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Trace Gas Emissions and Smoke-Induced Seed Germination

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Dormant seeds of a California chaparral annual were induced to germinate by smoke or vapors emitted from smoke-treated sand or paper. Nitrogen oxides induced 100 percent germination in a manner similar to smoke. Smoke-treated water samples inducing germination were comparable in acidity and concentration of nitrate and nitrite to nitrogen dioxide (NO_2)–treated samples. Vapors from smoke-treated and NO_2-treated filter paper had comparable NO_2 flux rates. Chaparral wildfires generate sufficient nitrogen oxides from combustion of organic matter or from postfire biogenic nitrification to trigger germination of *Emmenanthe penduliflora*. Nitrogen oxide–triggered germination is not the result of changes in imbibition, as is the case with heat-stimulated seeds.

Fire-prone mediterranean-climate regions are noted for their abundance of plant species whose germination and recruitment are restricted to postfire environments. For seeds of many species (such as Fabaceae, Convolvulaceae, and Rhamnaceae), heat shock during fire weakens the cuticle and loosens cells in localized regions, such as the hilum or strophiole, allowing imbibition and germination (*1*).

However, many species that restrict germination to postfire environments lack an impervious external cuticle and are not heat stimulated; instead, germination can be induced by chemicals released from the combustion of natural fuels (*2*). Incubation in the presence of charred wood has been shown to induce the germination of the Californian chaparral annual *Emmenanthe penduliflora* (*3*), as well as that of other species (*4*). Smoke triggers the germination of South African fynbos and savanna (*5*), western Australian heath (*6*), and Great Basin (Utah) scrub (*7*). Although 71 compounds have been identified from active fractions of smoke, none of these compounds were highly stimulatory in pure form (*7*), and other studies have also failed to identify the active components of smoke (*8*). Here we show that certain trace gases from smoke are sufficient to trigger germination and discuss mechanisms of how these gases may induce germination.

Emmenanthe penduliflora (Hydrophyllaceae) is an annual largely restricted to postfire sites, and its germination is cued not by heat (*2*) but chemically, by charred wood (*3*) or smoke (Fig. 1). For most chaparral populations, seeds exhibited deep primary dormancy: Controls uniformly gave 0% germination, whereas dormancy was overcome with as little as 1 min of smoke exposure at ambient temperature (*9*).

Smoke acts directly on seeds and indirectly through secondary transfer after fire (Fig. 1); 100% germination was induced by direct exposure of seeds to smoke or by incubation of untreated seeds on sand or filter paper previously exposed to smoke, with water previously exposed to smoke, or in the presence of gases emitted from smoke-treated sand or filter paper. For indirect treatments, seeds were sown on media between 1 and 4 hours after smoke treatment, and we have observed the same response with filter paper that was smoke-treated more than 2 months earlier. Seeds tolerate 10 min of direct exposure to smoke but are killed when sown with water exposed for that duration. Water samples that are lethal at 10 min of exposure will induce complete germination if diluted 10-fold (*10*).

We investigated the stimulatory effect of gaseous smoke emissions from the combustion of wood and foliage (*11*). Although CO_2 and C_2H_4 are known to induce germination in many species, they failed to affect *Emmenanthe* (Table 1). Nitrogen oxides induced 100% germination, and NO_2 was more stimulatory than NO_x (NO + NO_2). With increasing concentration, the exposure time needed to induce germination declined; NO_2 induced 100% germination with 3 min of exposure at 790 mg m^{-3} [500 parts per million by volume (ppmv)] or with 30 s of exposure at $\geq 1.5 \times 10^3$ mg m^{-3}.

NO_2 induced germination both directly and indirectly (Fig. 2), as did smoke (Fig. 1). Also, both smoke-treated and NO_2-treated water extracts were acidic (*12*). For water extracts inducing 100% germination, acidity and the concentration of nitrites and nitrates in smoke-treated samples were comparable to those of NO_2-treated samples (*12*). Trapping NO_2 (*13*) emitted over 24 hours [time sufficient to induce germination in vapor experiments (Figs. 1 and 2)] from smoked or NO_2-treated paper gave comparable NO_2 flux rates (26 to 38 ng m^{-2} s^{-1}). Thus,

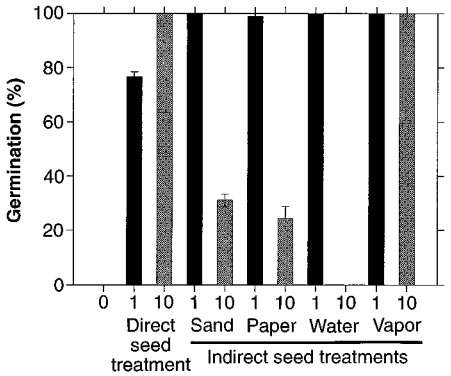

Fig. 1. Germination of *Emmenanthe* for control (0) and smoke treatments of 1- or 10-min exposures for direct treatment (smoke-treated seeds incubated on nontreated filter paper) and indirect treatments [untreated seeds incubated on smoke-treated sand (10 g of Fisher S25-3) or filter paper or untreated seeds incubated with smoked water or exposed in a 180-cm^3 chamber to gases emitted by smoke-treated filter paper (similar results occur when paper is replaced with sand)]. Bars, 1 SE (n = 3).

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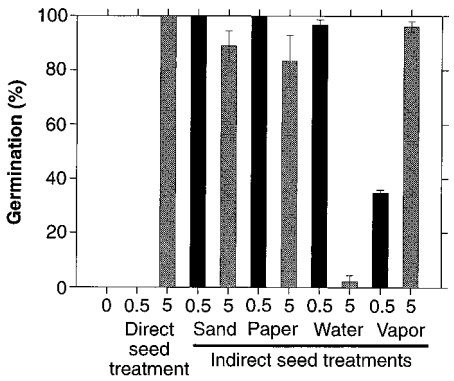

Fig. 2. Germination of *Emmenanthe* for control (0) and NO_2 (7.7 g m^{-3}) treatments of 0.5- or 5-min exposures for direct treatment (NO_2-treated seeds incubated on untreated filter paper) and indirect treatments [untreated seeds incubated on NO_2-treated sand or filter paper or on untreated filter paper with water exposed to NO_2 or untreated seeds exposed to vapors emitted from NO_2-treated filter paper (similar results occurred when sand was used)].

continuous exposure of wet seeds to low levels of NO_2 evolving off filter paper was as effective as brief exposure of dry seeds to higher concentrations (Table 1).

Comparing smoke emissions from chaparral fires (*14*) with our lab results presents an interesting scaling problem. NO_x emissions vary from 160 to 800 mg m^{-3} within the smoke plume to 160 to 2400 mg m^{-3} (100 to 1500 ppmv) closer to the fuel source (*15*). Although these NO_x levels are sufficient to account for experimental germination results (Table 1), they are generated aboveground and may be advected away from the site.

A better indicator of seed exposure to trace gases would be the NO_x from combustion of litter and soil organic matter, which is potentially >600 mg of N per square meter (*16*). Exposure of seeds to smoke generated from the heating of soil and litter will induce germination, and direct measurements of NO_2 produced from combustion of soil organic matter from chaparral sites gave minimum values of 80 to 160 mg of N per square meter (*17*). We calculate [from (*12*)] that direct application of 12 mg of N per square meter of NO_2 was sufficient to generate 100% germination.

Additionally, there is a postfire biogenic NO_x flux of 300 mg of N per square meter in the 6 months after a chaparral fire, which is three times greater than that in unburned soils, caused by nitrification of ammonium in ash and resulting in substantial $NO_3^- + H^+$ production (*18*).

We conclude that chaparral wildfires directly or indirectly generate levels of nitrogen oxides that are sufficient to induce germination of *E. penduliflora*. The importance of postfire biogenic production is suggested by the observation that in our vapor experiments (Figs. 1 and 2), wet seeds were triggered by NO_2 flux rates of the same magnitude as those observed in postfire chaparral but not in unburned chaparral (*18*).

These estimates raise some concerns about chaparral in highly polluted regions, such as the Los Angeles Basin, where there is the possibility that nitrogen oxides produced by automobiles may be sufficient to trigger germination in the absence of fire, because dry and wet nitrogen deposition rates of 500 to 900 mg of N per square meter per year (the vast majority of which are in oxidized forms) have been reported (*19*).

The physiological mechanism of how nitrogen oxides in smoke trigger germination in *Emmenanthe* is presently unknown, although we can exclude certain mechanisms. Substantial seed coat changes occur under the same smoke treatments that induce germination; for example, the subdermal cuticle is more permeable to dyes than that of untreated seeds (*20*). However, exclusion of these dyes does not always reflect exclusion of water (*21*), and imbibition rates and amounts are nearly identical between dormant and smoke-treated seeds (Fig. 3). Thus, nitrogen oxides in smoke do not affect imbibition (Fig. 3). Germination is also not induced by increased oxygen influx, because seeds failed to germinate when incubated either dry or moist under 100% oxygen for 1, 6, 12, 24, 48, 168, or 336 hours or when allowed to imbibe in water supersaturated with O_2 for similar lengths of time.

Dormant seeds have a semipermeable subdermal cuticle, and smoke increases the permeability of this membrane to solutes. We hypothesize that this change is induced by nitrogen oxides, which diffuse through and are desorbed at the inner surface of the cuticle (*22*). NO_2 treatment increases the solute permeability of *Emmenanthe* seeds (*20*). In addition, the cuticle may act as an NO_2 reservoir (20% by weight), and because nitrogen oxides demonstrate irreversible binding, cuticles can become complete-

Table 1. Gas concentration and exposure duration producing maximum germination ($\bar{x}$ + SD, n = 3) in *E. penduliflora* seeds (all gases were tested at 0.5-, 1-, 2-, 3-, 5-, 10-, 15-, 30-, 40-, 60-, 120-, 240-, 360-, 720-, and 1440-min duration). Germination means with the same superscript symbol are not significantly different at $P > 0.05$ with ANOVA on arc-sign transformed data. Dashes indicate all exposure durations.

Gas	Gas concentration (mg m^{-3})	Exposure duration (min)	Maximum germination (%)
Air control	–	–	0 ± 0*
CO_2	7.7×10^3	–	0 ± 0*
	1.5×10^6	10	5 ± 3*
CO	9.7×10^3	10	15 ± 4†
C_2H_4	98	–	0 ± 0*
CH_4	55	–	0 ± 0*
$NO + NO_x$	20.8×10^3	0.5	100 ± 0‡
NO_2	790	1	93 ± 6‡
	790	3	100 ± 0‡
	1.5×10^3	0.5	100 ± 0‡
	1.5×10^3	1	100 ± 0‡
	7.7×10^3	0.5	100 ± 0‡
N_2O	153	–	0 ± 0*
	15.3×10^3	10	9 ± 5*
SO_2	2.3×10^3	5	3 ± 2*
	23.0×10^3	10	23 ± 5†

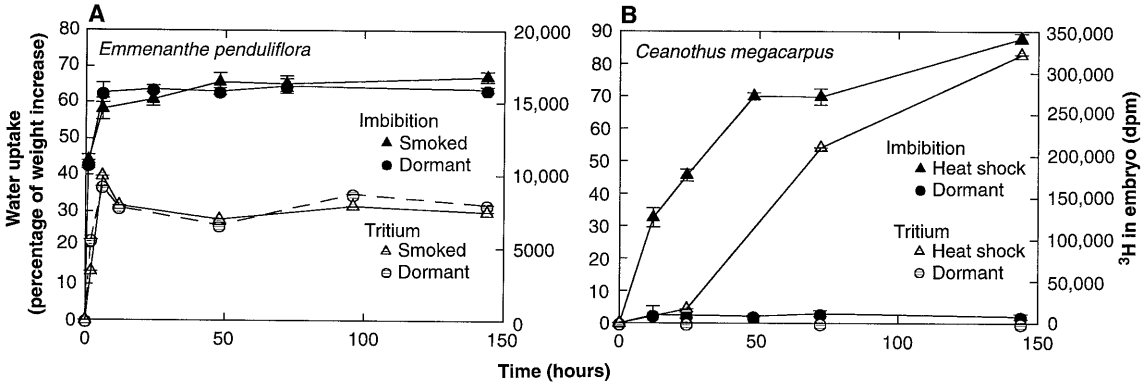

Fig. 3. Imbibition curves and accumulation of tritium in embryos of (**A**) *E. penduliflora* seeds (dormant and smoke-treated for 1 min) and, for comparison, (**B**) *Ceanothus megacarpus*, a hard-seeded chaparral species (dormant and heat-treated for 5 min at 110°C). For tritium uptake, seeds were soaked in ^3H_2O (18.5 megabecquerel ml^{-1}), blotted, and air-dried for 10 min before extraction of embryos. *Ceanothus* seeds have a mass ~40 × greater than *Emmenanthe* seeds.

ly saturated with nitrates during their lifetime (*22*). Thus, solute permeability could be increased by the delayed indirect transfer of gases from soil particles some time after fire (Figs. 1 and 2) or from postfire biogenic production (*18*).

Nitrogen oxides may alter the permeability of the subdermal cuticle, either through direct oxidation effects or after hydration as acids; HNO_3 is a strong acid that is capable of increasing the solute permeance of isolated cuticles (*23*), as well as the subdermal cuticle in *Emmenanthe* seeds (*20*), and induces germination at molarities comparable to those generated by smoke (*24*). Additionally, direct dry deposition of both nitric and acetic acids after fire may be important (*25*), but this is likely a short-lived effect as the high ammonium and alkalinity concentrations in ash eventually buffer these acids.

Although smoke-induced germination in *Emmenanthe* is associated with increased solute permeability of the subdermal cuticle, we cannot yet say if this is directly involved in triggering germination, and other roles for NO_2 have been proposed (*26*).

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13. Chambers had a center well of 2 ml of sulfanilic acid plus *N*-(1-naphthyl)ethylenediamine dihydrochloride absorbing reagent, and NO_2 was assayed spectrophotometrically according to B. E. Saltzman [*Anal. Chem.* **26**, 1949 (1954)].
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17. Soil (to a depth of 3 cm) and litter samples were collected from 315 cm^2 under chaparral and homogenized; subsamples were heated at 190°C for 10 min; and smoke, drawn by vacuum through dH_2O, was sampled for NO_2 spectrophotometrically as in (*13*). Deeper litter layers, higher temperatures, and longer burning durations would generate higher levels than those reported here.
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Discrete Determinants in Transfer RNA for Editing and Aminoacylation

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During translation errors of aminoacylation are corrected in editing reactions which ensure that an amino acid is stably attached to its corresponding transfer RNA (tRNA). Previous studies have not shown whether the tRNA nucleotides needed for effecting translational editing are the same as or distinct from those required for aminoacylation, but several considerations have suggested that they are the same. Here, designed tRNAs that are highly active for aminoacylation but are not active in translational editing are presented. The editing reaction can be controlled by manipulation of nucleotides at the corner of the L-shaped tRNA. In contrast, these manipulations do not affect aminoacylation. These results demonstrate the segregation of nucleotide determinants for the editing and aminoacylation functions of tRNA.

During aminoacylation of tRNAs for protein synthesis, errors of amino acid activation (by tRNA synthetases) can occur. These errors are corrected by translational editing reactions, some of which require the action of specific tRNAs. Major determinants for aminoacylation of many tRNAs are located in the acceptor stems and anticodons (*1*, *2*). These two regions of the L-shaped tRNA are in different domains that represent the individual arms of the "L." Editing reactions involve the transfer of a misactivated amino acid to the hydroxyl group of a water molecule. This is chemically similar to the transfer of an activated amino acid to the 3′-hydroxyl of a tRNA. Considering the similarity of the reactions and the early demonstration of the close relation between the editing and aminoacylation activity of a tRNA (*3*), nucleotides that are needed for aminoacylation may be sufficient to confer editing activity.

Errors occur as a result of the difficulty that aminoacyl tRNA synthetases have in discriminating between related amino acids. Pairs of amino acids such as valine and isoleucine, which differ by a single methylene group, or threonine and valine, which are isosteric, are difficult to discriminate (*4*). Isoleucyl–tRNA synthetase (IleRS) misactivates valine with a frequency of about 1/180 that of isoleucine activation (*5*). This misactivation is about two orders of magnitude less than the overall

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