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Mechanism of smoke-induced seed germination in a post-fire chaparral annual

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Summary

1 Smoke-stimulated germination in the post-fire flora of California chaparral does not appear to be triggered by nitrate. Application of freshly prepared unbuffered KNO_3 solutions (pH *c.* 6.2) failed to enhance germination of five populations of *Emmenanthe penduliflora* or one *Phacelia grandiflora* population, regardless of light or stratification conditions.

2 KNO_3 buffered at acidic pH (or unbuffered solutions equilibrated with atmospheric CO_2) did induce germination, but KNO_3 solutions at pH 7 failed to induce germination. Induction of germination is therefore not due to the nitrate ion *per se*, but rather to high $[H^+]$, although buffered controls gave weak germination at low pH, suggesting a role for H^+ plus nitrate. However, other anions such as sulphate were equally as effective as nitrate at breaking dormancy.

3 The germination response to KNO_3 was affected by the type of filter paper used and this may be linked to differences in pH.

4 NO_2, at concentrations present in biomass smoke, was highly effective at inducing germination, and other oxidizing agents also induced germination.

5 Several growth regulators, including nitrite and gibberellin, were stimulatory only at acidic pH, but KCN was stimulatory across a broad pH range.

6 Germination decreased at smoke exposures longer than a few minutes. Also, smoked water samples effective at breaking dormancy were acidic and were less effective when buffered to pH >7.

7 Physical scarification of the seed coat induced germination but the effect was not due to penetration of a water barrier, or to enhanced oxygen uptake or to wound responses such as CO_2 or ethylene production.

8 Different effects of the gibberellin inhibitor CCC (chlorocholine chloride) suggested that the mechanisms of scarification-induced and smoke-induced germination may differ.

9 We conclude that either oxidizing gases in smoke and/or acids generated on burnt sites play a role in germination of post-fire annuals in chaparral.

Keywords: acids, ammonium, *Emmenanthe penduliflora*, germination, nitrate, nitrite, nitrogen dioxide, *Phacelia grandiflora*, scarification, seeds, smoke

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Introduction

The California chaparral is remarkable in the number of annual species whose germination is closely tied to fire. It is apparent that for the majority of species, germination is not stimulated by heat-shock but rather is induced by chemicals from smoke or charred wood (Keeley 1991; Keeley & Fotheringham 1997b). Most attempts to determine the compounds responsible for triggering germination have not successfully identified any chemical from charred wood or smoke that is capable of inducing high germination in species from chaparral (Keeley & Pizzorno 1986) or other ecosystems (Baldwin *et al.* 1994; van Staden *et al.* 1995). Thanos & Rundel (1995) reported that nitrogenous ions stimulated germination of two post-fire annuals, *Emmenanthe penduliflora* and *Phacelia grandiflora*, and concluded that nitrate was the principal factor inducing germination in fire-following

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Californian chaparral species. However, similar nitrate levels (*c.* 10 mol m^{-3}) failed to induce germination in other charred wood or smoke-stimulated species typical of the post-fire flora (Keeley *et al.* 1985; Baldwin *et al.* 1994). Recently, nitric oxides in biomass smoke have been shown to induce germination of *E. penduliflora* (Keeley & Fotheringham 1997a), suggesting other forms of nitrogen may be the important trigger in charred wood or smoke-induced germination.

The focus of this study was to evaluate the importance of nitrate-stimulated germination as an explanation for charred wood/smoke-stimulated germination and to elucidate factors with a bearing on the mechanism of smoke-induced germination. Three classes of mechanisms proposed by Baldwin *et al.* 1994) are: (i) nutritive-mediated stimulation, a role sometimes ascribed to nitrate-induced germination (Karssen & Hilhorst 1992); (ii) chemical scarification of the seed coat; and (iii) a 'signal-mediated' stimulation of germination, a category encompassing a diversity of different chemical signals. In order to evaluate the relative importance of these mechanisms, germination experiments tested the effect of nitrogenous compounds, pH, known dormancy-breaking compounds and scarification of the seed coat.

SPECIES

Emmenanthe penduliflora Benth. and *Phacelia grandiflora* (Benth.) A. Gray are temporary post-fire annuals common in Californian chaparral. The former is a species with a widespread distribution in deserts and chaparral of the southwestern USA, although, on the Pacific Slope, both it and the endemic *P. grandiflora* are generally restricted to post-fire chaparral. *Emmenanthe penduliflora* was the first species shown to have charred wood-stimulated germination (Wicklow 1977; Jones & Schlesinger 1980) and recently smoke has been demonstrated to be even more effective in triggering its germination (Keeley & Fotheringham 1997a). Because of the long history of studies, we have focused most attention on the mechanism of germination in *Emmenanthe*.

Seeds were collected in summer 1994 from first-year post-fire chaparral in southern California and included five *Emmenanthe* populations (LS1 and S6 were from low-elevation coastal sites, CV2 and Sh7 were from higher elevation interior sites, and WS2 was intermediate to these extremes) and one low-elevation coastal population of *Phacelia*. Seeds were separated from debris and stored in closed bottles under room conditions at seed moisture content of 7–11% (of oven-dry mass) for *Emmenanthe* populations and 10% for *Phacelia*. Experiments were conducted between June 1995 and October 1996 and the response of controls and treatments remained unchanged during this period.

MATERIALS AND METHODS

All treatments were represented by three replicates of 30 seeds each, sown on a single 55-mm sheet of filter paper (VWR Grade 413, reported by VWR to be the same as Whatman No. 1) in 55-mm polystyrene Petri dishes. Some experiments were run with different grades of Whatman filter paper or with 'seed germination blotters' (Anchor Paper Co., St Paul, MN) and some experiments were conducted in glass Petri dishes without filter paper. Experiments were initiated with the addition of 2.0 ml purified H_2O or solution. Treatments were kept on separate trays and enclosed in 4-ml polyethylene ziplock bags to reduce desiccation and inadvertent transfer of gaseous emissions. All experiments reported here were given a 1-week cold stratification (4°C) in dim light on moist filter paper. Incubation was under a 12-h photoperiod (*c.* 100 μmol m^{-2} s^{-1}), 18°C light/12°C dark, except dark treatments, which were exposed to green light (<0.05 μmol m^{-2} s^{-1}) briefly when recording germination.

Solutions were made in freshly purified water (Barnstead NANOpure II, Barnstead (Subsidiary of Sybron Corp) 2555 Keeper Blvd. Dubuque, IO 52001, USA) and prepared daily. KNO_3 solutions were also prepared using air-equilibrated water or buffers adjusted to pH 3, 4, 5 (25 mol m^{-3} citrate phosphate), pH 6 (MES; Sigma M-5287, Sigma Chemical Co., 3300 S. 2nd St., St. Louis, MO 63118, USA), pH 7 and 8 (HEPES; Sigma H-7523), and pH 9 (TRIZMA; Sigma T-1503); pH was adjusted with HCl or NaOH. Inhibitors for endogenous biosynthesis of ethylene (AOA; aminooxy-acetic acid; Sigma A-4508) and gibberellin (CCC; chlorocholine chloride; Sigma C-4049) were prepared as unbuffered solutions and applied separately or in combination with other treatments.

Smoke treatment was applied to dry seeds on filter paper prior to incubation, and was generated by combusting foliage and wood from the common chaparral shrub *Adenostoma fasciculatum* on a hot plate and funneling smoke through a hose into the lid at one end of a 70-l glass chamber, with a vacuum attached to the opposite end. The temperature within the chamber did not increase >2°C above ambient. Relative humidity was 60–90%, dependent on fuel moisture. In one experiment dry commercial washed sea sand (Fisher S-25, Fisher Scientific Co., 755 U.S. Highway 202, Bridgewater, NJ 08807, USA), 10 g 55 mm^{-1} Petri dish, was subjected to smoke followed by sowing of untreated seeds and addition of 2.75 ml H_2O or buffer. In other experiments, dry seeds and filter paper were exposed to nitrogen dioxide (0.79 or 7.7 g m^{-3} in N_2) from a compressed gas tank, but in a separate glass chamber and without a vacuum.

Water samples (30 ml in 100-mm glass Petri dish bottoms) were exposed to either smoke or nitrogen dioxide for different durations, after which they were immediately titrated to pH 7.0 with 10 mol m^{-3}

NaOH. The molar concentration of protons was calculated following subtraction of titratable acidity from water controls.

Indirect exposure of seeds to gases emitted by solutions was conducted by placing a small Petri dish within a slightly larger enclosed chamber. Specifically, 5 ml of test solution was added to filter paper in a 100-mm Petri dish bottom. A 55-mm Petri dish with filter paper and seeds plus water was placed inside and the chamber made complete by placing an inverted 100-mm bottom on top and wrapping with parafilm. Chamber airspace was 180 cm^3.

Aqueous extracts of charred or fresh *Adenostoma* wood were prepared by grinding to pass a 2-mm screen, mixing 10 g 100 ml^{-1} purified H_2O, stirring overnight and filtering.

Seeds were physically scarified by piercing the seed coat at the edge with a needle-point probe. Imbibition of these and control seeds was determined by recording the mass of water taken up over the course of 1 week. Seeds were soaked in purified water, and at intervals blotted dry and weighed, then returned to the water.

One-way fixed-effects ANOVA and the Bonferroni multiple comparison test were applied to arc-sine transformed data.

Results

Exogenous application of freshly prepared unbuffered solutions of 1, 10 or 100 mol m^{-3} potassium nitrate (pH 6.0–6.5) failed to enhance germination significantly ($P > 0.05$) of any of the five *E. penduliflora* populations or *P. grandiflora*; however, germination was significantly ($P < 0.001$) enhanced by 5 min of

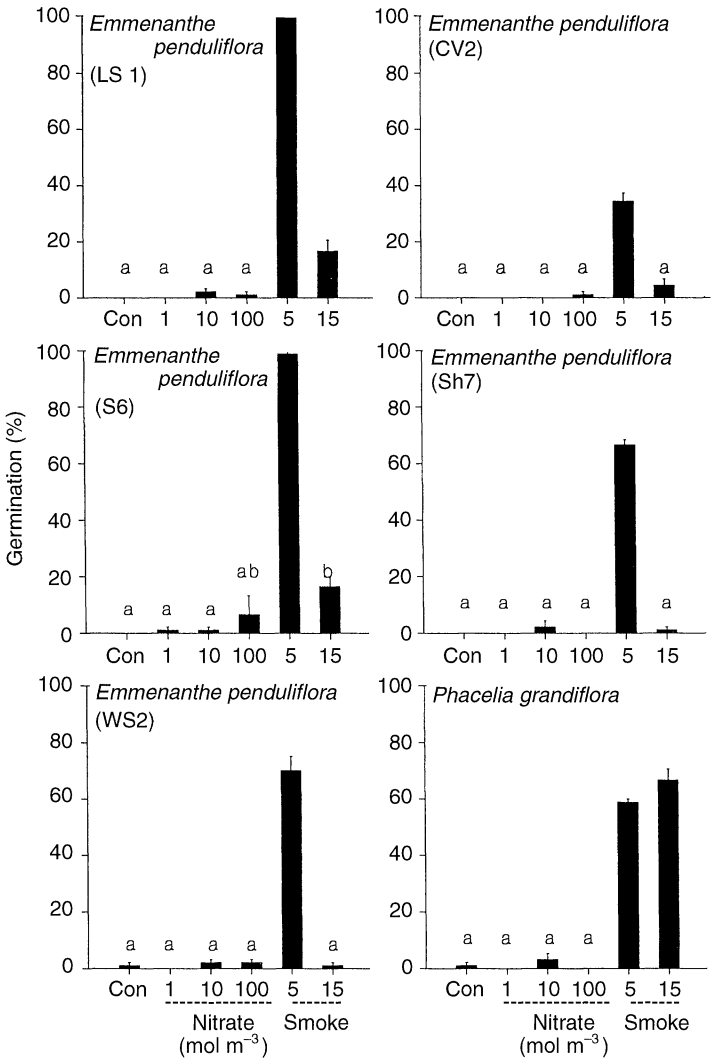

Fig. 1 Germination of five *Emmenanthe penduliflora* populations and *Phacelia grandiflora* for controls (Con) and in response to freshly prepared unbuffered KNO_3 application or following 5 or 15 min smoke exposure. Vertical lines = 1 SE, $n = 3$, treatments within a graph with the same letter are not significantly different ($P > 0.05$).

smoke (Fig. 1). Germination of all *Emmenanthe* populations was markedly lower at 15 min smoke; this was due to the lethal effects of smoke as these seeds rotted, whereas ungerminated control seeds did not.

For one population of *Emmenanthe* (LS1) these experiments were repeated under conditions of either no stratification or 1 month stratification, and in the dark (with and without stratification). The patterns observed in Fig. 1 remained unchanged (Table 1); seeds were light-insensitive and only slightly stimulated by cold stratification. Similar patterns of light-insensitive germination that was only weakly stimulated by cold stratification were observed for *P. grandiflora* (data not shown).

Although freshly prepared, unbuffered, 10 mol m^{-3} KNO_3 failed to stimulate germination (Fig. 1), this was not the case with solutions buffered at low pH (Fig. 2). *Emmenanthe* germination was complete in KNO_3 at pH 3 and not significantly different ($P > 0.05$) at pH 4. However, germination was markedly reduced at higher pH, and there was no significant germination at pH 7. This experiment has been repeated on two other populations of *Emmenanthe*, with similar results (data not shown). The pH response to KNO_3 was not due to interactions with the buffer, as evidenced by the fact that although KNO_3 prepared in freshly distilled unbuffered water (pH 6.0–6.5) failed to stimulate germination, KNO_3 prepared from unbuffered water air-equilibrated (pH 4.9–5.1 due to carbonic acid formed from CO_2 in the air) was highly stimulatory (Fig. 2).

pH dependence was also observed when seeds were subjected to 3–9-h pulses of 10 mol m^{-3} HNO_3 (Table 2). It is unlikely that the observed germination failure at pH 7 was due to negative effects of the pH or the buffer, since physically scarified seeds (which germinated readily in distilled H_2O), germinated optimally in the same buffered solutions at pH 7 (Table 2).

Lack of germination in any of these experiments requires careful analysis as it can be due to lack of stimulation or to inhibition resulting from phytotoxic effects. With respect to the HNO_3 treatments, germination failure at pH 7 was due to lack of stimulation and not to lethal effects, suggested by the fact that seeds were still viable and germinated if subsequently given smoke or other treatments. In contrast, germination failure with continuous exposure to unbuffered solutions of 10 mol m^{-3} HNO_3 was due to lethal effects, as evidenced by seeds rotting. This criterion is used throughout when designating treatments as lethal.

Brief pulses of other acids were also effective in breaking dormancy, although nitric acid was the most effective. In general, there was a lack of congruence between effectiveness of unbuffered acids at stimulating germination (nitric > sulphuric > acetic > hydrochloric) and the proton concentration of these solutions (pH 2.1, 2.0, 3.3 and 2.1, respectively).

The type of filter paper may influence pH when using unbuffered solutions. For example, aqueous extracts made from Whatman No. 1, 4 and 44 papers gave pH values of 6.0, 4.6 and 5.1, respectively. We have also observed a dramatic interaction effect between the filter paper and unbuffered KNO_3 solutions (Table 3). KNO_3 (made in aerated water) was highly stimulatory when applied to seeds on filter paper, or when an extract of KNO_3 + filter paper was applied on glass (without filter paper); however, KNO_3 alone on glass was far less stimulatory (Table 3).

$NaNO_2$ induced germination when 10 mol m^{-3} solutions at pH 5 or 6 were applied in pulses of 6 or 12 h, but when applied continuously in the incubation medium it was highly lethal, as was KNO_2 (Table 2). Germination was also induced by exposure of seeds to vapours emitted from nitrite solutions in an enclosed chamber (25% ± 4 with 100 mol m^{-3} $NaNO_2$ at pH 7; data not shown). At pH 3 or 5, nitrite emitted vapours that induced 100% of the seeds to initiate

Table 1 Effect of light and cold stratification on germination response of *Emmenanthe penduliflora* (LS1 population) in controls, unbuffered KNO_3 or smoke treatment. Mean ± SE, $n = 3$. No significant difference ($P > 0.05$) between means within a row is indicated by the same superscript letter and within columns by the same symbol

Cold stratification (days)	Light/ dark	Germination (%)		
		Control	KNO_3 (10 mol m^{-3})	Smoke
0	Light	0 ± 0[a]†	0 ± 0[a]†	86 ± 4‡
0	Dark	0 ± 0[a]†	0 ± 0[a]†	80 ± 6‡
7	Light	0 ± 0[a]†	3 ± 2[a]†	100 ± 0§
7	Dark	0 ± 0[a]†	2 ± 1[a]†	99 ± 1§
30	Light	1 ± 1[a]†	5 ± 3[a]†	99 ± 1§

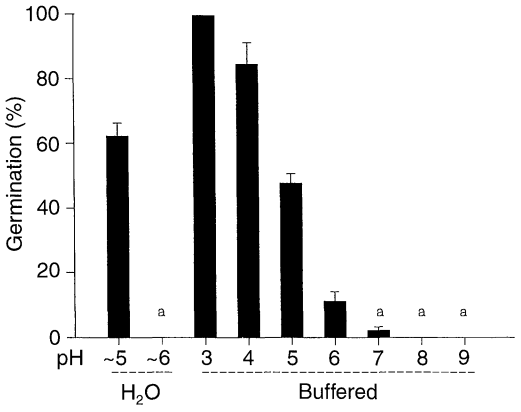

Fig. 2 Germination of *Emmenanthe penduliflora* (population LS1) incubated in KNO_3 solutions prepared in unbuffered air-equilibrated water (pH *c.* 5) or in freshly purified water (pH *c.* 6) or in solutions buffered to pH 3, 4, 5, 6, 7, 8 or 9. Vertical lines = 1 SE, $n = 3$, treatments with the same letter are not significantly different ($P > 0.05$).

Table 2 Germination response of *Emmenanthe* seeds to acids, bases and growth regulators (n = 1–9, dependent upon treatment). Seeds were treated continuously (cont) on filter paper or given a pulse of 3–24 h on glass and then washed repeatedly and transferred to filter paper in polystyrene dishes

Treatment (molarity)		Duration of exposure (continuous or pulse)	H_2O	Buffered medium pH 3	4	5	6	7	8
Control		–	0	30	15	5	0	0	0
Scarification		–	100	0	0	0	35	95	–
HNO_3	(1 mol m^{-3})	(cont)	62*	–	–	–	–	–	–
	(10 mol m^{-3})	(cont)	0	–	–	–	–	–	–
	(10 mol m^{-3})	(3 h)	100	77	63	30	7	20	0
	(10 mol m^{-3})	(6 h)	100	77	83	13	3	0	0
	(10 mol m^{-3})	(9 h)	100	97	97	70	0	0	0
HCl	(10 mol m^{-3})	(9 h)	60	–	–	–	–	–	–
H_3CCOOH	(10 mol m^{-3})	(6 h)	77	–	–	–	–	–	–
H_2SO_4	(10 mol m^{-3})	(24 h)	100	–	–	–	–	–	–
KNO_2	(10 mol m^{-3})	(cont)	–	0	0	0	0	0	–
$NaNO_2$	(10 mol m^{-3})	(cont)	–	0	0	0	20	10	–
	(10 mol m^{-3})	(6 h)	–	0	0	0	100	0	–
	(10 mol m^{-3})	(12 h)	–	0	0	60	90	0	–
NO_2 gas	(0.79 g m^{-3})	(0.05 h)	100	–	–	–	–	–	–
	(7.70 g m^{-3})	(0.008 h)	100	44	64	28	20	0	–
	(7.70 g m^{-3})	(0.03 h)	0	–	–	–	–	–	–
NH_4NO_3	(10 mol m^{-3})	(6 h)	–	0	0	0	0	0	–
NH_4Cl	(10 mol m^{-3})	(cont)	0	0	0	0	0	0	0
KOH	(1, 10, 100 mol m^{-3})	(3,6,9,24 h, cont)	0	–	–	–	–	–	–
NaOH	(1, 10, 100 mol m^{-3})	(3,6,9,24 h, cont)	0	–	–	–	–	–	–
KCl	(10 mol m^{-3})	(cont)	–	0	0	29	–	1	0
H_2O_2	(1 kmol m^{-3})	(24 h)	88	–	–	–	–	–	–
GA_3	(1 mmol m^{-3})	(cont)	3	–	–	–	–	–	–
	(5 mmol m^{-3})	(cont)	1	–	–	–	–	–	–
	(10 mmol m^{-3})	(cont)	3	–	–	–	–	–	–
	(100 mmol m^{-3})	(cont)	33	–	100	33	0	0	–
	(1 mol m^{-3})	(cont)	100	–	100	100	0	0	–
	(10 kmol m^{-3})	(cont)	100	–	100	100	40	0	–
KCN	(100 mmol m^{-3})	(cont)	43	–	47	57	20	43	–
	(1 mol m^{-3})	(cont)	73	–	70	69	47	63	–
	(10 mol m^{-3})	(cont)	50	–	93	100	97	100	–

*Seedlings died.

germination (seeds softened and radicles elongated) but radicles never emerged through the seed coat and eventually rotted. Vapours from nitrate (KNO_3) solutions failed to induce germination at either 10 or 100 mol m^{-3} and pH 3, 5 or 7.

Nitrogen dioxide is a gas potentially released from nitrite and was capable of inducing complete germination, although exposure to more than a few minutes was lethal (Table 2). This lethal effect may be tied to nitric acid deposition in the water samples, as acidity increased with increasing concentrations of NO_2 (Fig. 3a). However, under brief exposure to NO_2, seeds incubated in buffered solutions germinated best under acidic conditions (Table 2).

Increasing duration of smoke exposure also increased the acidity of exposed water samples (Fig. 3b).

Smoke-treated sand was stimulatory to untreated seeds and the effect was affected by pH, with marked drops in germination when the sand was buffered to higher pH (Fig. 4).

In addition to smoke, a 10% (w/v) aqueous leachate of charred foliage was nearly as effective in stimulating germination of both species (94%); however, a 10% leachate of fresh unburned foliage failed to induce germination in either the light or the dark (0–2%) and the same was observed for completely ashed foliage (0%).

The pH of these and other solutions made from *Adenostoma* foliage were: fresh unburned, 5.1; heated at 150°C for 1 h, 5.0; charred, 5.2; charred + ash, 8.9; ashed at 350°C, 11.2; ashed at 600°C, 11.3.

Ammonium had no effect on germination, regardless of pH, and other bases were also ineffective (Table 2).

Two other growth regulators demonstrated a marked pH effect. Gibberellic acid (GA_3 or $GA_{4/7}$) at 1–10 mmol m^{-3} concentrations, levels which are generally hormonally active, failed to induce germination, although much higher concentrations were effective (Table 2). It was quite effective at low pH but ineffective at higher pH. In contrast, potassium

Table 3 Germination of *Emmenanthe penduliflora* in response to a combination of KNO_3 (10 mol m^{-3} prepared from air-equilibrated water, final pH *c.* 5) and filter paper, either 'standard' Whatman No. 1 (used throughout this study) or Anchor germination paper. Mean ± SE, $n = 3$; means with same superscript are not significantly different at $P > 0.05$

	Glass	Whatman no. 1 filter paper	Anchor filter paper
Aerated water control	0 ± 0^a	0 ± 0^a	0 ± 0^a
Aerated KNO_3	12 ± 3	88 ± 5	0 ± 0^a
Aerated KNO_2	3 ± 0	68 ± 5	–
Extract of aerated KNO_3 ± Whatman paper	85 ± 1	–	–
Extract of aerated KNO_3 ± Anchor paper	5 ± 1	–	–

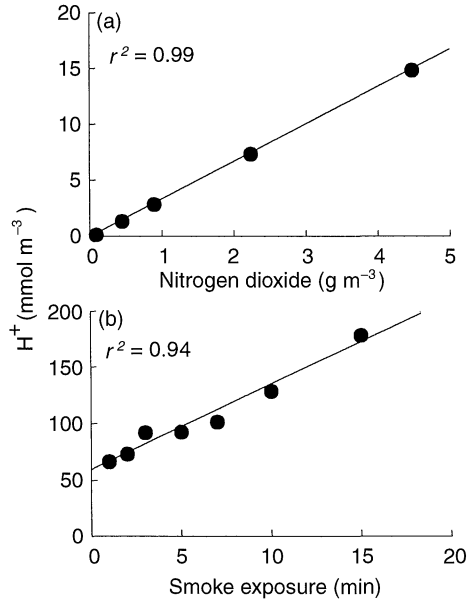

Fig. 3 Acidity absorbed by water samples exposed to (a) nitrogen dioxide for 3 min or (b) smoke for different durations.

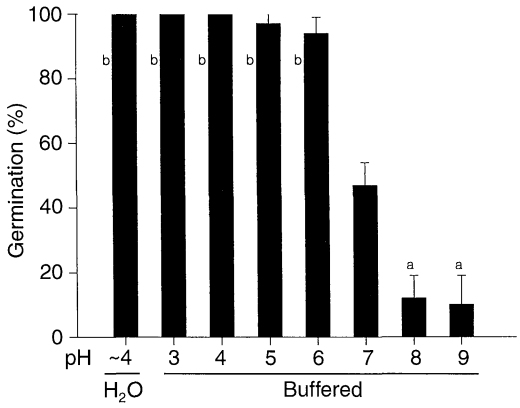

Fig. 4 Germination of *Emmenanthe penduliflora* (population LS1) incubated in sand exposed to 2 min of smoke and with water added (pH *c.* 4) or buffered solutions of pH 3, 4, 5, 6, 7, 8 or 9. Vertical lines = 1 SE, $n = 3$, treatments within a graph with the same letter are not significantly different ($P > 0.05$).

cyanide induced germination across a broad pH range, indicating that acidic conditions were unnecessary for activation of this dormancy-breaking compound (Table 2).

Physical scarification of the seed coat induced complete germination (Table 2). We tested the hypothesis that this response was due to a change in water uptake induced by scarification and found no significant difference in water uptake between dormant and scarified seeds ($P > 0.05$, $n = 5$ batches of 50 seeds each). We also tested the hypothesis that scarification induced germination by increasing oxygen uptake. If this were true, we predicted that unscarified seeds would germinate when allowed to imbibe in oxygen-saturated water. Such treatment for 1, 6, 12, 24, 48, 72 and 168 h failed to induce germination. Interestingly, although application of 100% oxygen to dry or moist seeds on glass failed to induce any germination, when applied to seeds on moist filter paper we repeatedly observed 10–20% germination.

Another potential mechanism for scarification triggered germination is wound-induced production of gases such as carbon dioxide and ethylene. We discount the role of carbon dioxide, since germination of scarified seeds was not inhibited when CO_2 was trapped, either by direct application of $1–10^5$ mmol m^{-3} of KOH, or by inclusion of 3 kmol m^{-3} KOH in an adjacent well of an enclosed chamber (Table 4); inhibition by direct application of 100 mol m^{-3} KOH was probably due to phytotoxic effects of this high dosage (Leather *et al.* 1992). These experiments have been repeated with smoke-treated seeds and the carbon dioxide trap again failed to reduce germination, indicating smoke does not induce germination by activating the endogenous production of CO_2.

Scarification also does not trigger germination by inducing endogenous ethylene production. This is supported by the fact that scarification plus the inhibitor AOA, which at 1 mol m^{-3} concentration will prevent endogenous ethylene production (Cranston *et al.* 1996), had no inhibitory effect on the germination of scarified *Emmenanthe* seeds (Table 4). This inhibitor also had no effect on germination of smoke-treated seeds (Table 4).

Application of CCC, an inhibitor of gibberellin biosynthesis (Adkins *et al.* 1984), provides some clue

Table 4 Effect of a KOH CO_2 trap applied directly to seeds or in the outer well of a 180-cm^3 chamber, application of AOA ethylene inhibitor and CCC gibberellic acid inhibitor to scarified and smoke-treated seeds of *Emmenanthe penduliflora*. Mean ± SE, $n = 3$. No significant difference ($P > 0.05$) between means within a row is indicated by the same superscript letter and within columns by the same symbol

		Control	Scarified	Smoke-treated
KOH	1 mmol m^{-3}	0 ± 0†	100 ± 0^{a}‡	100 ± 0^{a}‡
	10 mmol m^{-3}	0 ± 0†	91 ± 3^{a}‡	100 ± 0^{a}‡
	100 mmol m^{-3}	0 ± 0†	100 ± 1^{a}‡	100 ± 0^{a}‡
	10^3 mmol m^{-3}	0 ± 0†	97 ± 2^{a}‡	93 ± 2^{a}‡
	10^4 mmol m^{-3}	0 ± 0†	100 ± 0^{a}‡	100 ± 0^{a}‡
	10^5 mmol m^{-3}	0 ± 0^{a}†	0 ± 0^{a}†	52 ± 5
	1 kmol m^{-3} (outer well)	0 ± 0†	100 ± 0^{a}‡	100 ± 0^{a}‡
	3 kmol m^{-3} (outer well)	0 ± 0†	100 ± 1^{a}‡	100 ± 0^{a}‡
AOA	1 mol m^{-3}	0 ± 0†	92 ± 3^{a}‡	100 ± 0^{a}‡
CCC	10 mol m^{-3}	0 ± 0^{a}†	0 ± 0^{a}†	100 ± 0‡
	25 mol m^{-3}	0 ± 0^{a}†	0 ± 0^{a}†	92 ± 2‡
KNO_3	(10 mol m^{-3} at pH 3)			
	Control	100 ± 0‡	–	–
	10 mol m^{-3} CCC	45 ± 11§	–	–
	25 mol m^{-3} CCC	40 ± 12§	–	–
$NaNO_2$	(9 h pulse of 10 mol m^{-3} at pH 6)			
	Control	90 ± 6‡	–	–
	10 mol m–3 CCC	100 ± 0‡	–	–
	25 mol m^{-3} CCC	80 ± 11‡	–	–
KCN	(24 h pulse of 10 mol m^{-3} at pH 6)			
	Control	100 ± 0	–	–
	10 mol m^{-3} CCC	0 ± 0†	–	–
	25 mol m^{-3} CCC	0 ± 0†	–	–

that scarification and smoke may act through different mechanisms; CCC was highly inhibitory to scarified seeds but not to smoke-treated seeds (Table 4). The effect of CCC on the efficacy of dormancy-breaking compounds also suggests that nitrite and cyanide may be acting quite differently (Table 4). CCC was highly inhibitory to KCN-treated seeds, somewhat inhibitory to KNO_3-induced germination, but had no capacity to inhibit $NaNO_2$-stimulated germination.

Discussion

HYPOTHESIS OF NITRATE-STIMULATED GERMINATION

The attraction of this hypothesis is that nitrate increases briefly after fire (Christensen 1973) and is known to induce germination of many weedy species (Hendricks & Taylorson 1974; Pons 1989). However, nitrate-stimulated germination in *Emmenanthe* is a pH-dependent effect (Fig. 2). Since nitrate is the base of a strong acid, it would be completely dissociated across the pH range tested here (Fig. 2); i.e. at pH 7 this ion should be as abundant as at pH 4. Therefore, the marked difference in germination response cannot be attributed to the presence of the nitrate ion *per se*, but rather to the high H^+ concentration in the presence of the nitrate ion, a phenomenon observed for other species as well (Roberts 1963). Germination in *Emmenanthe* is also triggered by a high concentration of H^+ with anions such as sulphate (Table 2).

Post-fire soils are close to neutrality ($\bar{X} = 6.6 \pm 0.03$, $n = 90$ sites; J. E. Keeley, unpublished data) or, in microsites with heavy ash deposition, are even basic. Field studies are required to evaluate the extent to which nitrate persists in conditions acidic enough to induce germination of *Emmenanthe* seeds.

Other evidence inconsistent with nitrate being the germination trigger in smoke is the fact that KNO_3 solutions buffered at pH 6 and 7 are largely ineffective (Fig. 2), whereas smoked soils at those pH levels are highly stimulatory (Fig. 4). Also inconsistent with the hypothesis is the demonstration here (and elsewhere; Keeley & Pizzorno 1986) that an aqueous leachate from fresh *Adenostoma* wood fails to induce *Emmenanthe* germination, in contrast to the stimulatory effect of charred wood extracts, yet these wood fractions have nearly identical nitrate levels (Thanos & Rundel 1995) and pH values (see the Results). In addition, *Emmenanthe* seeds are light neutral, and nitrate is generally associated with breaking dark-imposed dormancy (Hilhorst & Karssen 1989). Finally, other post-fire chaparral species with charred wood/smoke-induced germination fail to respond to elevated nitrate levels (Keeley *et al.* 1985; Baldwin *et al.* 1994).

pH EFFECTS ON SEED GERMINATION

It is a common misconception that pH control in seed germination studies is of minimal importance. The idea that it is best avoided dates back to early studies that suggested inhibitory effects of buffers (Mayer & Evenari 1953). Germination studies reported here and elsewhere (e.g. Cohn *et al.* 1983; Cohn *et al.* 1987) illustrate the importance of pH, and the pH-dependent effect of nitrate (Fig. 2), calls into question conclusions previously made about *Emmenanthe*'s nitrate-induced germination response to light and cold (Thanos & Rundel 1995). In that study, pH was not controlled between experiments and reportedly varied from 4.1 to 5.5; the differences they reported in germination may therefore be attributable to pH effects (e.g. Fig. 2) rather than to treatment effects.

In addition to nitrate, several other dormancy-breaking compounds are pH dependent (Table 2). Of particular interest is our observation that gibberellic acid was effective at low pH but ineffective at higher pH, a pattern also noted for other species (Palevitch & Thomas 1976). It would therefore be of interest to check whether germination induced by the combination of smoked-water plus $GA_{4/7}$ observed by Thomas & van Staden 1995) was simply a pH effect, since smoke generates significant levels of acids (Fig. 3b).

When using unbuffered solutions, the effect on pH of type of filter paper used to absorb the incubation medium may explain the synergistic effect observed with the combination of filter paper and KNO_3 (Table 3).

ROLE OF THE SEED COAT IN SMOKE-INDUCED GERMINATION

Physical (and apparently chemical) scarification of the seed coat induces germination in *Emmenanthe* (Table 2; see also Jones & Schlesinger 1980) and in some, but not all, smoke-stimulated species (Keeley & Fotheringham 1997b). Whether or not this response provides clues to the mechanism of smoke-induced germination requires careful analysis.

In 'hard-seeded' heat-stimulated species (such as many Fabaceae and Rhamnaceae), artificial physical scarification induces germination by a mechanism similar to that of natural heat-shock from a fire. Dormant seeds have a water-impermeable seed coat that blocks imbibition, and both treatments alter the water permeability of the seed coat (Ballard 1973); the same may apply to certain non-hard-seeded species such as *Avena fatua* (Hsiao *et al.* 1983).

In contrast, *Emmenanthe*, and other species with smoke-induced germination, lack a water-impermeable seed coat and dormant seeds are capable of fully imbibing water (Keeley & Fotheringham 1997b). Neither smoke-treatment nor scarification alters the water uptake characteristics of these species. However, these treatments (as well as acids and NO_2) do affect permeability to solutes. Most species examined to date have a subdermal cuticle that is semi-permeable, and in dormant seeds this membrane blocks some solutes (Egerton-Warburton *et al.* 1997; L. M. Egerton-Warburton & J. E. Keeley unpublished data; Keeley & Fotheringham 1997b). It is not yet known whether or not changes in solute permeability of the subdermal cuticle, induced by smoke (or scarification), are causally related to germination.

Scarification-triggered germination may result from wound-induced production of gases such as ethylene and carbon dioxide (Leather *et al.* 1992; Cranston *et al.* 1996), but our data do not support this hypothesis (Table 4). In this respect smoke-induced germination is comparable to scarification-induced germination.

Application of CCC, an inhibitor of gibberellin biosynthesis (Adkins *et al.* 1984), provides some clue that scarification and smoke may act quite differently; CCC was highly inhibitory to scarified seeds but not to smoke-treated seeds (Table 4).

MECHANISMS OF SMOKE-INDUCED GERMINATION

Although germination of *Emmenanthe* has been more thoroughly studied than any other chaparral species, we are not yet certain of the physiological mechanism leading to germination. We considered three classes of compounds that could be involved in the induction of germination: (i) oxidizing compounds, (ii) protons and (iii) weak acids.

Oxidizing compounds

Nitrogen dioxide, at levels to be expected in smoke or from biogenic nitrification on burnt sites, will induce 100% germination in *Emmenanthe* and this trace gas has been proposed as the ecological trigger that initiates germination (Keeley & Fotheringham 1997a). Additionally, SO_2 (and possibly any other oxidizing agent, e.g. hydrogen peroxide; Table 2) will induce germination. These gases may also bind to soil particles and be indirectly transferred in aqueous or gaseous forms following fire (Keeley & Fotheringham 1997a). Oxidizing gases increase the permeability of the semi-permeable subdermal cuticle and this may play a role in the induction of germination.

I. Baldwin (personal communication) has found that an oxidation product of cellulose induces germination of smoke-stimulated *Nicotiana attentuata*. NO_2 may oxidize cellulose or other carbohydrates in the seed coat, producing a product that signals further changes leading to germination. Alternatively, sufficient levels of such an oxidation product may be directly generated from combustion (I. Baldwin, personal communication). Degradation products of cellulose or other polysaccharides, such as certain oli-

gosaccharides, are known to have hormone-like activity and signal various developmental processes (Ryan & Farmer 1991). This sort of mechanism could explain the interactions we observed between filter paper and KNO_3 or between filter paper and O_2.

Protons

Biomass smoke has a high water vapour content (Ohlemiller *et al.* 1987; Dollard *et al.* 1987), and consequently hydration of oxidizing gases evolved during fire are responsible for substantial oxy-acid deposition (Lacaux *et al.* 1991; LeBel *et al.* 1991). Also, nitrogen oxides may adsorb onto the outer seed coat or soil particles and later be hydrated to nitric or nitrous acid (Cohn & Hugh 1986).

In the laboratory, complete germination is triggered by strong acids such as nitric or sulphuric acid (Table 2), which, over the pH range used, would be completely dissociated into protons and the respective anion. Understanding the mechanism of this acid-induced germination response requires knowledge of whether protons and anions need be present simultaneously, or whether they act in series. For example, protons may first 'acid scarify' the subdermal cuticle or increase the permeability of cell membranes, thus allowing entry of anions or other solutes (Sanders *et al.* 1981). Alternatively, protons and anions may act in unison. Since nitrate assimilation is coupled with an internal release of hydroxyl ions (Raven 1988), nitrate uptake may be limited by the availability of excess protons for cellular ion balance, or protons may serve as a transporter, one that needs to be coupled with an anion.

In the field there are other sources of acids on burned sites, such as those generated by pyrolysis of soil organic matter (Blank *et al.* 1996) and biogenic nitrification (Christensen 1973; Anderson *et al.* 1988). The effect of acids in these soils is likely to be short-lived as they are potentially neutralized by the highly alkaline nature of ash and, perhaps as a consequence, post-fire chaparral soils tend to be near pH 7.

Weak acids

Uptake of weak acids (in an associated or protonated state) is likely to result in dissociation at cellular pH, leading to internal acidification, and the breaking of dormancy in seeds and other 'resting' stages (Cohn 1989, 1996). This model holds for a great many dormancy-breaking compounds (Cohn *et al.* 1983, 1987; Cohn & Hughes 1986) and is possibly due to increased membrane permeability of the associated form (Jackson & St John 1980). Thus, it is not surprising that the effectiveness of many dormancy-breaking chemicals is correlated with their degree of lipophilicity (Cohn 1989). This acid-loading model is supported by the pH-dependent germination response of *Emmenanthe* to growth regulators such as nitrite, gibberellic acid and potassium cyanide (Table 2).

Other, more complex roles for weak acids have been proposed (Adkins *et al.* 1985). For example, Hamabata *et al.* 1994) showed that protonation of GA_3 was required for induction of I-amylase and mobilization of the endosperm reserves, but concluded that it was unclear if this was due to enhanced uptake of the protonated form or to direct pH effects on a tissue targeted by GA_3.

In conclusion, both oxidizing gases and acids are potential triggers inducing germination of *Emmenanthe* and other post-fire species with smoke-induced germination. In light of the complex chemistry resulting from biomass burning, the complexity of soil chemistry changes following fire and the myriad of events that take place during germination, sorting out the precise physiological mechanism may be difficult. On a community level this is made even more daunting by the likelihood that the number of internal barriers to germination may differ between smoke-stimulated species (Keeley & Fotheringham 1997b).

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