SMOKE-CUED EMERGENCE IN PLANT SPECIES OF PONDEROSA PINE FORESTS: CONTRASTING GREENHOUSE AND FIELD RESULTS

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ABSTRACT

Smoke-cued seed germination and emergence is common in some frequent-fire ecosystems, but this process is little studied in frequent-fire conifer forests of the southwestern United States. To assess whether aqueous smoke promotes plant emergence in frequent-fire ponderosa pine (*Pinus ponderosa*) forests in northern Arizona, I conducted three experiments at different scales (seed, seed bank, and plant community) in both greenhouse and field settings. In the first greenhouse experiment, aqueous smoke significantly increased ($P < 0.05$) emergence of seeds of 13 % of 61 assayed species. Five of eight (63 %) *Penstemon* species exhibited significant increases ranging from two- to ten-fold. In the second greenhouse experiment, aqueous smoke increased emergent density by 67 % and species richness by 60 % in soil seed bank samples collected from nine sites that had undergone tree thinning. Contrary to these greenhouse experiments, spraying aqueous smoke on 0.05 ha field plots at nine sites in the third experiment had no effect on plant species cover, richness, or composition relative to control plots 15 mo after treatment. Many factors, such as time since fire or interactions with other cues, could have contributed to this lack of response. While aqueous smoke appears promising for some applications such as enhancing seed germination for plant production, its potential for promoting emergence in field settings is currently uncertain.

Keywords: aqueous smoke, fire effects, germination, *Penstemon*, soil seed bank

INTRODUCTION

Seed germination and emergence are key components of terrestrial ecosystems, partly regulating plant regeneration and many ecosystem processes affected by seed-based plants. One of the many effects of fire is exposing seeds, either on plants or in the soil seed bank, to smoke (Van Staden *et al.* 2000). While smoke does not affect germination of all species, it stimulates germination of a variety of species in both frequent- and infrequent-fire ecosystems (Bhalla and Sabharwal 1973, Dixon *et al.* 1995, Pierce *et al.* 1995). The mechanism by which smoke stimulates germination is not fully understood and can vary among species. Smoke may serve as a chemical cue to increase seed coat permeability or stimulate metabolic activity (Baldwin *et al.* 1994, Keeley and Fotheringham 1998). Smoke contains

many different compounds, and the specific component of smoke responsible for promoting germination puzzled researchers as smoke's extensive promotive effect became more well documented (Baldwin *et al.* 1994, Baxter *et al.* 1994, Brown and Van Staden 1997). In a recent breakthrough, Flematti *et al.* (2004) isolated a butenolide compound in smoke responsible for smoke's effect and thought to occur in any plant-derived smoke. Several studies have found that both airborne and liquid forms of smoke can promote germination, although the magnitude of increase can vary among forms, concentrations, and timing of applications (Roche *et al.* 1998, Lloyd *et al.* 2000).

Smoke has been assessed as a germination or emergence cue in both laboratory and field settings, and at three different scales: individual seeds, soil seed bank samples, and in field plots. Seeds have been screened for responses to smoke in a variety of species from South African fynbos communities (Brown 1993), Australian eucalyptus and other communities (Dixon *et al.* 1995, Thomas *et al.* 2007), chaparral in California, USA (Keeley and Fotheringham 1998), and arable weed communities (Adkins *et al.* 2003, Daws *et al.* 2007). Smoke has sharply increased emergence from soil seed bank samples in greenhouse assays, many of which have been conducted in Australian vegetation communities (e.g., Enright *et al.* 1997, Read *et al.* 2000). Results of smoke applications to field plots have been mixed. In Australia, where nearly all of the research has been conducted, smoke effects on plant emergence have ranged from dramatic increases (e.g., 48-fold increases; Dixon *et al.* 1995, Roche *et al.* 1997) to no effect (Coates 2003). The importance of smoke cues in field settings could hinge upon many factors. For example, smoke cues may only be effective on sites containing sufficient seed densities of responsive species (Rokich *et al.* 2002, Coates 2003). Smoke cues might interact with other cues (e.g., moisture, light), and seeds might only respond if the timing of exposure is appropriate or if levels of these other cues are suitable (Roche *et al.* 1998, Baker *et al.* 2005). Whether smoke-stimulated seeds subsequently result in established plants also depends on many factors, such as grazing or tree density in forested ecosystems (Bakker and Moore 2007).

In addition to smoke's potential importance to plant regeneration following fire, smoke technology could have a wide variety of applications. For example, smoke can be used to pre-treat seeds to increase germination for revegetation projects, an application used in Australia since the early 1990s to revegetate open-pit mines on former *Eucalyptus* forest sites (Roche *et al.* 1997). In areas where burning cannot be conducted, aqueous smoke could be useful for stimulating emergence (Van Staden *et al.* 2000). As Keeley and Baer-Keeley (2000) pointed out, however, there have been few assessments of smoke effects in field settings compared to smoke effects in greenhouse settings. There also have been relatively few studies examining more than one of the seed, seed bank, or field scales (Dixon *et al.* 1995, Lloyd *et al.* 2000).

Based on a network of fire-history study sites, many semi-arid ponderosa pine (*Pinus ponderosa*) forests in the southwestern United States are frequent-fire forests (Swetnam and Baisan 1996). For example, before Euro-American settlement in the late 1800s and subsequent institutional fire exclusion, fire-return intervals commonly averaged <5 years in northern Arizona ponderosa pine forests (e.g., Van Horne and Fulé 2006). These observations suggest that smoke-cued emergence could be prevalent in ponderosa pine forests, but this potential has been little studied in these forests (Fulé *et al.* 2001). Understanding fire effects is important in these and other western forests, because wildfires and the use of fire are increasing (Collins and Stephens 2007). Increased fire activity is further anticipated in

southwestern forests due to warmer temperatures and earlier spring snow melts in climate change scenarios (Westerling *et al.* 2006). Fire was a major factor structuring historical forests and will likely again become a dominant influence in future southwestern forests (Laughlin and Fulé 2008).

The purpose of this study was to determine the effects of aqueous smoke on (1) seeds of a variety of species native to ponderosa pine forests, (2) soil seed bank samples collected at nine northern Arizona ponderosa pine sites, and (3) field plots at those same sites. I tested the hypothesis that aqueous smoke increases emergence at all three of these scales, and with or without exposure to grazing by large herbivores in the field experiment.

METHODS

Seed Experiment

In a two-factor experiment, I screened seeds of 61 species native to northern Arizona ponderosa pine forests for potential aqueous smoke stimulation of emergence. Species were selected based on seed availability through a local vendor in northern Arizona (Flagstaff Native Plant and Seed, Flagstaff, Arizona, USA) and spanned a range of growth forms, longevities, and genera. Although the specific locations of the seed sources are not known, all of the seeds were collected from ponderosa pine forests. Of the 61 species, 92 % were forbs, 7 % were grasses, and 2 % were trees (NRCS 2007). Ninety-seven percent were perennials and 3 % were annuals-biennials. Forty-eight genera representing 21 families were included. I weighed 50 to100 air-dry seeds of each species on a balance accurate to the nearest 0.001 g.

I stored seeds at -5 °C for four months before initiation of the experiment. For each species, I lightly pressed 16 seeds (arranged in four rows of four seeds) in each of eight 700 cm³ square plastic pots filled with 500 cm³ of sterile potting soil (Black Gold Potting Soil, Sun Gro Horticulture Distribution, Bellevue, Washington, USA). Next, I applied 60 ml of 10 % (vol/vol, diluted with tap water) concentrated aqueous smoke (Wright's Brand, Roseland, New Jersey, USA) to the soil surface containing seeds of four randomly selected pots of each species. The other four pots of each species were untreated controls. I randomly arranged pots on benches in a greenhouse maintained at ≈ 24 °C with supplemental lighting to provide 14 hr of light each day. I kept soils moist by daily watering, at which time I also checked pots to ensure that seedling mortality was not missed in between periodic seedling counts. Every 15 days for 60 days, I counted emerging seedlings.

Seed Bank and Field Experiment Study Area

Field sites where seed bank samples were collected and experimental treatments were performed were in the 9000 ha southern half of the Northern Arizona University Centennial Forest, 10 km southwest of the city of Flagstaff, Arizona, USA. Nine ponderosa pine sites, each in a separate soil mapping unit, were randomly selected from a pool of candidate sites following methods detailed in Abella and Covington (2006). Linear distances between sites averaged 8 km, and ranged from 2 km to 16 km. Elevations of sites ranged from 2166 m to 2272 m. Based on a weather station approximately 15 km to the east (2137 m elevation), annual precipitation averaged 54 cm, mean daily July high temperatures were 28 °C, and mean daily January low temperatures were -9 °C (1950 to 2006 records; Western Regional Climate Center, Reno, Nevada, USA). Most precipitation fell either as snow or as summer monsoon rains from July to September. Sites occupied basalt, benmoreite, or limestone soil parent material and contained soils classified as Typic, Lithic, or Mollic Eutroboralfs (Miller

et al. 1995). Large grazing animals in the study area included livestock (cattle and sheep) and Rocky Mountain elk (*Cervus elaphus*). By analyzing fire-scarred trees and stumps, Fulé *et al.* (1997) recorded a mean fire interval (all fires) of 3.7 yr from 1637 to 1883 for the area immediately adjacent to my study area. Another study within my study area recorded a fire interval of 1.7 yr from 1682 to 1881 (Van Horne and Fulé 2006).

I established two 20 m × 25 m (0.05 ha) plots, both located in densely treed patches (White *et al.* 1991) and within approximately 100 m of each other, at each site in 2003. In September 2003, the trees (all ponderosa pine) on these plots were thinned by hand using chainsaws (no mechanized equipment drove over the sites) from an average density of 1362 trees ha^{-1} (SD = 824 ha^{-1}) to either 60 ha^{-1} or 80 trees ha^{-1} (3 or 4 trees per plot). The slight difference in density among plots resulted from tree sizes (all trees >40 cm diameter at 1.4 m height were retained) or from the presence of wildlife nests in a tree's crown that resulted in retention. The aqueous smoke field experiment was conducted on these plots after thinning. In many ponderosa pine forests, mechanical thinning is used to prepare stands for prescribed burning or to reduce crown fuels (Covington 2003). We conducted this experiment in thinned stands to test whether aqueous smoke would augment understory vegetation establishment.

Seed Bank Experiment

Prior to tree thinning, I obtained mineral soil seed bank samples by collecting the top 5 cm of soil at each of the two plots at the nine sites. A 70 cm^3 sample core was collected at 15 total points in plots at 0.5 m, 5 m, 12.5 m, 20 m, and 24.5 m along the perimeters of the long axes and the centerline. I combined the 15 sample cores into one sample for each plot. Sampling occurred in June and July 2003.

Based on a phenological study (Clary and Kruse 1979), sampling occurred prior to post-monsoon seed dispersal in August to October and predominately represents the persistent seed bank. It is possible that current-year seeds of some early flowering species were deposited in samples.

After collection, I stored samples at -5 °C for 5 mo to 6 mo before applying treatments and beginning an emergence assay. From each plot, I placed 120 cm^3 of seed bank soil on top of 500 cm^3 of sterile potting soil in each of two 700 cm^3 square plastic pots. Subsequently, one of the two pots from each plot received a randomly allocated application of 60 ml of 10 % (vol/vol, diluted with tap water) concentrated aqueous smoke to the soil surface. Eight pots containing only potting soil, half of which received smoke, served as checks for greenhouse seed contamination (no contamination was detected). I randomly arranged all pots on a bench in a greenhouse maintained at ≈ 24 °C with supplemental lighting to provide 14 hr of light per day. I watered samples daily until moist. I tallied emerging seedlings every 15 days during a 10 mo period. Additionally, samples were checked for any seedling mortality that may have occurred between the 15 day inventories. Seedlings were removed after they could be reliably identified, while seedlings not able to be readily identified were left in place until maturity. A species that had numerous glabrous, slightly lobed, basal leaves never flowered and was possibly a *Veronica* sp., but was not positively identified. These specimens were included in seed density counts and were designated as "unknown forb" in compositional analyses.

Field Experiment

To measure plant community responses to aqueous smoke application, I randomly selected one of the two plots to receive aqueous smoke at each of the same nine sites from

which seed bank samples were collected. The experimental design was a randomized complete block split plot, with aqueous smoke treatment (two levels: present or absent) as the whole plot factor and grazing treatment (two levels: grazed or grazing excluded) as the subplot factor. Grazing was included as a treatment in this experiment because grazing could interact with smoke application in a number of ways. For example, smoke could increase forage availability and make the plots more attractive to herbivores. A grazing exclosure, 3.16 m × 3.16 m (10 m²) and 2 m tall consisting of four metal fence posts and 1 cm thick wire with 5 cm × 10 cm openings, was installed in the center of half of each 20 m × 25 m plot. An area also 3.16 m × 3.16 m (10 m²) in size, but without an exclosure, was delineated in the center of the other half of the plot. I applied undiluted aqueous smoke (Regen Direct, Forest Flavors, Glasgow, Kentucky, USA) by backpack spraying on 22-23 June 2005 to entire 20 m × 25 m plots at an even rate of 100 mL m⁻². This application rate followed manufacturer recommendations and was used in previous studies in Australia (Lloyd *et al.* 2000, Rokich *et al.* 2002). I chose the June application time to be within the May to early September period when most fires historically occurred in these forests (Fulé *et al.* 1997).

Pre-treatment data, immediately following exclosure establishment and before aqueous smoke application, were collected in mid-September 2003 within a 9 m² area inside exclosures and within a 9 m² area inside grazed sample areas on each plot. I divided each area into nine 1 m × 1 m subplots. I visually categorized areal cover of each plant species rooted in each subplot. Between 0% and 1%, I used 0.1%, 0.25%, 0.5% and 1.0% categories. Between 1% and 10%, I used 1% intervals, and above 10%, I used 5% intervals. The same data were collected in mid-September 2006, 15 mo after smoke application. This sampling time is post-monsoon and is consid-

ered to be the period of peak understory biomass in northern Arizona ponderosa pine forests (Laughlin *et al.* 2006). We followed plant nomenclature from the PLANTS database (NRCS 2007).

Statistical Analysis

I analyzed the seed experiment using a two-factor analysis of variance, with 61 levels of species and two levels (presence or absence) of aqueous smoke. Because of the large number of possible multiple comparisons, I used individual two-sample *t* tests (two-tailed) to compare control and smoke mean emergence within species only for the 20 species that exhibited the largest average differences between treatments. These tests were designed to evaluate each species independently, so I did not adjust the experiment-wise error rate (Sankoh *et al.* 1997). Using Pearson's *r*, I examined the correlation between percent emergence and species mean seed weight.

To analyze the seed bank experiment, I averaged data from the two plots to calculate a site mean. I compared mean seed density and species richness between aqueous smoke and control treatments using two-tailed paired *t* tests (*n* = 9 pairs of samples). Using relative emergent density, calculated by expressing the density of each species as a percentage of the density of all species on a plot, I compared seed bank composition between treatments using blocked multi-response permutation procedures (Sørensen distances). This nonparametric analysis tests the multivariate hypothesis of no difference between two or more groups (Zimmerman *et al.* 1985). I did not employ average distance function commensuration because data were already relativized (McCune and Mefford 1999), but I did perform median alignment.

Response variables in the field experiment consisted of plant cover (calculated by summing the cover of individual species), species

richness per m^2 and 9 m^2, and multivariate species composition. The univariate variables in this split-plot experiment were analyzed using analysis of variance with pre-treatment data as a covariate. To test if composition of aqueous smoke and untreated plots diverged from each other from pre- to post-treatment, I computed a Sørensen similarity separately for grazed and ungrazed areas between each pair of smoked and unsmoked plots at each site ($n = 9$ pairs of plots). Sørensen similarities were based on species importance values (average of relative cover and relative frequency derived from occurrences in nine 1 m^2 subplots). The relative cover and frequency values were computed as the percent of cover or frequency each individual species contributed to the total cover or frequency of all species for each plot. I used two-tailed paired t tests to evaluate the hypothesis that the mean pre-treatment similarity between pairs of plots did not differ from the mean post-treatment similarity between pairs of plots. I also graphically examined pre- and post-treatment composition (importance values) for pairs of plots using successional vectors within a non-metric multidimensional scaling ordination (NMS). To account for pre-treatment differences in community composition, I translated vectors to a common origin by subtracting the ordination score of the tail from the scores of both the head and tail (McCune and Mefford 1999).

I conducted univariate analyses using JMP version 5, which accommodates random effects needed to analyze the split-plot field experiment (SAS Institute 2004). I performed multivariate compositional analyses (computation of Sørensen similarities, multi-response permutation procedures, and NMS) with PC-ORD version 5 (McCune and Mefford 1999). For non-metric multidimensional scaling, I employed PC-ORD's autopilot, thorough mode.

RESULTS

Seed Experiment: Responses of 61 Species

In the seed experiment, emergence varied with both species and aqueous smoke, with these factors interacting significantly (Table 1). Smoke significantly increased emergence of eight of 61 (13 %) species and did not significantly decrease emergence of any species (Table 2, Appendix). Five of the eight species benefiting from smoke were *Penstemon* spp., with increases ranging from two- to ten-fold. Sixteen species (26 %) exhibited <10 % emergence in both control and smoke treatments, and six of those species did not emerge in either treatment. There was no relationship between mean seed weight of species and either smoke emergence percent ($r = -0.12$) or smoke minus control emergence percent ($r = -0.09$).

Seed Bank Experiment: Effects on Richness, Density, and Composition

Aqueous smoke significantly increased both the density and the species richness of emergents from soil seed bank samples (Table 1). Smoke increased density by 67 % and richness by 60 % (Figure 1). This increase was at the community level, and was not associated with any particular species or group of species. Species composition did not differ between smoke and control samples (Table 1), which were 75 % similar (Sørensen index) based on relative seed density. Thirty-two total species were detected in samples, with 30 species in smoke samples and 21 species in control samples. Predominant species in both treatments included common mullein (*Verbascum thapsus*), trailing fleabane (*Erigeron flagellaris*), thyme-leaf sandmat (*Chamaesyce serpyllifolia*), rough bent (*Agrostis scabra*), spreading fleabane (*Erigeron divergens*), rabbit tobacco (*Pseudognaphalium macounii*), and White Mountain sedge (*Carex geophila*) (Table 3).

Table 1. Summary of statistical results for three experiments examining seed, seed bank, and field responses to aqueous smoke in ponderosa pine forests, northern Arizona.

Effect	Response variables and statistics							
Seed experiment[a]	Emergence							
	F	*P > F*	df					
Species	45.3	<0.001	60					
Smoke	43.6	<0.001	1					
Species × smoke	3.4	<0.001	60					
Seed bank experiment[b]	Density			Richness				
	t	*P > t*	df	*t*	*P > t*			
Smoke	-2.6	0.034	8	-2.4	0.043			
	Composition							
	T	*A*	*P > T*					
Smoke	-0.2	0.006	0.358					
Field experiment[c]	Plant cover			Richness/m²			Richness/9 m²	
	F	*P > F*	df	*F*	*P > F*		*F*	*P > F*
Smoke	0.1	0.728	1	0.2	0.667		1.5	0.239
Plot [smoke]	1.7	0.153	16	1.2	0.391		2.0	0.096
Grazing	4.9	0.043	1	3.3	0.088		3.7	0.074
Smoke × grazing	0.6	0.461	1	0.5	0.489		3.7	0.074
Covariate	15.8	0.001	1	9.4	0.008		5.6	0.032
	Composition							
	t	*P > t*	df					
Smoke: grazed	-1.6	0.145	8					
Smoke: ungrazed	-1.2	0.255	8					

[a] Two-factor analysis of variance with percentage of seeds emerging as the response variable.
[b] Density and species richness of emergents analyzed using two-tailed paired *t* tests. Species composition (importance values) of emergents compared between treatments using blocked-multi-response permutation procedures (T = test statistic, A = chance-corrected within-group agreement, which provides a measure of within-group homogeneity; McCune and Grace 2002).
[c] Univariate response variables analyzed as a split plot (aqueous smoke treatment as the whole-plot factor and grazing treatment as the subplot factor) with pre-treatment data as a covariate using analysis of variance. Species composition compared between control and aqueous smoke treated plots separately for grazed and ungrazed areas within plots by computing a Sørensen similarity (based on species importance values) between each pair of control and treated plots before and after treatment. A two-tailed paired *t* test evaluated the null hypothesis that the mean pre-treatment similarity between pairs of plots did not differ from the mean post-treatment similarity between pairs of plots.

Field Experiment: Effects on Grazed and Ungrazed Areas

In contrast to the overall promotive effect in the seed and seed bank experiments, aqueous smoke had no effect on plant cover and richness 15 mo after spraying field plots (Table 1, Figure 2). Smoke and grazing exclusion also did not interact. Similarly, smoke did not induce significant changes in species composition on either grazed or ungrazed plots. On plots that were grazed, there was a 51 % similarity in species composition between smoke and control plots. For ungrazed plots, the similarity was 45 %. This similarity changed <8 % 15 mo after application (grazed: 44 % similar; ungrazed: 40 % similar). Successional vectors in a NMS ordination were consistent with

Table 2. Species exhibiting significantly ($P < 0.05$, two-tailed t tests) greater emergence after treatment with 10 % (vol/vol) aqueous smoke.

Species	Control	Smoke	Seed wt.[b]
	Emergence (%)[a]		(mg)
Artemisia ludoviciana	20±19	55±11	0.14
Erigeron speciosus	8±6	27±6	0.05
Penstemon barbatus	8±9	61±13	0.76
Penstemon pachyphyllus	3±4	30±8	1.76
Penstemon palmeri	41±6	81±10	0.86
Penstemon rostriflorus	3±6	23±3	0.42
Penstemon virgatus	19±9	61±6	0.86
Symphyotrichum falcatum	13±9	30±18	0.18

[a] Mean ± 1 SD.
[b] Mean weight of individual seeds; $n = 50$ for seeds averaging >0.5 mg and $n = 100$ for seeds averaging <0.5 mg.
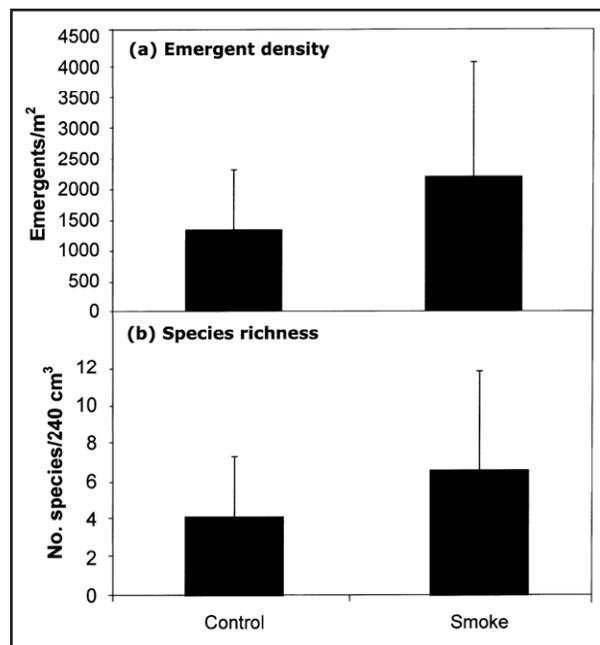
Figure 1. Mean density and species richness of emergents from 0 cm to 5 cm soil seed bank samples either untreated or treated with aqueous smoke. Means within (a) and (b) differed significantly ($P < 0.05$), with error bars representing 1 SD. Samples were collected from nine sites in ponderosa pine forests, northern Arizona.

these results, where vectors of control and smoke plots showed no apparent tendency to differ in their direction or amount of compositional change from pre- to post-treatment (Figure 3). There also were no clear patterns in the relative cover between treatments among the 87 total species detected on plots (Table 3). The two most dominant species, White Mountain sedge and bottlebrush squirrreltail (*Elymus elymoides*), differed in relative cover by <4 % between treatments. *Verbascum thapsus* averaged 9 % greater cover in control plots, but this difference resulted from exceptionally high relative cover (48 % and 85 %) on just two ungrazed plots.

DISCUSSION

The three experiments in this study each have limitations that could have affected results. Although seeds in the seed experiment were obtained from a local seed vendor, the specific population genetic origin of the seeds is unknown. Differences in response to fire cues among populations have not been extensively studied; however, Baldwin *et al.* (1994) found that smoke differentially affected germination percentages among six populations of coyote tobacco (*Nicotiana attenuata*) in southwestern Utah. Germination patterns commonly differ among populations (Baskin and Baskin 1998). For example, intermountain *Penstemon* populations have varying responses to chilling (Meyer *et al.* 1995). Although the seeds I used were subjected to cold storage to

Table 3. Relative cover of the 10 most dominant plant species in control and aqueous smoke treated field plots 15 mo after treatment, compared to the relative density of the 10 most abundant species in 0 cm to 5 cm soil seed bank samples in ponderosa pine forests, northern Arizona.

	Field		Seed bank	
	Control	Smoke	Control	Smoke
Species[a]	Relative cover (%)[b]		Relative density (%)[c]	
Carex geophila	18	19	4	5
Elymus elymoides	14	18	0	0
Festuca arizonica	7	4	0	0
*Verbascum thapsus**	10	1	12	11
Erigeron formosissimus	5	5	0	0
Poa fendleriana	4	5	2	2
Muhlenbergia montana	2	3	0	1
Packera multilobata	1	4	0	0
*Poa pratensis**	1	3	2	2
Erigeron divergens	2	1	5	7
Pinus ponderosa	1	2	0	2
*Taraxacum officinale**	2	1	2	0
Chenopodium graveolens	<1	2	4	4
Chamaesyce serpyllifolia	<1	<1	9	9
Cyperus fendlerianus	<1	<1	4	1
Erigeron flagellaris	<1	<1	11	12
Muhlenbergia minutissima	<1	<1	4	2
Pseudognaphalium macounii	<1	<1	7	4
Arenaria lanuginosa	0	0	4	2
Agrostis scabra	0	0	12	5
Other species[d]	32	31	18	31

[a] Species arranged in order of decreasing field relative cover. Asterisks denote exotic species (NRCS 2007).
[b] Calculated as the percent of cover that each individual species contributed to the total cover of all species for each plot.
[c] Calculated by expressing the emergent density of each species as a percentage of the density of all species from a sample.
[d] Including the species listed in the table, the total numbers of species recorded were as follows: control field plots = 71, smoke-treated field plots = 73, control seed bank samples = 21, and smoke-treated seed bank samples = 30.

simulate winter conditions, the unknown storage period prior to acquiring the seeds could have affected germinability (Baskin and Baskin 1998). I randomly selected seeds for treatment from seed lots to account for potential non-uniform storage periods. Similarly, seed bank samples were processed uniformly between aqueous smoke and control treatments, but greenhouse conditions may not have been optimal for all species. This is a well-known po-

tential limitation of the emergence method for seed bank assays (Baskin and Baskin 1998). However, I did provide a 10 mo emergence period that exceeded the suggestion by Warr *et al.* (1993) of a minimum period of six months. Species composition also was similar to composition emerging from samples with a different greenhouse regime (e.g., no supplemental lighting) in a previous seed bank study in this region (Abella *et al.* 2007)
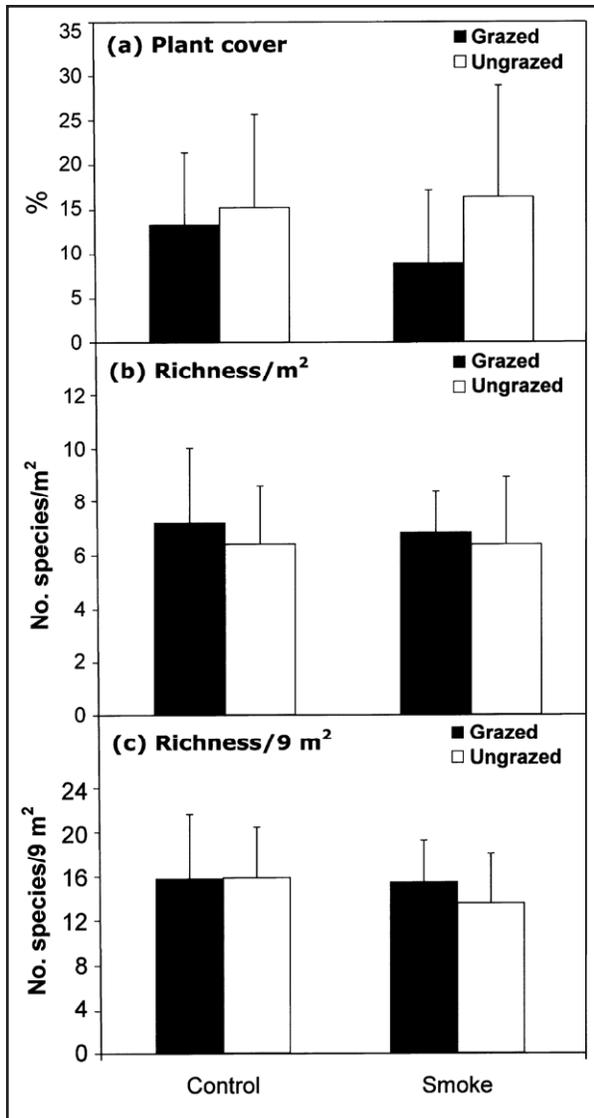
Figure 2. Mean plant cover and species richness in control and aqueous smoke treated plots at nine sites in ponderosa pine forests 15 mo after treatment, northern Arizona. Results are presented for 10 m^2 areas that were either grazed or ungrazed within each treatment plot. Error bars represent 1 SD.
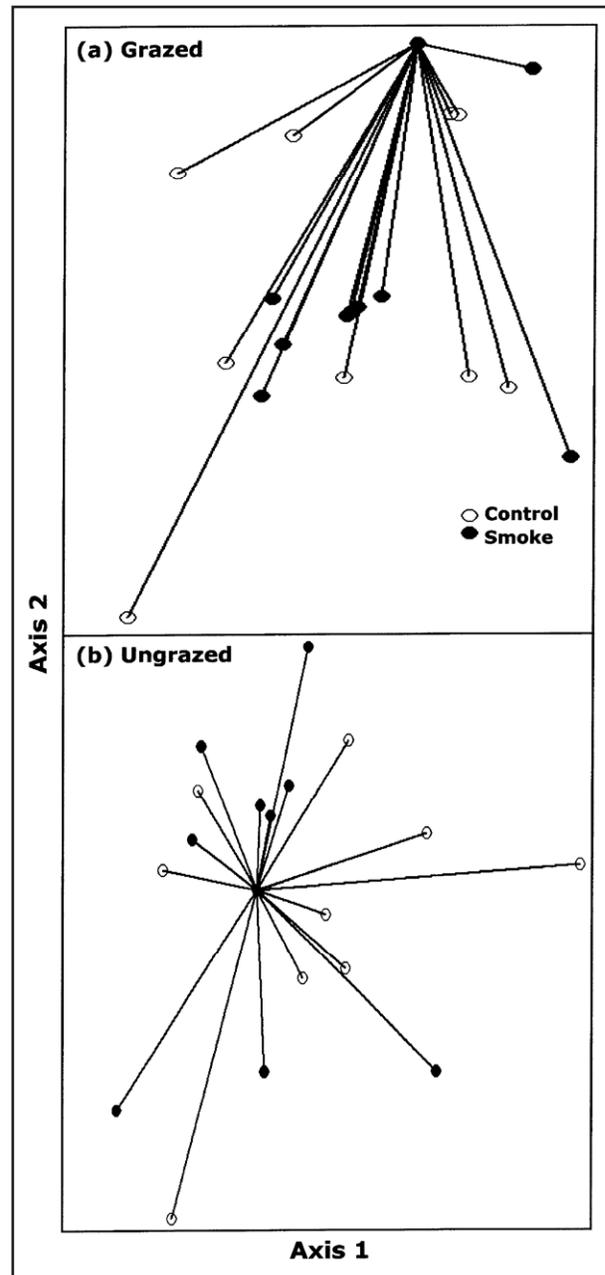

Figure 3. Non-metric multidimensional scaling ordination showing plant species composition (importance values) in control and aqueous smoke treated plots at nine sites in ponderosa pine forests 15 mo after treatment, northern Arizona. Results are presented separately for 10 m^2 areas that were either (a) grazed or (b) ungrazed within each treatment plot. Successional vectors leading to each plot on the graph are standardized to a common origin to account for pre-treatment differences.

The sources and batches of aqueous smoke could have affected results of the experiments. The seed and seed bank experiments used a different brand of smoke than the field experiment because the large quantity of smoke needed for the field experiment required that smoke be obtained from a wholesale vendor. Baldwin *et al.* (1994) compared the effects of two different brands of aqueous smoke on the

smoke-stimulated coyote tobacco and found no significant differences in laboratory seed

germination between brands. Rokich *et al.* (2002) found that two different commercially available brands of aqueous smoke both increased plant recruitment at an Australian woodland site. Nevertheless, the smoke products in my respective experiments came from a single manufacturer, and specifically testing potential brand or batch effects may help enhance the generality of results. Furthermore, some studies have found that aerosol forms of smoke more effectively stimulate germination than aqueous smoke (e.g., Lloyd *et al.* 2000, Rokich *et al.* 2002), and this possibility also could be further evaluated. However, aerosol smoke is currently more difficult to apply over large field sites than aqueous smoke (Rokich *et al.* 2002).

The aqueous smoke application rate, timing, or weather could have further affected the field experiment. My application rate (100 mL m^{-2}) was identical to that used in two Australian studies that found aqueous smoke stimulation of plant establishment (Lloyd *et al.* 2000, Rokich *et al.* 2002) and was within the range used in other Australian studies that recorded varying responses (e.g., Burne *et al.* 2003, Coates 2003). It seems unlikely that my application rate was potent enough to inhibit emergence because the much higher application rate I applied to pots in the seed and seed bank experiments (6 mL of concentrated aqueous smoke pot^{-1}, or 417 mL m^{-2}) improved emergence while not significantly reducing that of any samples (Table 2, Figure 1). It is possible that the application rate was too low to elicit a response. However, this rate was sufficient to stain soils brown immediately after application, similar to the observations of Roche *et al.* (1997) following their aqueous smoke application that promoted emergence. The late June application was timed to correspond to the middle of the period when fires historically occurred in the study area (late April to early September; Fulé *et al.* 1997). Earlier or later applications within this period, however, may

have corresponded with different germination cues (e.g., light, water) that could affect responses to smoke (Roche *et al.* 1998). A rain of 0.4 cm fell the day after the smoke application, followed by no rain until 1.3 cm fell 23 days after application. It is unclear if this precipitation pattern affected smoke incorporation or residence time in soil. In constructed sand columns, Stevens *et al.* (2007) found that simulated 0.4 cm rainfall events allowed the butenolide compound in smoke to penetrate 18 cm deep into the column. This depth would be more than sufficient for exposing the compound to the 0 cm to 5 cm depth that contains >60 % of germinable seeds in the mineral soil seed bank in the study area (Abella *et al.* 2007). Precipitation falling after fire was probably not uncommon historically, with ignitions associated with lightning storms during the late summer monsoon season (Fulé *et al.* 1997). In the longer term, precipitation in the year prior (2004) to smoke application was 110 % of the long-term mean, 112 % during the smoke application year (2005), and 72 % in 2006 post-treatment (Western Regional Climate Center, Reno, Nevada, USA). Although overall precipitation was below average in 2006, monsoonal precipitation in July and August preceding the September post-treatment measurement in that year was 128 % of normal.

Seed bank ecology and site history are two additional factors that may have resulted in the lack of a field response to aqueous smoke compared to the strong overall response of seeds and seed banks in the greenhouse experiments. The species most responsive to smoke in the seed experiment (e.g., *Penstemon* spp.) were not detected in seed bank samples and are likely currently uncommon in seed banks in these forests (Korb *et al.* 2005). Seed banks also become depleted in densely treed stands (Wienk *et al.* 2004) such as those occupying the experimental sites prior to tree thinning. The experimental sites have not experienced fire for

probably 120 yr (Fulé *et al.* 1997, Van Horne and Fulé 2006). It is possible that the seed bank density, and particularly that of smoke-stimulated species, was not sufficient to produce a field response. Although smoke elicited an overall response in seed bank samples in the greenhouse, other cues might be more important in the field. Heavy tree thinning (e.g., reducing densities to near presettlement densities) typically stimulates emergence and results in an increase in understory cover in ponderosa pine forests (Laughlin *et al.* 2006). Rokich *et al.* (2002) found that sites disturbed (and likely stimulating emergence) prior to aqueous smoke application in Australia *Banksia* woodlands had a diminished response to smoke compared to undisturbed sites. This could be analogous to thinning providing a flush of emergence in my study that was not further increased by subsequent smoke application. However, smoke is unlikely to be effective in densely treed, unthinned sites because these sites support little plant cover based on correlational and experimental planting studies (Korb and Springer 2003). These observations suggest that tree thinning might be a stronger cue in current forests than smoke.

Smoke is only one component of fire, and a lack of response to smoke does not necessarily translate to a lack of response to prescribed or wildland fires in these forests. Heat has increased emergence of seeds of some species (e.g., *Ceanothus fendleri* [Fendler ceanothus]) in ponderosa pine forests in greenhouse experiments (Blank and Young 1998, Huffman 2006), but did not increase emergence when applied to seed bank samples (Abella *et al.* 2007). Ponderosa pine charred wood reduced emergence of *Penstemon barbatus* (beardlip penstemon) seeds (Abella 2006) and of seed bank samples (Abella *et al.* 2007). The importance of direct fire cues relative to an altered post-fire environment (e.g., light, moisture) in affecting emergence of ponderosa pine forest plant species is uncertain.

The lack of smoke stimulation of the soil seed bank in the field experiment differs with some field experiments in other ecosystems (e.g., Roche *et al.* 1997), but is consistent with others (e.g., Coates 2003). However, the strong overall response to aqueous smoke of seeds and seed bank samples in the greenhouse experiments suggests that smoke technology may have practical applications. For example, seeds of smoke-stimulated species, such as *Penstemon*, could be treated with smoke prior to seeding in revegetation projects. Accurately quantifying the composition and density of soil seed banks also is useful for a variety of applications in exotic species management, ecological restoration, and evaluating potential contributions of seed banks for vegetation succession following fire or mechanical fuel reduction (Wienk *et al.* 2004). My study found that aqueous smoke is useful for this purpose by stimulating emergence of >50% more seeds and species to allow their detection. Further research could attempt to elucidate factors that limited smoke-stimulation in the field, as grazing by large herbivores was not a limiting factor.

Although not a focus of this research, aqueous smoke stimulation of some species in the seed and seed bank experiments raises questions about whether this stimulation resulted from an evolutionary response to fire in ponderosa pine forests or from a more general response to a germination stimulant. Understanding the effects of smoke, and fire in general, as a selective force in structuring the species composition and abundance of ponderosa pine forest understories remains an important research need, especially given the prognosis for increased fire activity and longer fire seasons with climate change in southwestern forests (Westerling *et al.* 2006). The seeds and seed banks of forest understory species will receive increased exposure to smoke and other fire-related cues if fire activity increases.

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Appendix. Emergence after treatment with 10 % (vol/vol) aqueous smoke of 61 plant species native to ponderosa pine forests.

Species[a]	Control	Smoke	Seed wt.
Smoke stimulated	— Emergence (%)[b] —		– mg[c] –
Artemisia ludoviciana	20±19	55±11	0.14
Erigeron speciosus	8±6	27±6	0.05
Penstemon barbatus	8±9	61±13	0.76
Penstemon pachyphyllus	3±4	30±8	1.76
Penstemon palmeri	41±6	81±10	0.86
Penstemon rostriflorus	3±6	23±3	0.42
Penstemon virgatus	19±9	61±6	0.86
Symphyotrichum falcatum	13±9	30±18	0.18
No significant smoke effect			
Antennaria rosulata	42±29	69±5	0.04
Arabis fendleri	95±3	81±22	0.12
Arnica chamissonis	14±6	3±4	0.56
Asclepias tuberosa	55±29	38±7	5.30
Bahia dissecta	5±6	42±34	0.04
Castilleja integra	48±28	52±23	0.20
Chamerion angustifolium	14±9	11±8	0.04
Commelina dianthifolia	86±14	83±11	3.00
Elymus elymoides	88±11	90±7	5.60
Erigeron formosissimus	64±21	50±10	0.16
Eriogonum jamesii	50±14	42±11	2.68
Eriogonum racemosum	31±9	34±8	2.64
Erysimum capitatum	72±4	81±7	0.46
Festuca arizonica	79±14	67±7	1.24
Geranium caespitosum	31±13	31±11	6.90
Helianthella quinquenervis	56±14	71±15	7.38
Heliomeris multiflora	30±13	38±11	0.56
Heterotheca villosa	48±9	42±8	0.28
Hymenoxys bigelovii	39±3	45±11	1.96
Hymenoxys richardsonii	41±15	53±8	0.64
Ipomopsis aggregata	38±15	59±15	1.20
Linum lewisii	92±6	88±5	1.42
Lupinus argenteus	23±7	35±10	23.24
Machaeranthera canescens	50±13	45±14	0.42
Machaeranthera tanacetifolia	56±14	72±11	1.28
Muhlenbergia wrightii	52±8	63±14	0.03
Oenothera elata ssp. *hookeri*	14±16	30±8	0.68
Penstemon clutei	53±12	64±14	1.08

Species[a]	Control	Smoke	Seed wt.
Penstemon rydbergii	20±20	20±11	0.12
Penstemon whippleanus	20±6	22±11	0.24
Pinus ponderosa	20±14	20±3	35.94
Pseudocymopterus montanus	63±22	58±12	2.08
Senecio spartioides	53±21	41±19	0.12
Thalictrum fendleri	42±11	64±11	3.20
Thlaspi montanum	56±22	84±6	0.52
Townsendia exscapa	13±10	28±11	1.76
Vicia americana	71±12	64±14	13.72
Emergence >1% <10%			
Achnatherum hymenoides	5±6	0±0	3.50
Agoseris glauca	6±5	9±6	2.16
Allium cernuum	2±3	2±3	3.44
Allium geyeri	3±5	8±10	4.50
Anaphalis margaritacea	3±4	2±3	0.06
Calylophus hartwegii	2±3	3±4	0.54
Monardella odoratissima	3±4	6±5	0.84
Oxytropis lambertii	2±3	2±3	3.74
Potentilla crinita	5±6	6±5	0.50
Potentilla subviscosa	2±3	2±3	0.24
No emergence			
Clematis hirsutissima	0±0	0±0	6.72
Iris missouriensis	0±0	0±0	18.48
Nicotiana attenuata	0±0	0±0	0.12
Sisyrinchium demissum	0±0	0±0	0.78

[a] Species classified as smoke stimulated if emergence was significantly greater in the smoke than the control treatment at $P < 0.05$ (two-tailed t tests).
[b] Mean ± 1 SD.
[c] Mean weight of individual seeds; $n = 50$ for seeds averaging ≥ 0.5 mg and $n = 100$ for seeds averaging <0.5 mg.