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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.

Contents

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.

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).

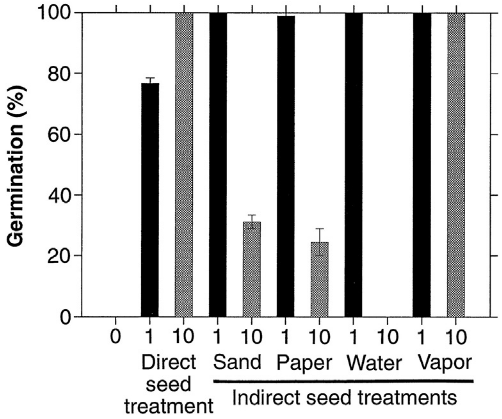

Fig. 1. Germination of the chaparral pyroendemic *Emmenanthe pendulifera* for control (O) and smoke treatments of 1- or 10 min exposures for direct treatments (smoke-treated seeds incubated on nontreated filter paper) and indirect treatments (untreated seeds incubated on smoke-treated sand or filter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

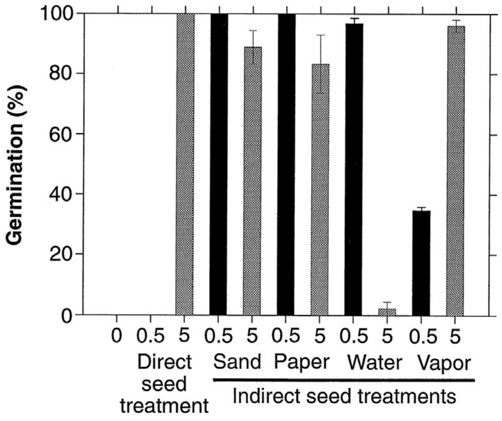

Fig. 2. Germination of the chaparral pyroendemic *Emmenanthe pendulifera* for control (O) and NO_2 (7.7 g m^{-3}) treatments of .5 or 5 min exposures for direct treatment or indirect treatments, untreated seeds incubated on NO_2 treated sand or filter paper on untreated filter paper with water exposed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

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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Table 1
Examples of genera with species that show germination stimulated by smoke and in which both N-based compounds and karrikins have been tested.

Response to karrikins	Response to N-based compounds	
	Yes	No
Yes	*Andersonia* (Ericaceae) [1] *Emmenanthe* (Boraginaceae) [1] *Ficinia* (Cyperaceae) [1] *Conostylis* (Haemodoraceae) [4]	*Stylidium* (Stylidiaceae) [1] *Blancoa* (Haemodoraceae) [2] *Conostylis* (Haemodoraceae) [4]
No	*Rhodocoma* (Restionaceae) [1] *Anigozanthos* (Haemodoraceae) [1,2,3] *Capsella* (Brassicaceae) [5]	*Gyrostemon* (Gyrostemonaceae) [3]

1 Flematti et al., 2011.
2 Downes et al., 2014.
3 Downes et al., 2013.
4 Downes et al., 2015.
5 Daws et al., 2007.

months after fire from burned soils (Anderson and Levine, 1988). In light of all of these factors, we need to be prudent in extrapolating these laboratory results, particularly those involving agricultural species, to the role of smoke in the germination of pyroendemics.

3. Evolution

Bradshaw et al. (2011) have contended that postfire recruitment species do not represent an adaptation to fire but rather an exaptation arising from deep seated evolutionary change in primary metabolism. They point out that 2500 species in a vast phylogenetic range have been shown to respond to smoke; though it should be noted that only a small subset of these species exhibit a dependence on smoke for germination as many merely exhibit increased germination or increased growth rates with smoke. Studies that show a mere increase in germination in response to smoke are very hard to interpret in terms of their ecological significance since doses experienced under field conditions are critical to understanding their adaptive significance. In addition, finding a positive smoke response in species that do not live in a fire-prone ecosystem (e.g., Pierce et al., 1995; Daws et al., 2007), may be interesting from a physiological point of view, but can hardly be used to explain the evolutionary relevance of how pyroendemics have exploited this signal for postfire recruitment.

Bradshaw et al. (2011) further contend that all postfire species also respond to soil disturbance and therefore there is some common factor in recently burned and recently disturbed soil. However, communities following simple soil disturbance are different from postfire communities (e.g., Roche et al., 1997; Keeley et al., 2012). Bradshaw et al. further speculate that the postfire response is related to ethylene generation, although they cite no data to support such a conclusion and ignore reports of smoke-induced responses shown not to be due to ethylene (Keeley, 1993). They then go on to propose a model of smoke-induced germination that hypothesizes that after disturbance of any type, microbes produce karrikinolide, "*the* responsible chemical in smoke." They speculate that, after any disturbance, microbes generate karrikinolide and this triggers germination. In their words, because of the very wide phylogenetic spread of species responding to karrikinolide, which includes major clades from monocots to dicots, this trait probably was an early development in the evolution of angiosperms. Thus, this response has been present for hundreds of millions of years but just since the middle Cenozoic, when in their view fire suddenly appeared on the scene, it was pre-empted for cueing germination to postfire conditions (this issue of adaptation vs exaptation is explored more fully in Keeley et al., 2011).

A somewhat similar model was proposed by Flematti et al. (2015), although they accept the evidence that fires have been a feature of the Earth since land plants evolved, over 400 million years ago (Pausas and Keeley, 2009). Consistent with previous work by this Flematti, it is assumed that there is only one chemical in smoke that postfire species respond to, namely karrikin. They point out that the KA12 gene responsible for the signaling system in plants can be traced back to the earliest plants. In their view this gene first evolved in response to either karrikins or the closely related strigolactones (following soil disturbance) and has been passed down throughout land plant evolution and is not a trait specifically evolved in response to fire.

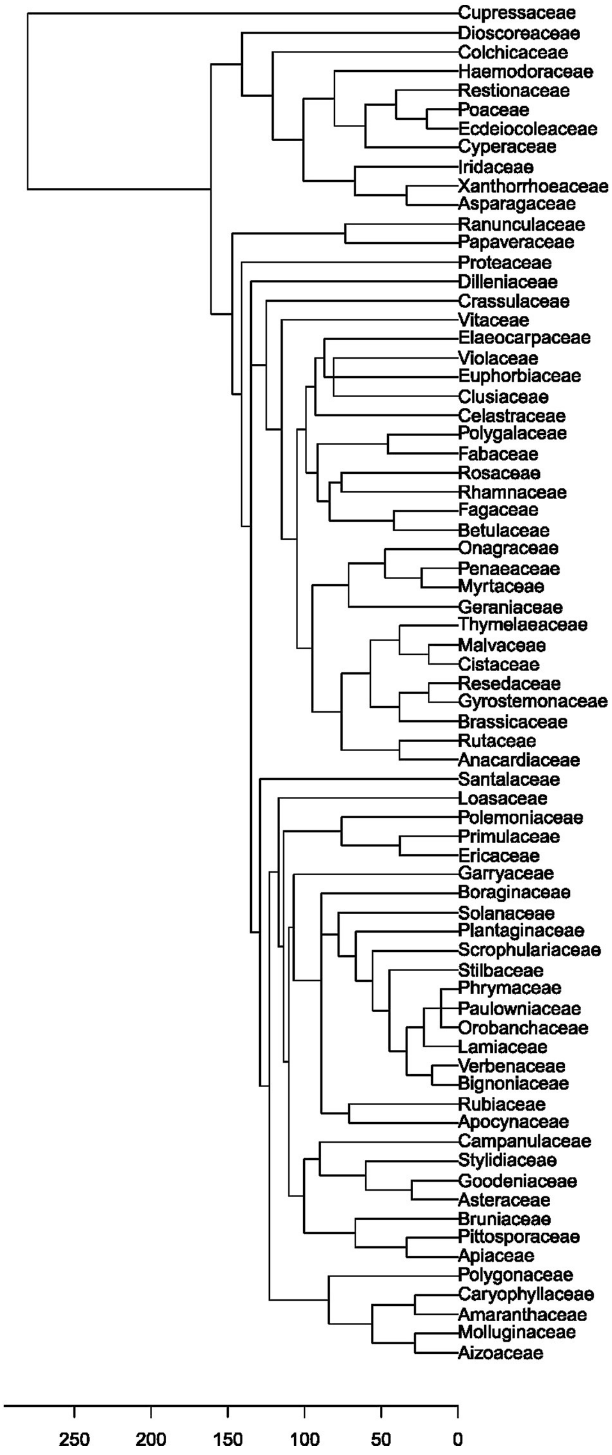

Fig. 3. Phylogenetic tree of plant families with evidence of smoke-stimulated germination suggests that this trait is phylogenetically widespread (x-axis in millions of years). Figure updated from Pausas and Keeley (2009); phylogenetic relatedness based on APG-III (2009).

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An alternative hypothesis is that smoke-stimulated germination is an example of convergent evolution that has evolved multiple times in response to a variety of organic and inorganic chemical triggers in smoke (Keeley and Bond, 1997; Pausas and Keeley, 2009). The fact that the trait occurs in several of the geographically separate mediterranean climate ecosystems and in a wide diversity of taxa suggests a convergent evolution mode (Fig. 3). To support this view is the experimental evidence that multiple products of smoke are shown to trigger germination, including many unidentified organic compounds that are not associated with the karrikin elution in separation techniques.

Other evidence supporting the convergent evolution model come from the remarkable similarity between the mode of action of karrikins and the structurally similar strigolactones (Daws et al., 2008), chemical triggers important in the germination of root parasites. The very recent report by Conn et al. (2015) shows that the karrikin gene KA12 underwent convergent evolution enabling developmental responses to strigolactones in angiosperms and host detection in parasites on multiple independent origins. We hypothesize the same is true for the evolution of both karrikin dependent and non-karrikin dependent germination of pyroendemics.

Of perhaps greater importance is the fact that a vast majority of adaptive traits in plants have been shown to be the result of convergent evolution. For example, metabolic pathways such as C_4 photosynthesis share many similarities across unrelated clades but the specific pathways are sufficiently different to support a hypothesis of multiple origins. When it comes to other fire related traits such as serotiny, heat stimulated germination of hard seeded species and others, the evidence is over-whelming that globally these are the result of convergent evolution (Keeley et al., 2011).

We support the adaptive evolution model for smoke stimulated germination as suggested by the evolutionary perspectives in Van Staden et al. (2000); Pausas and Keeley (2009), and Lamont and He (2012). This trait is found in widely disparate parts of the world in widely separate clades and the better supported hypothesis is this evolved in response to different mutations that occurred in different clades at different times as selected by changes in local fire regimes.

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