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Evolution of 'smoke' induced seed germination in pyroendemic plants

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ABSTRACT

Pyroendemics are plants in which seedling germination and successful seedling recruitment are restricted to immediate postfire environments. In many fire-prone ecosystems species cue their germination to immediate postfire conditions. Here we address how species have evolved one very specific mechanism, which is using the signal of combustion products from biomass. This is often termed 'smoke' stimulated germination although it was first discovered in studies of charred wood effects on germination of species strictly tied to postfire conditions (pyroendemics). Smoke stimulated germination has been reported from a huge diversity of plant species. The fact that the organic compound karrikin (a product of the degradation of cellulose) is a powerful germination cue in many species has led to the assumption that this compound is the only chemical responsible for smoke-stimulated germination. Here we show that smoke-stimulated germination is a complex trait with different compounds involved. We propose that convergent evolution is a more parsimonious model for smoke stimulated germination, suggesting that this trait evolved multiple times in response to a variety of organic and inorganic chemical triggers in smoke. The convergent model is congruent with the evolution of many other fire-related traits.

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1. Introduction

Since the middle of the 20th century fire-induced seed germination has been widely reported in at least four of the five Mediterranean climate ecosystems of the world (Keeley et al., 2012). In California many annual species are almost entirely restricted to the immediate year or two after fire and thus have been described as pyroendemics as many of these species are present only in the first year or two after fire. Many Mediterranean woody species also show germination to be restricted to the immediate postfire environment and their lifetime recruitment comprises a single pulse of germination in the first postfire year.

The earliest studies on fire-stimulated germination focused on the role of heat in breaking seed coat permeability (e.g., Sweeney, 1956; Mott and McKeon, 1979; Jefferey et al., 1988; Trabaud and Oustric, 1989a,b). However, the world changed in 1977 with the report of charred wood stimulated germination of the postfire chaparral annual *Emmenanthe penduliflora* (Boraginaceae) (Wicklow, 1977), later confirmed by Jones and Schlesinger (1980) and Keeley and Nitzberg (1984). Wicklow's study was the first report of chemicals from biomass combustion playing a role in stimulating germination of postfire species.

In 1990 De Lange and Boucher reported the same phenomenon of combustion products simulating the germination in a species from the family Bruniaceae in South African fynbos, but used smoke or a leachate

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of smoke as the medium of transfer rather than water leached from charred wood. He noted the similarity between his studies and those from California. Subsequent studies have revealed that the combustion products from burning biomass of a wide variety of woody plants in California chaparral and South African fynbos will stimulate germination of species restricted to postfire environments. In addition, it is apparent that the response is the same with both smoke and charred wood (Brown, 1993; Keeley and Bond, 1997; Van Staden et al., 2000). Indeed, it has been shown that the postfire *Emmenanthe penduliflora*, which is deeply dormant, will germinate readily in response to direct application of ground up charred wood, a water extract of charred wood, smoke, a water extract of smoke, or vapors from smoke-treated sediments (Fig. 1). This has also been demonstrated for South African fynbos species. The role of combustion products in stimulating germination has now also been widely demonstrated in Australia (e.g, Dixon et al., 1995) and the Mediterranean Basin (e.g., Moreira et al., 2010). Since de Lange and Boucher's report, researchers have used the term 'smoke'-stimulated germination, and because it is more succinct than 'combustion product' stimulated germination we will follow that convention here.

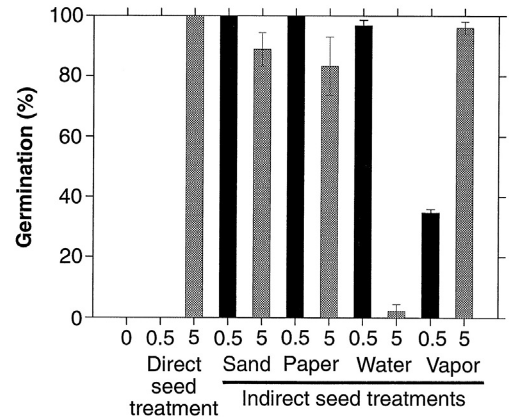

Fig. 2. Germination of the chaparral pyroendemic *Emmenanthe pendulifera* for control (O) and NO_2 (7.7 g m^{-3}) treatments of .5 or 5 min exposures for direct treatment or indirect treatments, untreated seeds incubated on NO_2 treated sand or filter paper on untreated filter paper with water exposed to NO_2 or untreated seeds or exposed to vapors emitted from NO_2 treated filter paper.
From Keeley and Fotheringham (1997).

2. Combustion products that stimulate germination

In recent years, a lot of effort by numerous labs has gone into trying to determine which components in smoke stimulate germination. It is now apparent, after two decades of work, that many chemicals in smoke stimulate germination. There is clear evidence that there are both inorganic and organic chemicals generated or released by smoke that will stimulate germination of seeds of plants that exhibit fire-stimulated germination.

The first report of a compound in smoke that stimulated germination was in 1997 and it showed that nitrogen dioxide, at levels that occur in smoke, can generate 100% germination in the chaparral annual *Emmenanthe penduliflora* (Keeley and Fotheringham, 1997). Seeds of this species are deeply dormant but brief treatment with smoke can trigger 100% germination (Fig. 1), and comparable germination with 500 ppm NO_2 produces a remarkably similar response (Fig. 2). However, it was also found that not all pyroendemics in chaparral responded to this gas and thus it was apparent that other chemicals were also active germination stimulants in smoke and charred wood (Keeley and Fotheringham, 2000). A number of lines of evidence support the idea that nitrogen oxides affect the differential permeability of a sub-testa cuticle (Keeley and Fotheringham, 1997; Egerton-Warburton, 1998), [however, Baldwin et al. (2005), discounted the idea]). It has been shown that both smoke in which nitrogen oxides are removed, or of insufficient fire intensity to generate nitrogen oxides, will still stimulate germination of *Emmenanthe* and other smoke-stimulated species (Light and Van Staden, 2003; Preston et al., 2004). Such studies, of course, do not rule out a role for nitrogen oxides in smoke stimulated germination, but rather support the hypothesis that multiple chemicals in smoke are involved. Other nitrogenous compounds resulting from biomass combustion appear to have a role in smoke-stimulated germination of pyroendemics include glyceronitrile (cyanohydrin), which can lead to nitrogen oxide formation (Flematti et al., 2011; Downes et al., 2014).

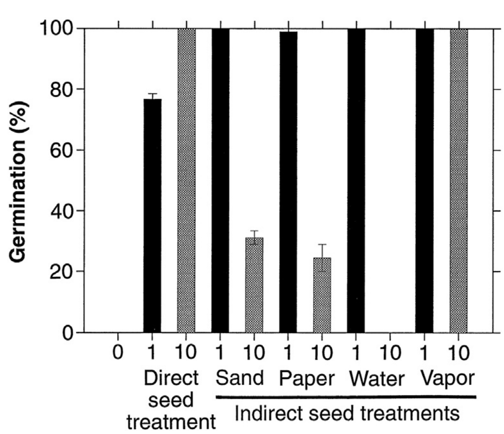

Fig. 1. Germination of the chaparral pyroendemic *Emmenanthe pendulifera* for control (O) and smoke treatments of 1- or 10 min exposures for direct treatments (smoke-treated seeds incubated on nontreated filter paper) and indirect treatments (untreated seeds incubated on smoke-treated sand or filter paper or untreated seeds incubated with smoke water or exposed to gases emitted by smoke-treated filter paper.
From Keeley and Fotheringham (1997).

Organic chemists searched for organic molecules in smoke responsible for germination, and finally, in 2004, two independent studies reported the finding of an organic molecule in a class known as butanolides, which had highly stimulatory activity in triggering germination of postfire recruiting species (Flematti et al., 2004; Van Staden et al., 2004). This chemical known as karrikin has stirred huge interest because, in addition to triggering germination of *some* deeply dormant pyroendemics, it enhances germination and changes light dependent germination characteristics of many agriculturally important weeds and domesticated species (Daws et al., 2007).

This karrikin compound has gained attention as "*the* compound in smoke" responsible for promoting seed germination of postfire species (Flematti et al., 2007, 2009). However, much of the literature suggests that smoke-stimulated germination is a far more complex trait and supports the idea that multiple compounds in smoke can stimulate germination. Indeed, there are a number of species that are stimulated to germinate in response to smoke, but karrikin is clearly not the responsible compound (Daws et al., 2007; Downes et al., 2010, 2014).

Karrikin is not responsible for this response in many species (Table 1), and additionally elutions of stimulatory compounds from smoke demonstrate clearly that there are many other organic compounds in smoke that trigger germination (van Staden et al., 1995). Although under laboratory conditions karrikin is more active, this difference may not be meaningful under field conditions than many of the other elutions showing stimulatory activity. Also, karrikin has been shown to be broken down when exposed to solar irradiation (Scaffidi et al., 2012), further raising questions of its efficacy in the field. In addition to the role of inorganic compounds in smoke, there are complex ecological interactions between stimulatory compounds in smoke and the presence of soil inhibitors which are degraded by fire (Egerton-Warburton and Ghisalberti, 2001; Krock et al., 2002). Furthermore, signals such as nitrogen oxides may be generated for six

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