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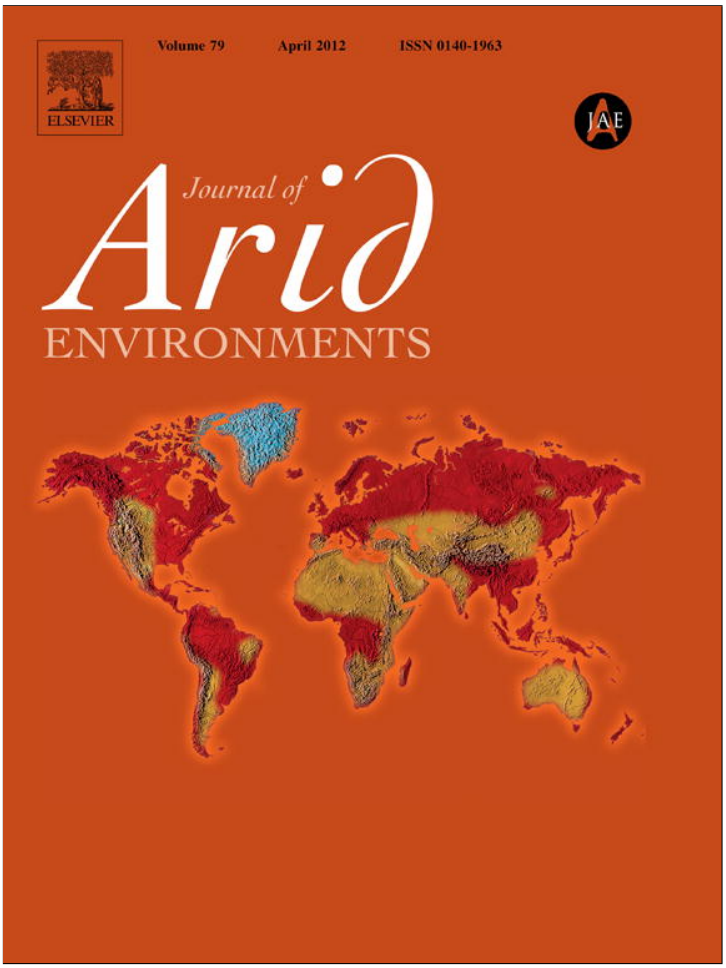

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Short communication

Germination response of grassland species to plant-derived smoke

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ABSTRACT

In fire-prone ecosystems, many species require signals such as heat or smoke to cue seedling establishment to the relatively favorable post fire environment. Grassland ecosystems are often maintained by recurring fire and many grassland species are considered well adapted to fire. Despite this, smoke-induced germination has been studied much less in grasslands than in shrublands subject to crown fire. We tested 15 species native to the southern Great Plains and Edwards Plateau of Texas for smoked-stimulated or heat stimulated germination. Smoke and heat treatments were followed by either a true wet stratification or a dry cold period. Four species exhibited smoke-stimulated germination while the others exhibited no response or were inhibited by smoke. In two of the species that showed a positive response, smoke acted as a substitute for wet cold stratification. Heat treatments proved lethal to all species tested.

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1. Introduction

Seed dormancy is a common trait among plant species that inhabit environments characterized by periodic disturbance such as fire. In fire-prone ecosystems, many species require signals such as heat or smoke to cue seedling establishment to the relatively favorable post fire environment. Smoke-stimulated seed germination has been widely reported from mediterranean-climate ecosystems (de Lange and Boucher, 1990; Dixon et al., 1995; Keeley and Fotheringham, 1998; Roche et al., 1998). Researchers in these systems have identified possible important chemical components of such cues (Flematti et al., 2004; Keeley and Fotheringham, 1997, 1998). Outside of mediterranean-climate ecosystems, smoke as a germination cue has been less often investigated. A recent study in fire-prone upland Florida habitats found smoke-cued germination to be rare (present in three of 20 species, Lindon and Menges, 2008). Grassland ecosystems are often maintained by recurring fire and many grassland species are considered well adapted to fire. Despite this, until quite recently, smoke-induced germination had not been investigated in North American grasslands. Recent work, however, demonstrated that several species of the northern Great Plains show increased germination when exposed to smoke (Jefferson et al., 2008).

Semi-arid grasslands in the southern Great Plains and rolling plains of west Texas were historically maintained by frequent fire and a reduction in fire frequency since EuroAmerican settlement is thought to be one cause of increased shrub encroachment (McPherson et al., 1988). Although the importance and prevalence of fire in these grasslands might suggest an adaptive role for smoke-stimulated seed germination, if fire was historically frequent (every several years), then fire may have been less useful as a cue. Under short fire return intervals, fire may provide less information to a seed regarding the suitability of current conditions than fire does in mediterranean-climate shrublands prone to crown fire with fire return intervals of several decades. In mediterranean-type climates, fires occur during summer or early fall, and smoke-stimulated species often require a subsequent cold stratification period to break dormancy (Keeley and Fotheringham, 1998). In the grasslands of the southern Great Plains and the mixed woodlands and savannas of Texas, fires can occur at any time of year. The lack of a dendrochronology record makes reconstructing prehistoric fire seasons difficult (Ford and McPherson, 1996). Although dormant season (winter and spring) was the preferred season for prescribed burning historically, there has been increasing interest in summer burning (Engle and Bidwell, 2001). Although many species are known to require cold stratification (Jefferson et al., 2008; Jordan and Haferkamp, 1989), depending upon the historic timing of fire relative to the growing season, it is possible that a fire-cued grassland species may require no cold wet stratification after the smoke cue.

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We tested 15 herbaceous species native to Texas, USA, for seed dormancy and smoked-stimulated germination. All species selected belong to genera in which smoke-stimulated seed germination has been previously reported elsewhere. In addition to tests for the smoke cue, we investigated the role of true cold stratification vs a dry cold period following smoke exposure and we tested these species for response to heat treatment of seeds.

2. Materials and methods

We tested 15 herbaceous species for smoke-stimulated seed germination. The species were *Coreopsis basalis* (A Dietr) S.F. Blake, *Coreopsis lanceolata* L., *Coreopsis tinctoria* Nutt. *Echinacea angustifolia* DC., *Echinacea purpurea* L. (Moench) (Asteraceae); *Liatris mucronata* DC., *Liatris pycnostachya* Michx. (Lamiaceae); *Monarda citriodora* Cerv. Ex Lag., *Salvia coccinea* Buc'hoz ex Etl., *Salvia farinacea* Benth., *Salvia penstemonoides* Kunth and Bouché (Lamiaceae); *Bouteloua curtipendula* (Michx.) Torr., *Bouteloua eriopoda* (Torr.) Torr., *Bouteloua gracilis* (Willd. Ex Kunth) Lag ex Griffiths (Poaceae); *Penstemon cobaea* Nutt. (Scrophulareaceae). All nomenclature follows Diggs et al. (1999) for species contained therein and Correll and Johnston (1979) for the remainder of the species. Forb seeds were obtained from commercial seed sources (Native American Seed, Junction, Texas) and grass seed was collected by the authors from sites on the Edwards Plateau near Junction, Texas. *B. curtipendula* was collected from three additional sites on the Llano Estacado of west Texas and the rolling plains — these populations were examined separately in trials outlined below, but results did not differ among populations. Seeds were separated from flower and fruit material and were subjected to a cold storage of three weeks at 4 °C and 10% relative humidity prior to smoke and heat treatments. We conducted two separate experiments on these species to test the effect of smoke or heat effects combined with either of two alternative stratification treatments. We conducted a final, third small experiment on those species that exhibited positive germination responses to smoke in the second experiment to test smoke effects when combined with no stratification.

2.1. Experiment 1

In the first experiment, we tested if smoke or heat can increase germination. Seeds were subjected to smoke exposure treatments of four or eight minutes and two heat treatments of 30 or 60 s in an oven at 100 °C. For smoke treatments, seeds in glass Petri dishes were placed in a glass chamber (60 cm × 44 cm, 18 cm high) with a plexiglass top sealed with a cork gasket. The chamber was connected to a beekeeper's smoker via an inlet tube which released smoke 3 cm from the bottom of the chamber and to an outlet tube connected to a vacuum pump. When exposing the seeds to smoke, a single replicate of each species was placed into the smoke-chamber for each trial. Smoke was produced by burning *Buchloe dactyloides* (Nutt.) Engelm. (buffalo grass) and was pumped into the chamber by drawing vacuum on the outlet tube for one minute of filling time. After this one minute, the pump was turned off and the seeds were left for the treatment time of four or eight minutes. During the smoke exposure time, the temperature inside the chamber was monitored to ensure that it did not rise more then 0.5 °C above ambient (25 °C). The treatment was ended by venting the chamber through removal of the entire plexiglass top underneath a fume hood. Four replicates of 25 seeds each were used per treatment per species. The first experiment was conducted Nov–Dec 2008.

The seeds were sown on filter paper (Whatman No. 1) in 55 mm polystyrene Petri dishes following smoke or heat exposure (Keeley and Fotheringham, 1998). They were then watered and placed in a stratification period for one month at 4 °C. The seeds were checked daily for germination starting with stratification. The three *Bouteloua* species (all warm season C4 grasses) were not included in the first stratification experiment because these species are reported to not require wet cold stratification (Jordan and Haferkamp, 1989; Knipe, 1968).

2.2. Experiment 2

In the second experiment, smoke treatments, heat treatments and controls were identical to those in Treatment 1, but the post treatment stratification differed. Seeds were not subjected to a true wet cold stratification, but were placed in dry cold (4 °C) for a period of 1 month at a relative humidity of 10%. The seeds were checked daily for germination starting with stratification. The second experiment was conducted in March–April 2009.

2.3. Experiment 3

We conducted a final follow up smaller-scale smoke exposure experiment on three species that exhibited positive responses to smoke in Experiment 2. This was identical to Experiment 2 except that seeds were placed in incubation immediately following smoke treatments (or control) rather than receiving any cold stratification (seeds had been cold-stored, however, prior to the experiment). This experiment was conducted in April–May 2011.

2.4. Measuring germination and analyses

In all three experiments, after the dry or wet cold period, the seeds were watered, and incubated at 24 °C with light and dark periods of 12 h each. Seeds were checked twice-weekly for germination for one month. Germination was defined to be the initial presence of an epicotyl.

We tested for treatment effects on germination proportions using generalized linear models with a binomial link function using the glm package in R (Pinheiro et al., 2011; R Development Core Team, 2011). If there was no difference between the two smoke treatments or two heat treatments, we combined both treatments for comparison with the control. Separate models were analyzed for each species and reported *p*-values were adjusted across the 15 species tested to maintain an experiment-wide false discovery rate of 0.05 (Benjamini and Hochberg, 1995). The *p*-value adjustment was conducted separately for each of the three experiments. We reported treatment effects as significant in the results if logistic glm treatment coefficient *p*-values were less than 0.05 after experiment-wide adjustment for multiple tests.

3. Results

In Experiments 1 and 2, heat treatments (30 and 60 s at 100 °C) resulted in zero germination for all species. We therefore dropped heat treatments from further analyses because zero variance would invalidate linear modeling.

3.1. Experiment 1

In the first experiment, in which treatment and controls were followed by wet cold stratification, one species exhibited increased germination following smoke exposure while four exhibited negative responses (Fig. 1). Three species showed no significant response to smoke (Fig. 2) and four species had no germination in treatments or controls (*C. tinctoria*, *E. angustifolia, L. pycnostachya*, and *S. penstemonoides*).

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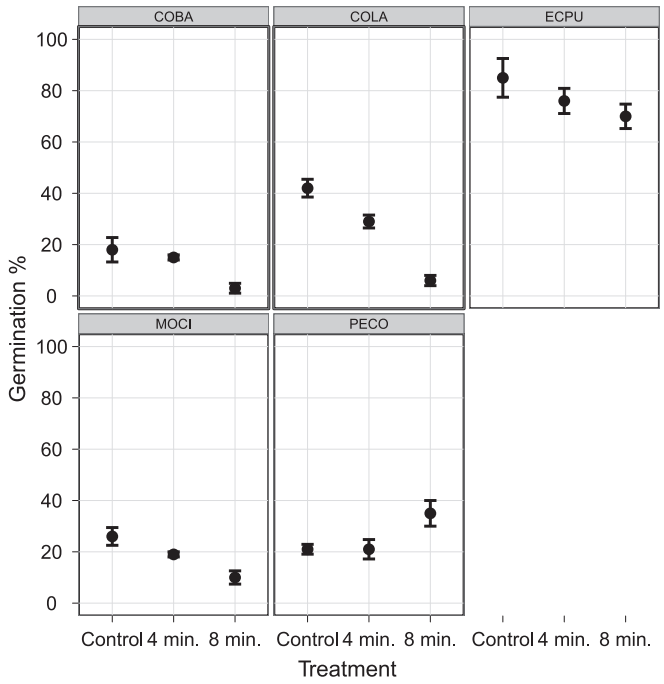

Fig. 1. Germination responses of five species that exhibited significant smoke treatment effects in Experiment 1 (wet cold stratification). Dots and bars indicate mean ± standard error. A significant treatment response was indicated by logistic generalized linear model treatment coefficient p-values <0.05 after experiment-wide adjustment for multiple tests. COBA = *Coreopsis basalis*, COLA = *Coreopsis lanceolata*, ECPU = *Echinacea purpurea*, MOCI = *Monarda citriodora*, PECO = *Penstemon cobea*.

3.2. Experiment 2

In the second experiment, in which seeds were subjected to a dry cold period following treatment or controls, four species exhibited increased germination and one exhibited decreased germination in smoke treatments relative to controls (Fig. 3). Eight species showed no significant response to smoke (Fig. 4) and two species had little or no germination (*L. pycnostachya*, and *S. penstemonoides*) in controls or treatments.

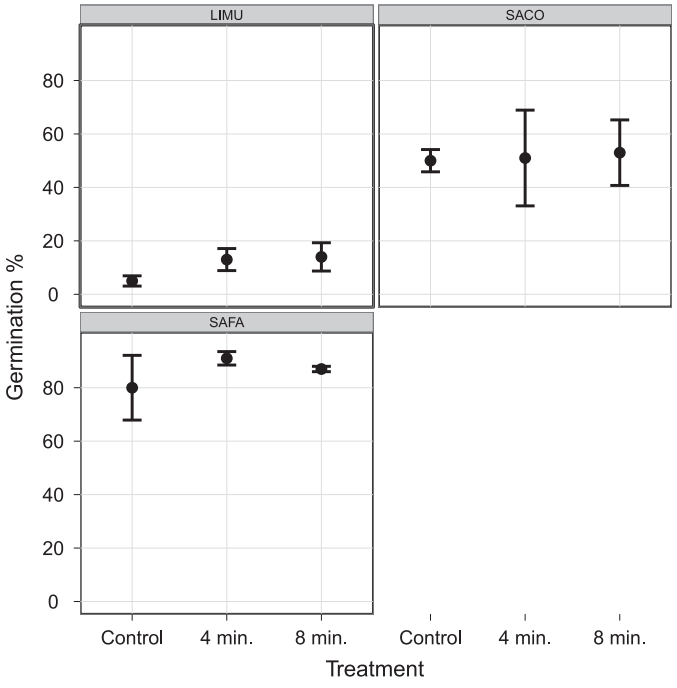

Fig. 2. Germination responses of three species that showed no smoke treatment effects in Experiment 1 (wet cold stratification). LIMU = *Liatris mucronata*, SACO = *Salvia coccinea*, SAFA = *Salvia farinacea*.

3.3. Experiment 3

We examined smoke response in three species, *B. gracilis*, *S. coccinea* and *S. farinacea*, in a final experiment in which any stratification was omitted. For all three species, the response was very similar to that in Experiment 2: significantly increased germination in combined smoke treatments relative to controls (*B. gracilis*: 41% vs 78%, *S. coccinea*: 20% vs 53%, *S. farinacea*: 22% vs 39%).

4. Discussion

Historically, fire acted as a recurring disturbance in the southern Great Plains and mixed southern grasslands and these fires probably assisted in maintaining the grasslands by excluding woody vegetation (Ford and McPherson, 1996). Currently, fire is an important tool for rangeland restoration (Ansley and Castellano, 2006). Despite this, fire-cued seed germination appears to be relatively rare in this system. Smoke increased germination relative to wet stratified controls in only one species and our heat treatment proved at least inhibitory and probably lethal to all seeds — although similar heat treatments have successfully broken dormancy in other species (e.g. Keeley and Bond, 1997). Rapid fungal colonization of heat treated seeds (Zavala, personal observation) suggests that heat was lethal. A study of species from a fire-prone subtropical grassland similarly found no evidence of heat stimulated germination (Overbeck et al., 2006).

A novel result of our study is that in two *Salvia* species, aerosol smoke can act substitute for wet cold stratification in cueing seed

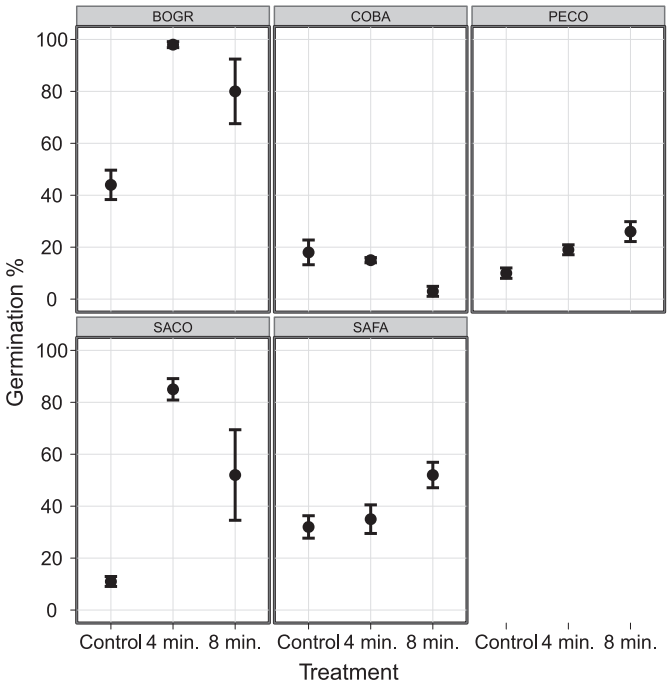

Fig. 3. Germination responses of five species that exhibited significant smoke treatment effects in Experiment 2 (dry cold stratification). Dots and bars indicate mean ± standard error. A significant treatment response was indicated by logistic generalized linear model treatment coefficient p-values <0.05 after experiment-wide adjustment for multiple tests. BOGR = *Bouteloua gracilis*, COBA = *Coreopsis basalis*, PECO = *Penstemon cobea*, SACO = *Salvia coccinea*, SAFA = *Salvia farinacea*.

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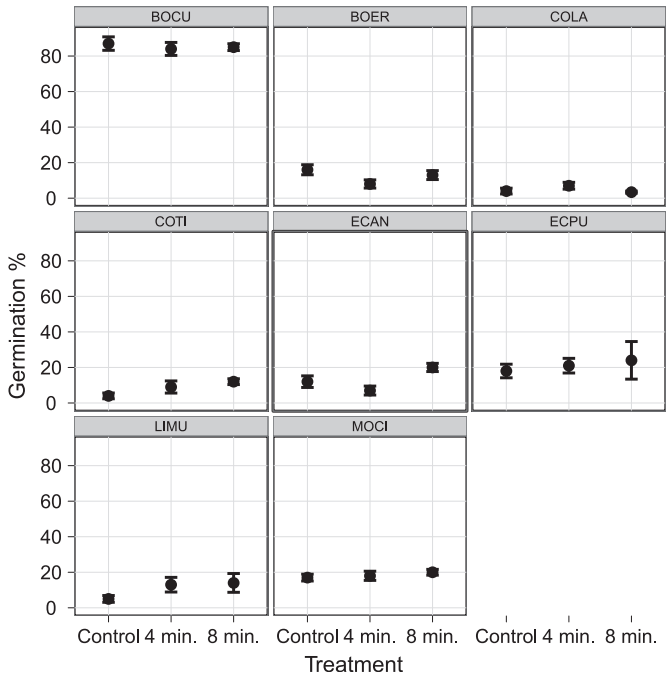

Fig. 4. Germination responses of eight species that showed no smoke treatment effects in Experiment 2 (dry cold stratification). BOCU = *Bouteloua curtipendula* (Texas panhandle population shown), BOER = *B. eriopoda*, COLA = *Coreopsis lanceolata*, COTI = *Corepsis tinctoria*, ECAN = *Echinacea angustifolia*, ECPU = *Echinacea purpurea*, LIMU = *Liatris mucronata*, MOCI = *Monarda citriodora*.

germination. This substitution effect may have implications on the timing of germination in the species: late summer fires could potentially trigger late summer germination in species which would otherwise require a winter/spring stratification period. If autumn germination is non-adaptive for these species, then this suggests that late summer or autumn fires could be detrimental for these species. Interestingly, the two *Salvia* species exhibiting the smoke cue response belong to the same clade within the large *Salvia* complex (Walker et al., 2004). Our small third experiment suggests that for these two *Salvia*, increased germination following smoke exposure occurs even if smoke exposure is immediately followed by incubation. This experiment, however, is not a true test of no stratification because all seeds were stored in cold prior to the experiments. More completely factorial experiments investigating interactions among smoke and other germination cues are needed.

Smoke-stimulated seed germination has only been rarely reported among the Poaceae (Baxter et al., 1994). One previous paper, however, reported that a common North American C4 grass, *B. curtipendula*, exhibited smoke-stimulated germination (Jefferson et al., 2008). We were unable to duplicate this result with any of our seeds collected from three sites in central and west Texas, however. All three populations had very high germination under all treatments, although there were some differences in germination proportions among populations (results not shown). *B. curtipendula* is a widespread species, and Jefferson et al. (2008) tested seed from tallgrass prairie populations, whereas we gathered seed from shortgrass systems at three disparate locations. This discrepancy suggests that there is variation in seed germination response among populations of this widespread grass. Additionally, as Jefferson et al. (2008) reported unadjusted *p*-values, the value they report would not have shown up as significant in our study after adjustment. Although we were originally skeptical of smoke-stimulated germination in a C4 grass, we did however, see a strong positive response to smoke in *B. gracilis* — another C4 grass species not tested by Jefferson et al. (2008). The positive response in *B. gracilis* is interesting given that this species, like other warm season grasses of the Great Plains, is not known for dormancy and does not require cold stratification (Jordan and Haferkamp, 1989; Knipe, 1968). In Experiment 2, Four minutes of smoke brought germination rates to near 100% while controls had germination rates closer to 50% (Fig. 3). The germination rates in controls were consistent with the relatively low germination rates previously reported for this species (Blake, 1935). We only tested one source population, so we cannot say how widespread this smoke response is within *B. gracilis*.

Overall, smoke-stimulated seed germination is not common among these grassland species. Even those cases of increased germination following smoke exposure do not indicate any absolute reliance on a smoke as is seen in some species from mediterranean-type climates. Smoke may not be a necessary cue, but may substitute for other cues such as cold wet stratification. This suggests that the seasonal timing of prescribed burning may influence the germination and population dynamics of some species. This is especially relevant for *B. gracilis* — most considerations of fire seasonality in *B. gracilis* grasslands have focused on the survivorship and vigor of the adult plants, but if smoke can increase germination, then fire my have additional unexplored effects on *B. gracilis* populations.

Acknowledgments

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