaki and Miyoshi, 2009; Ghebrehiwot et al., 2012; Gonzalez and Ghermandi, 2012; Schwilk and Zavala, 2012) and tropical zones (Razanamandranto et al., 2005; Dayamba et al., 2010; Le Stradic et al., 2015). In our study, only S. byzantina positively responded to the smoke treatment, and this is the first record of smoke-stimulated germination in Central Anatolian steppe. Although it is hard to mention any trend about the smoke-enhanced germination in grassland

ecosystems worldwide, our study showed that seedling vigor enhanced by the smoke treatment in some species we studied. In grassland ecosystems, the seedling growth of plant species in response to fire cues has been rarely studied (but see; Blank and Young, 1998). Our results suggest that the use of seedling vigor can be a useful tool to understand the plant regeneration responses to various treatments (in our case, to heat shock and smoke) rather the use of the germination percentage or seedling size alone. Many studies have effectively used seedling vigor to determine the establishment success of seedlings (e.g., Van Staden et al., 2006; Abdelgadir et al., 2012). Moreover, seedling vigor is evaluated as a component of vegetative performance or fitness of a plant species

(Darmency et al., 2014). Therefore, the use of the seedling vigor as a function of germination and seedling growth may be a better way to explain the regeneration dynamics in plant communities. In crown-fire ecosystems, as distinct from the post-fire germination and seedling growth patterns in surface fire ecosystems such as grasslands, most seeder species in the plant community have positive response to fire-related germination cues, and seeds of plant species can resist to higher heat-shock temperatures than those can be tolerated by grassland species (Paula et al., 2009). For instance, most of the plant species give positive germination response to moderate- and high-intensity heat shocks and smoke treatments in Mediterranean Turkey (Çatav et al., 2012; Moreira et al., 2012; Çatav et al., 2014), just a few hundred kilometers south of the Central Anatolian plain. This difference suggests that the regional fire regime is responsible for the observed post-fire germination and seedling growth properties of plant species in a specific region. The distinction in fire response of plants found in crown fire and surface fire ecosystems also supports the idea that local or regional fire regimes shape regeneration traits in plant species (Keeley et al., 2011; Moreira et al., 2012).

In conclusion, the seeds of most of the species considered in the study were resistant to low intensity fires, and moreover we found evidence of the presence of fire-stimulated germination or seedling growth in some species. Since the species with seeds that can resist to low-intensity fires would have an advantage over the ones in surface fire ecosystems, the resistance can be evaluated as a 'positive' response to fire in such ecosystems. Our results contribute to understanding the role of fire in temperate grassland ecosystems and show the importance of fire regimes in shaping plant traits.

One limitation of our study is that our results are based on one representative study site in Central Anatolian steppes. However, we believe in that the results are still valuable since this is the first fire-related germination study performed in Anatolian grassland ecosystems. Further studies including more species and more study sites are needed before generalization about the ecosystem-level response to fire could be drawn for Central Anatolian steppes.

Acknowledgments

References

Abdelgadir, H.A., Kulkarni, M.G., Arruda, M.P., Van Staden, J., 2012. Enhancing seedling growth of *Jatropha curcas*—a potential oil seed crop for biodiesel. South Afr. J. Bot. 78, 88–95.

Amraoui, M., Liberato, M.L.R., Calado, T.J., DaCamara, C.C., Coelho, L.P., Trigo, R.M., Gouveia, C.M., 2013. Fire activity over Mediterranean Europe based on information from Meteosat-8. For. Ecol. Manag. 294, 62–75.

Archibald, S., Lehmann, C.E.R., Gómez-Dans, J.L., Bradstock, R.A., 2013. Defining pyromes and global syndromes of fire regimes. Proc. Natl. Acad. Sci. 110, 6442–6447.

Asouti, E., Fairbairn, A., 2002. Subsistence economy in Central Anatolia during the Neolithic: the archaeobotanical evidence. In: Thissen, L., Gerard, F. (Eds.), The Neolithic of Central Anatolia. Internal Developments and External Relations during the 9th-6th Millennia ca. B.C. Proceedings of the International CANeW Round Table, Istanbul, 23–24 November 2001. Ege Yayınları, Istanbul.

Blank, R.R., Young, J.A., 1998. Heated substrate and smoke: influence on seed emergence and plant growth. J. Range Manag. 51, 577–583.

Çatav, Ş.S., Bekar, İ., Ateş, B.S., Ergan, G., Oymak, F., Ülker, E.D., Tavşanoğlu, Ç., 2012. Germination response of five eastern Mediterranean woody species to smoke solutions derived from various plants. Turkish J. Bot. 36, 480–487.

Çatav, Ş.S., Küçükakyüz, K., Akbaş, K., Tavşanoğlu, Ç., 2014. Smoke-enhanced seed germination in Mediterranean Lamiaceae. Seed Sci. Res. 24, 257–264.

Clarke, S., French, K., 2005. Germination response to heat and smoke of 22 Poaceae species from grassy woodlands. Aust. J. Bot. 53, 445–454.

Dantas, V.L., Pausas, J.G., Batalha, M.A., Loiola, P.P., Cianciaruso, M.V., 2013. The role of fire in structuring trait variability in Neotropical savannas. Oecologia 171, 487–494.

Darmency, H., Menchari, Y., Le Corre, V., Délye, C., 2014. Fitness cost due to herbicide resistance may trigger background evolution. Evolution 69, 271–278.

Davis, P.H., 1965-1985. Flora of Turkey and the East Aegean Islands, vol. 1–9. Edinburgh University Press, Edinburgh.

Daws, M., Davies, J., Pritchard, H., Brown, N., Van Staden, J., 2007. Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. Plant Growth Regul. 51, 73–82.

Dayamba, S.D., Tigabu, M., Sawadogo, L., Oden, P.C., 2008. Seed germination of herbaceous and woody species of the Sudanian savanna-woodland in response to heat shock and smoke. For. Ecol. Manag. 256, 462–470.

Dayamba, S.D., Sawadogo, L., Tigabu, M., Savadogo, P., Zida, D., Tiveau, D., Oden, P.C., 2010. Effects of aqueous smoke solutions and heat on seed germination of herbaceous species of the Sudanian savanna-woodland in Burkina Faso. Flora 205, 319–325.

Fırıncıoğlu, H.K., Seefeldt, S.S., Şahin, B., Vural, M., 2009. Assessment of grazing effect on sheep fescue (*Festuca valesiaca*) dominated steppe rangelands, in the semi-arid Central Anatolian region of Turkey. J. Arid Environ. 73, 1149–1157.

Gashaw, M., Michelsen, A., 2002. Influence of heat shock on seed germination of plants from regularly burnt savanna woodlands and grasslands in Ethiopia. Plant Ecol. 159, 83–93.

Ghebrehiwot, H.M., Kulkarni, M.G., Kirkman, K.P., Van Staden, J., 2012. Smoke and heat: influence on seedling emergence from the germinable soil seed bank of mesic grassland in South Africa. Plant Growth Regul. 66, 119–127.

Giglio, L., Descloitres, J., Justice, C.O., Kaufman, Y.J., 2003. An enhanced contextual fire detection algorithm for MODIS. Remote Sens. Environ. 87, 273–282.

Gonzalez, S.L., Ghermandi, L., 2012. Fire cue effects on seed germination of six species of Northwestern Patagonian grasslands. Nat. Hazards Earth Syst. Sci. 12, 2753–2758.

Jäger, A.K., Light, M.E., Van Staden, J., 1996. Effects of source of plant material and temperature on the production of smoke extracts that promote germination of light-sensitive lettuce seeds. Environ. Exp. Bot. 36, 421–429.

Kaniewski, D., Paulissen, E., De Laet, V., Waelkens, M., 2008. Late holocene fire impact and post-fire regeneration in the Bereket basin, Taurus mountains, southwest Turkey. Quat. Res. 70, 228–239.

Keeley, J.E., Fotheringham, C.J., 1998. Smoke-induced seed germination in California chaparral. Ecology 79, 2320–2336.

Keeley, J.E., Pausas, J.G., Rundel, P.W., Bond, W.J., Bradstock, R.A., 2011. Fire as an evolutionary pressure shaping plant traits. Trends Plant Sci. 16, 406–411.

Keeley, J.E., Bond, W.J., Bradstock, R.A., Pausas, J.G., Rundel, P.W., 2012. Fire in Mediterranean Ecosystems: Ecology, Evolution and Management. Cambridge University Press, Cambridge.

Kulkarni, M.G., Sparg, S.G., Light, M.E., Van Staden, J., 2006. Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide. J. Agron. Crop Sci. 192, 395–398.

Kulkarni, M.G., Sparg, S.G., Van Staden, J., 2007. Germination and post-germination response of *Acacia* seeds to smoke-water and butenolide, a smoke-derived compound. J. Arid Environ. 69, 177–187.

Le Stradic, S., Silveira, F.A.O., Buisson, E., Cazelles, K., Carvalho, V., Fernandes, G.W., 2015. Diversity of germination strategies and seed dormancy in herbaceous species of *campo rupestre* grasslands. Austral Ecol. http://dx.doi.org/10.1111/aec.12221.

Moran, M.D., 2003. Arguments for rejecting the sequential Bonferroni in ecological studies. Oikos 100, 403–405.

Moreira, B., Tormo, J., Estrelles, E., Pausas, J.G., 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean basin flora. Ann. Bot. 105, 627–635.

Moreira, B., Tavsanoglu, Ç., Pausas, J.G., 2012. Local versus regional intraspecific variability in regeneration traits. Oecologia 168, 671–677.

Mutlu, B., Erik, S., Tarıkahya, B., 2008. New contributions to the flora of Beytepe campus (Ankara) and floristic comparison with neighboring floras and other campus floras. Hacettepe J. Biol. Chem. 36, 181–195.

NASA, 2012. Global fire maps. http://lance-modis.eosdis.nasa.gov/cgi-bin/imagery/firemaps.cgi (accessed 09.12.2012).

Naveh, Z., 1975. The evolutionary significance of fire in the Mediterranean region. Plant Ecol. 29, 199–208.

Overbeck, G.E., Müller, S.C., Pillar, V.D., Pfadenhauer, J., 2006. No heat-stimulated germination found in herbaceous species from burned subtropical grassland. Plant Ecol. 184, 237–243.

Paula, S., Arianoutsou, M., Kazanis, D., Tavsanoglu, Ç., Lloret, F., Buhk, C., Ojeda, F., Luna, B., Moreno, J.M., Rodrigo, A., Espelta, J.M., Palacio, S., Fernández-Santos, B., Fernandes, P.M., Pausas, J.G., 2009. Fire-related traits for plant species of the Mediterranean basin. Ecology 90, 1420.

Pausas, J.G., Alessio, G., Moreira, B., Corcobado, G., 2012. Fires enhance flammability in *Ulex parviflorus*. New. Phytol. 193, 18–23.

Razanamandranto, S., Tigabu, M., Sawadogo, L., Odén, P.C., 2005. Seed germination of eight savanna-woodland species from West Africa in response to different cold smoke treatments. Seed Sci. Technol. 33, 315–328.

Ribeiro, L.C., Borghetti, F., 2014. Comparative effects of desiccation, heat shock and

high temperatures on seed germination of savanna and forest tree species. Austral Ecol. 39, 267—278.

Schwilk, D.W., Zavala, N., 2012. Germination response of grassland species to plant-derived smoke. J. Arid Environ. 79, 111—115.

Şekercioğlu, Ç.H., Anderson, S., Akçay, E., Bilgin, R., Can, Ö.E., Semiz, G., Tavşanoğlu, Ç., Yokeş, M.B., Soyumert, A., İpekdal, K., Sağlam, İ.K., Yücel, M., Dalfes, H.N., 2011. Turkey's globally important biodiversity in crisis. Biol. Conserv. 144, 2752—2769.

Tavşanoğlu, Ç., Çatav, Ş.S., 2012. Seed size explains within-population variability in post-fire germination of Cistus salviifolius. Ann. Bot. Fenn. 49, 331—340.

Tavşanoğlu, Ç., Gürkan, B., 2014. Long-term post-fire dynamics of co-occurring woody species in Pinus brutia forests: the role of regeneration mode. Plant Ecol. 215, 355—365.

Tompsett, P.B., Pritchard, H.W., 1998. The effect of chilling and moisture stress on the germination, desiccation tolerance and longevity of Aesculus hippocastanum L. seeds. Ann. Bot. 82, 249—261.

Trabaud, L., 1979. Etude du comportement du feu dans la Garrigue de Chêne kermes à partir des températures et des vitesses de propagation. Ann. For. Sci. 36, 13—38.

Tsuyuzaki, S., Miyoshi, C., 2009. Effects of smoke, heat, darkness and cold stratification on seed germination of 40 species in a cool temperate zone in northern Japan. Plant Biol. 11, 369—378.

Turner, R., Roberts, N., Jones, M.D., 2008. Climatic pacing of Mediterranean fire histories from lake sedimentary microcharcoal. Glob. Planet. Change 63, 317—324.

Turner, R., Roberts, N., Eastwood, W.J., Jenkins, E., Rosen, A., 2010. Fire, climate and the origins of agriculture: micro-charcoal records of biomass burning during the last glacial—interglacial transition in Southwest Asia. J. Quat. Sci. 25, 371—386.

Van Staden, J., Sparg, S.G., Kulkarni, M.G., Light, M.E., 2006. Post-germination effects of the smoke-derived compound 3-methyl-2H-furo[2,3-c]pyran-2-one, and its potential as a preconditioning agent. Field Crops Res. 98, 98—105.

Vannière, B., Power, M.J., Roberts, N., Tinner, W., Carrión, J., Magny, M., Bartlein, P., Colombaroli, D., Daniau, A.L., Finsinger, W., Gil-Romera, G., Kaltenrieder, P., Pini, R., Sadori, L., Turner, R., Valsecchi, V., Vescovi, E., 2011. Circum-Mediterranean fire activity and climate changes during the mid-Holocene environmental transition (8500—2500 cal. BP). Holocene 21, 53—73.

Williams, P.R., Congdon, R.A., Grice, A.C., Clarke, P.J., 2003. Fire-related cues break seed dormancy of six legumes of tropical eucalypt savannas in north-eastern Australia. Austral Ecol. 28, 507—514.

Zar, J.H., 1996. Biostatistical Analysis, third ed. Prentice Hall, New Jersey.