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Effects of smoke and fire-related cues on Panstemon barbatus seeds

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Notes and Discussion

Effects of Smoke and Fire-related Cues on Penstemon barbatus Seeds

ABSTRACT.—Previous research has found that exposure to fire-related cues enhances germination of some plant species, and such species may exist in frequent-fire southwestern United States *Pinus ponderosa* forests. I performed four greenhouse experiments with *Penstemon barbatus*, a perennial forb common in *P. ponderosa* forests, testing seed responses to liquid and air smoke, charred *P. ponderosa* wood and leachate, heat and emergence substrates. Liquid smoke increased *P. barbatus* emergence to as high as 63%, 44% greater than controls, and enhanced emergence in all 4 experiments. Air smoke produced by burning *P. ponderosa* litter for 15 min appeared to increase emergence similar to liquid smoke. In contrast, *P. ponderosa* charred wood and charred wood leachate did not improve emergence, and sometimes inhibited positive effects of smoke. Heating samples at 100 C for 30 min did not affect emergence. Substrate and liquid smoke interacted in one experiment, with smoke increasing emergence more sharply on basalt and potting soil than on limestone soil. These greenhouse findings have practical implications for germinating *P. barbatus*, but need testing under field conditions to evaluate their importance in this species' population biology after fire in *P. ponderosa* forests.

INTRODUCTION

Seeds of some plant species inhabiting fire-prone ecosystems are thought to have evolved responses to fire cues triggering germination (Keeley, 1991; Dixon *et al.*, 1995; Van Staden *et al.*, 2000). These cues presumably cause germination to coincide with post-fire environments favorable for seedling survival and growth (Baldwin *et al.*, 1994; Baskin and Baskin, 1998; Bell, 1999). In frequent-fire Australian heath ecosystems, for example, Burne *et al.* (2003) found that spraying soil with liquid smoke induced emergence of the shrub *Grevillea rudis* Meisn. from the soil seed bank whereas no emergence occurred on untreated plots. In greenhouse experiments with 45 species from frequent-fire California chaparral, Keeley (1987) reported that *Adenostoma fasciculatum* Hook. & Arn. charred wood enhanced germination of 25% of the species, while 25% of the species exhibited heat-stimulated germination.

Fire-germination relationships may also be important in the plant ecology of frequent-fire southwestern United States *Pinus ponderosa* P. & C. Lawson forests, but little is known about these relationships in these forests. Fire-return intervals for presettlement *P. ponderosa* forests before fire exclusion in the late 1800s, for example, averaged 3.7 y near Flagstaff in northern Arizona (Fulé *et al.*, 1997). More than 800 plant species occur in northern Arizona *P. ponderosa* forests (McDougall, 1973), and it seems plausible that some species have evolved germination strategies in accordance with frequent surface fires long characteristic of these forests. In preliminary experiments I found that emergence of the perennial forb *Penstemon barbatus* (Cav.) Roth inhabiting southwestern *P. ponderosa* forests responded strongly to liquid smoke, so I conducted several experiments to more closely examine its responses to fire-related cues. *Penstemon barbatus* has small seeds (≈ 0.8 mg) thought to be primarily wind dispersed, and as with many *Penstemon* is believed to be an opportunistic species colonizing disturbed habitat (Fulé *et al.*, 2001). This paper reports the results of 4 greenhouse experiments testing emergence responses of *P. barbatus* seeds to liquid and air smoke, charred *P. ponderosa* wood and leachate, heat and emergence substrates.

METHODS

General experimental procedures.—*Penstemon barbatus* seeds collected around Flagstaff, Arizona, were stored at −5 C for 12–17 mo, and used in all 4 experiments. Based on tetrazolium testing of 25 seeds (Association of Official Seed Analysts, 2000), the seed lot used in these experiments had a viability of 88%. For all experiments, 16 *P. barbatus* seeds per pot were lightly pressed in 4 rows of 4 seeds on top of either natural or potting soil in 700-cm^3 square plastic pots. I filled pots with 300 cm^3 of sterile potting soil (United Industries Co., St Louis, Missouri). If required in a specific experiment, I also placed 120 cm^3 of natural soil on top of the potting soil. Commercially available liquid smoke (Wright's Brand, Roseland, New Jersey), diluted to desired concentrations using deionized water and applied on top of

seeds in soil, was used for experiments requiring liquid smoke. I arranged pots randomly on a greenhouse bench in a Northern Arizona University greenhouse (temperature ≈ 24 C) and kept samples moist by daily watering with tap water. To check for seed contamination, which did not occur, 2–4 pots per experiment containing only soil were randomly interspersed with treated pots.

I counted emerging *Penstemon barbatus* every 10 d and ran experiments for 60 d, with 90–95% of emergence in all experiments occurring in the first 30 d. Experiment 1 began on 12 March, Experiment 2 on 5 May, Experiment 3 on 28 July and Experiment 4 on 22 August 2004. All experiments occurred under natural lighting except for Experiment 1, which also occurred under 2 h of artificial lighting daily to supplement natural lighting. I used the percent of 16 seeds (all seeds including non-viable seeds) emerged per pot as the response variable for analysis of variance (ANOVA) performed with the software SAS JMP (SAS Institute, 2002). For mean separation, I used Fisher's protected least significant difference with $\alpha = 0.05$. Raw data approximated equal variance (Levene test) and normality assumptions (Shapiro-Wilk test), and arcsin transformations did not change statistical conclusions. Specific procedures and ANOVA models for each experiment are given in the following sections.

Experiment 1.—This experiment was a 2-factor, split-plot factorial design with 2 levels (none, exposure to 100 C for 30 min) of the whole plot factor heat, and 2 levels (none, 60 ml of 10% liquid smoke) of the subplot factor liquid smoke. Locally collected (35°16′14″N, 111°42′35″W) native soil derived from basalt was placed on top of potting soil, and seeds were sown on top of the basalt soil. Pots including soil and seeds were placed in an electric oven, heating was performed on all heated samples at once, and then liquid smoke was applied to smoke samples. I randomly assigned treatments to pots, and used 3 pots (replicates) per treatment combination for a total of 12 pots.

Experiment 2.—This experiment was a single-factor, completely random design with 6 treatments (60 ml of 5, 10 or 20% liquid smoke, 30 ml charred wood, 10% liquid smoke + 30 ml charred wood and control) and 5 replicates per treatment for a total of 30 pots. Charred wood (blackened but not ashed with no visible bark remaining) was prepared by burning *Pinus ponderosa* logs in a wood-burning stove and grinding burned pieces to pass a 4-mm sieve. About 5.5–6.5 g of charred wood comprised 30 ml by volume. I used potting soil for this experiment, placed the charred wood on top of the potting soil forming a discontinuous layer of charred wood averaging 1 mm thick, and sowed seeds intermixed with the charred wood.

Experiment 3.—To asses relative effects of air and liquid smoke and *Pinus ponderosa* charred wood leachate, this experiment employed a 3-factor, split-plot factorial design with 2 levels each of air smoke (none, 15 min exposure), liquid smoke (none, 60 ml of 10% concentration) and charred wood leachate (none, 60 ml of 10 g/100 ml concentration). Because air smoke treatments were applied to all air-treated samples at once, the whole plot factor was air smoke and the subplot factors were liquid smoke and charred wood leachate. Treatments were randomly assigned to pots, and 3 replicates were used for each of the 8 treatment combinations. I used potting soil overlaid with native soil derived from limestone (35°07′13″N, 111°39′29″W) for this experiment.

Air smoke treatments were performed on pots containing soil and seeds in a home-made smoker. The smoker consisted of a 40 × 20 cm closed barbecue grill containing *Pinus ponderosa* needles, bark, twigs and cones for fuel, 3 m of coiled 1.3-cm diameter copper wire to carry smoke and minimize heat transfer to the chamber, a 62 × 75 cm enclosed plywood and glass chamber for housing samples, and a fan enclosed at the top of the chamber to pull smoke into the chamber. I monitored temperatures within the chamber using a thermometer, and temperatures remained within ± 1 C of ambient temperature (≈25 C) during smoker operation. Smoke was pumped into the chamber continuously and remained inside the enclosed chamber for the duration of the exposure time. I prepared charred wood leachate by soaking pieces (<3 cm diameter) of charred *P. ponderosa* wood for 24 h in deionized water, and added leachate or liquid smoke to randomly assigned samples after air smoke treatments.

Experiment 4.—This experiment tested whether effects of liquid smoke were consistent across emergence substrates, and consisted of 3 levels (basalt, limestone or potting soil) of soil and 2 levels of liquid smoke (none, 60 ml of 10% concentration) in a completely random factorial design with 5 replicates per treatment combination for 30 total pots. The same locally collected basalt and limestone soils (both at ≈2200 m elevations) used in Experiments 1 and 3 were used in this experiment. I analyzed the upper 15 cm of these soils for texture (hydrometer method), pH (1:2 soil:0.01 M $CaCl_2$) and organic
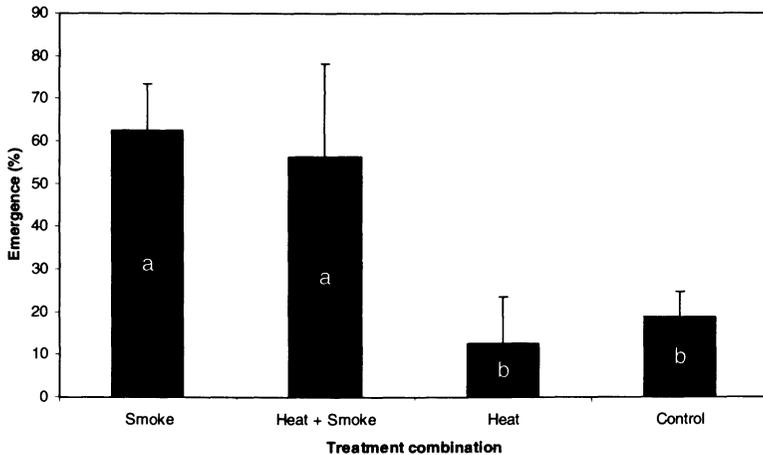
FIG. 1.—Mean emergence of *Penstemon barbatus* across treatment combinations in a 2-factor, split-plot factorial experiment using basalt soil with 2 levels (none, exposure to 100 C for 30 minutes) of the whole plot factor heat, and 2 levels (none, 60 ml of 10% liquid smoke) of the subplot factor liquid smoke. Means without shared letters differ at P < 0.05 (Fisher's LSD). Error bars are 1 SD

C and total N (instrumental C/N analyzer). The basalt soil was a pH 6.08 loam (40% sand, 47% silt, 13% clay) with 1.9% organic C and 0.13% total N. The limestone soil was a pH 6.45 sandy loam (62% sand, 32% silt, 6% clay) with 1.3% organic C and 0.08% total N.

RESULTS

Experiment 1: heat and liquid smoke.—Main effects were significant for liquid smoke ($F = 26.72$, P < 0.01), but not for heat ($F = 0.75$, P = 0.43). Liquid smoke at 10% concentration increased *Penstemon barbatus* emergence by 44% above the control to 63% emergence (Fig. 1). Heat and liquid smoke did not interact ($F = 0.0$, P = 1.0), and emergence in the heat + smoke treatment did not differ from the smoke only treatment.

Experiment 2: liquid smoke concentration and charred wood.—One-way ANOVA was significant ($F = 15.20$, P < 0.01), with emergence in treatments incorporating only liquid smoke at least 36% greater than emergence in *Pinus ponderosa* charred wood and control treatments (Fig. 2). Emergence did not differ among 5, 10 and 20% liquid smoke concentrations, ranging from 44–54% emergence. Charred wood, charred wood + 10% smoke and control treatments resulted in emergence <8%.

Experiment 3: air smoke, liquid smoke and charred wood leachate.—Liquid smoke was the strongest main effect ($F = 10.09$, P < 0.01), followed by air smoke ($F = 5.31$, P = 0.08) and charred wood leachate ($F = 2.93$, P = 0.11). Interactions were not important (P > 0.48) except for a marginally significant liquid smoke × charred wood leachate ($F = 3.51$, P = 0.08). The air smoke + liquid smoke treatment induced the highest emergence (58%), but because of large variation did not differ significantly from a 35% mean emergence in the control (Fig. 3). Air smoke and liquid smoke emergence means differed by 6%, an insignificant difference. Treatments containing charred wood leachate exhibited significantly lower emergence than the air smoke mean (50%) only in treatment combinations where liquid smoke was not applied in combination with charred wood leachate.

Experiment 4: substrate and liquid smoke.—Substrate used as the emergence medium was not a significant main effect ($F = 0.35$, P = 0.71), in contrast to liquid smoke ($F = 31.51$, P < 0.01). Substrate and liquid smoke interacted ($F = 3.99$, P = 0.03), however, with liquid smoke promoting emergence more strongly on potting and basalt soil than on limestone soil (Fig. 4). Potting soil with liquid smoke produced the highest emergence (54%), and emergence did not differ between basalt and limestone soil for smoke or non-smoke treatments.
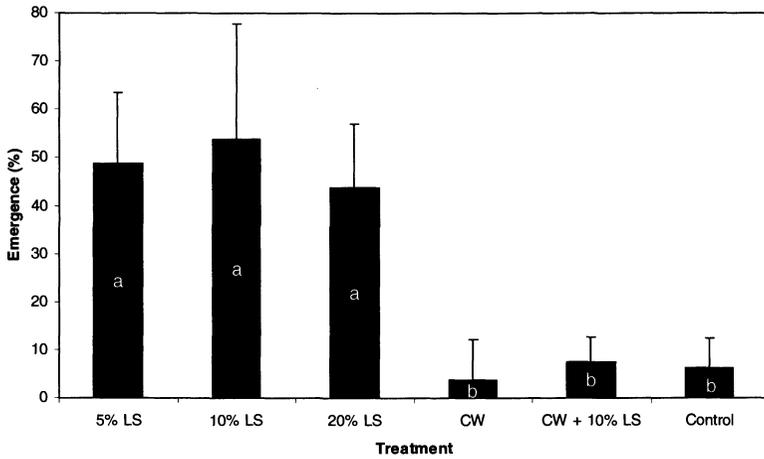
FIG. 2.—Mean emergence of *Penstemon barbatus* across treatments in a one-way experiment using potting soil testing effects of liquid smoke (LS) concentrations and *Pinus ponderosa* charred wood (CW). Means without shared letters differ at P < 0.05 (Fisher's LSD). Error bars are 1 SD

DISCUSSION

Smoke improved *Penstemon barbatus* emergence in all 3 factorial experiments, and increased emergence by more than 36% above a control treatment in the one-way experiment. Experiment 3 suggested that similar emergence was induced by liquid smoke and more natural air smoke produced by burning *Pinus ponderosa* litter. Several studies have found that different types of smoke (air or liquid) and materials burned to produce smoke (native or not native to the ecosystem in which a study species occurs)
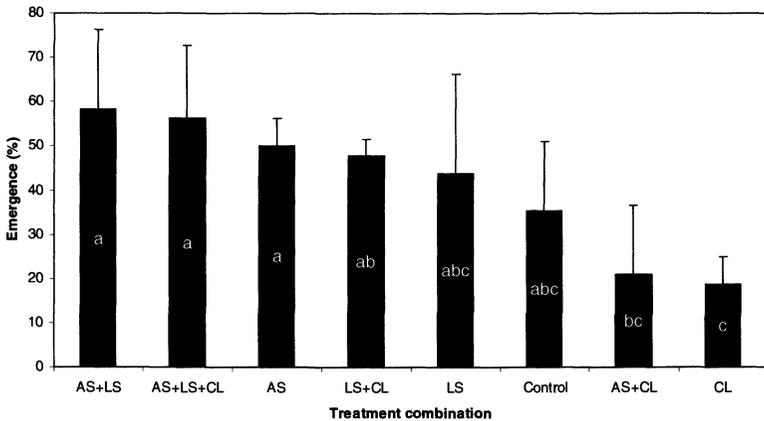

FIG. 3.—Mean emergence of *Penstemon barbatus* across treatment combinations in a 3-factor, split-plot factorial experiment using limestone soil with 2 levels of the whole plot factor air smoke (none, 15 min exposure) and 2 levels each of the subplot factors liquid smoke (none, 60 ml of 10% concentration) and *Pinus ponderosa* charred wood leachate (none, 60 ml of 10 g/100 ml concentration). AS = air smoke, LS = liquid smoke and CL = charred wood leachate. Means without shared letters differ at P < 0.05 (Fisher's LSD). Error bars are 1 SD
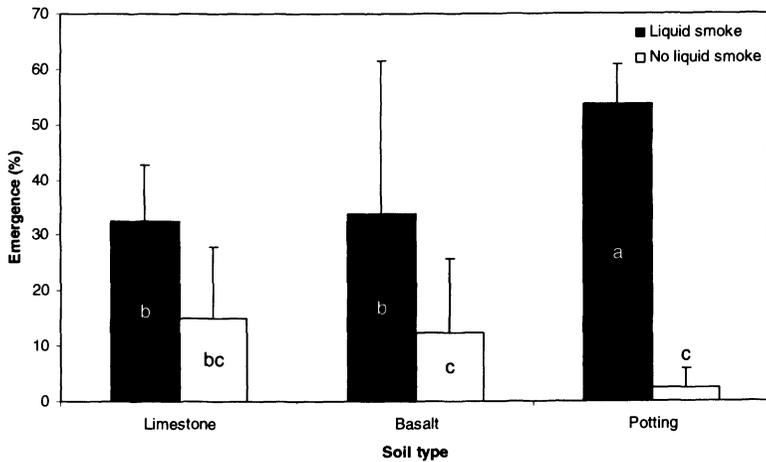
FIG. 4.—Mean emergence of *Penstemon barbatus* across treatment combinations in a factorial experiment with three levels of soil (basalt, limestone or potting soil) and 2 levels of liquid smoke (none, 60 ml of 10% concentration). Means without shared letters differ at P < 0.05 (Fisher's LSD). Error bars are 1 SD

promote emergence similarly for many species benefiting from smoke (Brown and Van Staden, 1997; Blank and Young, 1998; Van Staden *et al.*, 2000). Flematti *et al.* (2004) recently isolated a butenolide compound contained in any cellulose-derived smoke thought to cause smoke's cueing effects.

In contrast to smoke, *Pinus ponderosa* charred wood and charred wood leachate did not improve emergence in either of the experiments where they were applied. Charred wood counteracted positive effects of liquid smoke in Experiment 2, and in Experiment 3, charred wood leachate + air smoke resulted in lower emergence than air smoke only. Keeley *et al.* (1985) found that charred wood from the shrub *Adenostoma fasciculatum* in California chaparral increased germination of some forbs, while reducing germination of others. Although charred wood from species other than the *P. ponderosa* used in these experiments may benefit *P. barbatus* seeds, *P. ponderosa* is the dominant or only tree in local forests. Lodhi and Killingbeck (1982) identified caffeic acid and other allelochemicals in *P. ponderosa* needles and bark, which reduced germination of *Schizachyrium scoparium* (Michx.) Nash and *Andropogon gerardii* Vitman in North Dakota *P. ponderosa* forests. Their findings suggest allelopathic chemicals in *P. ponderosa* wood and leachate may have resulted in the observed reductions in *P. barbatus* emergence after exposure to these materials in the present experiments. Organic matter in the charred wood, however, may also have absorbed and "de-activated" smoke cues.

Across experiments including Experiment 4 testing substrate effects, liquid smoke more sharply increased *Penstemon barbatus* emergence above controls on potting and basalt soil than on limestone soil. Basalt soil used in these experiments exhibited organic C concentrations 0.6% higher and pH 0.37 units lower than limestone soil, and potting soil likely contained the most organic C. Keeley (1987) also found that substrate (filter paper or potting soil) affected germination of 23/36 (64%) California chaparral species in experiments examining influences of heat and charred wood. It is uncertain if smoke cues interacted with soil pH or other soil characteristics affecting *P. barbatus* emergence in my experiments.

Greenhouse findings from these experiments need to be tested in the field, and predict that *Penstemon barbatus* emergence in *Pinus ponderosa* forests is enhanced when seeds are exposed to smoke during fires, but not killed by intensive heat, and are located away from *P. ponderosa* charred wood. Fires in presettlement Arizona *P. ponderosa* forests occurred almost exclusively from May–August (Fulé *et al.*, 1997), and it is difficult to assess whether contemporary prescribed burns often conducted in September–October (Abella, 2004) differentially affect *P. barbatus* emergence. *Penstemon barbatus* flowers primarily in May–June with seeds maturing by mid-late summer, and emergence in some *Penstemon*

species is enhanced by ≥30 d of chilling (Meyer *et al.*, 1995). The ecological importance of fire cueing in *P. barbatus* population biology and whether it is affected differently by summer or fall burns could depend on: (1) if fire influences immature or mature on-plant seeds the same as seeds on or in the soil, (2) whether chilling is required before smoke can serve as a cue or if smoke enables seeds to bypass any chilling requirements, (3) how long smoke cues persist with seeds for triggering emergence, (4) whether seed presence on-site coincides with a period of exposure to air or soil-persistent smoke and (5) possible interactions of smoke cueing with other cues not directly related to fire such as alternating temperature, precipitation or light. These other cues may also influence *P. barbatus* emergence and subsequent seedling survival (Meyer *et al.*, 1995). Greenhouse findings from these experiments have practical implications for germinating *P. barbatus*, and future research could examine if and under what environmental conditions smoke cueing affects recruitment of this species in native communities.

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