Regulation of Seed Germination and Seedling Growth by Chemical Signals from Burning Vegetation

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Abstract

It is well known that burning of vegetation stimulates new plant growth and landscape regeneration. The discovery that char and smoke from such fires promote seed germination in many species indicates the presence of chemical stimulants. Nitrogen oxides stimulate seed germination, but their importance in post-fire germination has been questioned. Cyanohydrins have been recently identified in aqueous smoke solutions and shown to stimulate germination of some species through the slow release of cyanide. However, the most information is available for karrikins, a family of butenolides related to 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one. Karrikins stimulate seed germination and influence seedling growth. They are active in species not normally associated with fire, and in *Arabidopsis* they require the F-box protein MAX2, which also controls responses to strigolactone hormones. We hypothesize that chemical similarity between karrikins and strigolactones provided the opportunity for plants to employ a common signal transduction pathway to respond to both types of compound, while tailoring specific developmental responses to these distinct environmental signals.

Contents

THE REGENERATION OF PLANT ECOSYSTEMS AFTER FIRE

Fire and Plant Evolution

Fire became a feature of life on Earth with the establishment of land plants, which provided the fuel and sufficiently high oxygen levels to support fire. The oldest charcoal deposits found are in Wales and have been dated to approximately 420 Mya, when the first vascular plants were evolving (45). During the Carboniferous period (359–299 Mya), as gymnosperms evolved, fires became more prevalent, possibly because oxygen levels were particularly high (up to 35%) (97). The first angiosperms appeared at the beginning of the Cretaceous (145–65 Mya) and quickly overtook gymnosperms as the dominant land plants. The evolution of land plants and the occurrence of wildfires have necessarily gone hand in hand, albeit with modulations imposed by climatic changes, including periodic ice ages (97). We could therefore expect vascular plants of all groups to have evolved to respond to the fires that they fuel. Over the past several thousand years, human activity has led to the deliberate setting of fires as a tool used in agriculture and to manage the environment (87). This in turn may have imposed added selective pressures for genotypes adapted to fire.

The frequency and distribution of natural wildfires depend upon natural cycles of rainfall, drying, and lightning. They occur throughout the world, even in northern latitudes of

Alaska, Scandinavia, and Siberia. Wildfires are prevalent in hot and dry Mediterranean-type environments, including those of Southern Europe, California, Chile, South Africa, and Australia. However, in some parts of the world—such as in the Amazon rain forest, the Cerrado savannah, and agricultural land in Africa, Russia, and Southeast Asia—both intentional and accidental human-triggered fires dominate (16).

Plant Regeneration After Fire

Plants recover from or exploit fire by at least five different mechanisms (13, 61): (*a*) resprouting from shoot meristems that are not damaged by the fire; (*b*) serotony (or bradyspory), in which viable seeds are stored in cones or woody fruits in the canopy, potentially for decades, before being released by fire; (*c*) breakage of physical dormancy in seeds by fire damage to the tough seed coat, which otherwise prevents germination; (*d*) post-fire flowering (or pyrogenic flowering) in which the fires trigger flowering, typically of plants with underground storage organs (geophytes); and (*e*) smoke-induced or chemically-induced seed germination (the subject of this review).

The local environmental conditions after a fire differ markedly from those before a fire; these differences include changes in temperature range, water availability, wind, light spectrum, light intensity, soil nutrients, allelochemicals, plant competition, microbial activity, and animal behavior. Therefore, plants adapted to fire could potentially sense one or more signals from such changes in the environment. Their development and physiology might then be tuned to optimize growth and reproductive success in the new conditions created by the fire, giving them an advantage over potential competitors. Some species are even so highly adapted that they are essentially dependent on fire: For example, species classified as fire ephemerals and obligate fire followers germinate, grow, and flower only after a fire. Their new seeds remain dormant in the soil until the next fire event, which could be several decades in the future. Such a strategy means that soils, particularly in Mediterranean-type ecosystems, can house a species-rich bank of dormant seeds that can be triggered to germinate in the months following a fire (24, 69).

DISCOVERY OF BIOACTIVE COMPOUNDS FROM PYROLYSED PLANT MATERIAL

Recognition That Products of Pyrolysis Stimulate Seed Germination

The first clear evidence for fire-generated chemical stimulants of seed germination came from studies of the dormant seeds of post-fire annuals from the California chaparral, including *Emmenanthe penduliflora* (Hydrophyllaceae) and *Eriophyllum confertiflorum* (Asteraceae). In these studies, burned or charred woody material was able to stimulate germination (60, 121), and aqueous extracts prepared from such charred material were also active, indicating the presence of one or more water-soluble stimulants (62). Heating plant material at 175°C for 10–30 min was sufficient to produce stimulatory activity. One important finding was that cellulose and hemicelluloses were highly effective sources of the germination stimulant, implying that the active compound(s) did not contain nitrogen or require nitrogenous compounds to form (62).

The next key discovery was that smoke from burning plant material stimulates germination of the South African species *Audouinia capitata* (Bruniaceae) (23). This provided evidence that the stimulatory molecule (or molecules) was volatile, and a convenient way to collect the stimulant (or stimulants) was by bubbling smoke through water to produce "smoke water" (SW). Purification and determination of the chemical structures of active compounds appeared to be an ambitious task because plant-derived smoke may contain as many as 5,000 compounds (99). Moreover, Baldwin et al. (5) had concluded that germination cues for seeds of *Nicotiana attenuata* (Solanaceae) could be active at concentrations of less than 1 pg per

seed, which suggested that the compound (or compounds) was highly potent.

Keeley & Fotheringham (57) reported that trace gases in smoke induced germination of *E. penduliflora*, and provided evidence that nitrogen dioxide (NO_2) and potentially nitric oxide (NO) were responsible. They proposed that although smoke contains sufficient NO_x to stimulate seed germination, a more relevant source of NO_x could be that generated in the soil surface layer by the heat of the fire, which in the presence of water would dissolve and establish equilibrium with nitrite. They further suggested that fire produces ammonium salts that are subsequently washed into the soil, where nitrifying microorganisms can oxidize it to NO_2, nitrite, and nitrate (**Figure 1**).

Thanos & Rundel (108) reported that soil nitrate content is higher at burned sites than at unburned sites, but Baldwin & Morse (4) showed that nitrate is not sufficient to reproduce an SW effect. Keeley & Fotheringham (58) later reported that nitrite and nitrate could break dormancy in *E. penduliflora* but only at acid pH levels, and concluded that oxidizing gases in smoke and/or acids play a role in germination of post-fire annuals in chaparral. A role for acids has also been identified in studies of dormancy breaking in *Oryza sativa* (red rice; Poaceae) (17). However, the importance of acids in smoke was not supported by Baldwin et al. (5), who tested many organic acids.

Although NO is recognized as a plant signaling compound (7, 10, 50) and a promoter of seed germination (44), the proposed role of NO_x as the dormancy-breaking cue in smoke was challenged in a number of studies. Smoke-responsive plant species did not respond to NO_x generated from solutions of sodium nitroprusside (SNP) or sodium nitroso-*N*-acetylpenicillamine (SNAP) (90), and the stimulatory effect of smoke could not be inhibited with a specific nitrogen-oxide scavenger (71). Furthermore, nitrite and nitrate could not be detected at relevant levels in SW (26, 90), supporting the idea that smoke-induced germination does not arise from NO_x, nitrite, or nitrate. Preston et al. (90) additionally showed that SW prepared by burning pure cellulose was highly active in the breaking of seed dormancy, and concluded that nitrogenous compounds are not required. They further argued that NO_x does not have the correct attributes to provide a specific signal to trigger germination of fire followers.

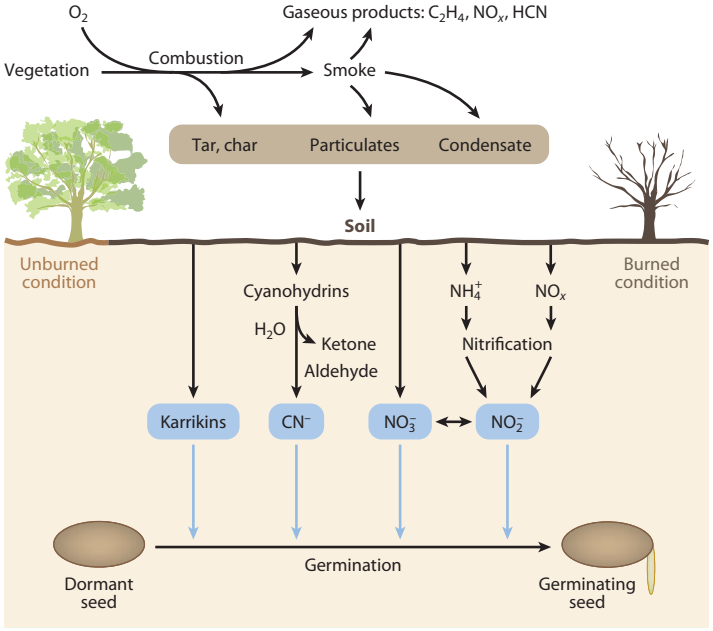

Figure 1

Known germination stimulants derived from combustion of plant material. Substances in blue boxes are known to stimulate seed germination (32, 39, 57), here depicted by blue arrows. NO_x represents NO or NO_2 and could theoretically be derived by combustion or by microbial activity in the soil. Oxidation of NH_4^+ or NO_x to NO_2^- (nitrite) or NO_3^- (nitrate) can occur by microbial nitrification. Chemicals would normally be eluted into the soil by rain. Other factors such as light and temperature may also regulate seed germination.

Discovery of Karrikins

During the search for the germination stimulant(s), Baldwin et al. (5) identified 71 compounds in active smoke fractions and tested a total of 233 compounds, none of which significantly promoted germination in the post-fire annual *N. attenuata*. Fractions collected from a 45-min gas-chromatographic separation found significant germination in at least three distinct portions of the chromatogram, suggesting

that more than one compound was active (5). Further work established that similar types of bioactive compounds form in smoke extracts obtained from burning different plant materials (8, 116). Extracts prepared by heating agar and cellulose gave compounds that stimulated the germination of *Lactuca sativa* (Grand Rapids lettuce; Asteraceae) (52), and a comparison of chromatographic parameters suggested that the same bioactive factor was present.

Following the initial leads that burning cellulose produced SW with dormancy-breaking activity (5, 62), Flematti et al. (32, 33) used bioassay-guided fractionation procedures—including solvent partitioning, high-performance liquid chromatography, gas chromatography–mass spectrometry, and nuclear magnetic resonance—to identify the active compound from burned filter paper (99% cellulose) that stimulated germination of *L. sativa* and 15 other smoke-responsive species from Australia, North America, and South Africa. The stimulant's structure was confirmed by chemical synthesis as 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one (**Figure 2*a***) (34), a new class of compound containing a butenolide (a four-carbon lactone) fused to a pyran ring. Initially, this compound was trivially called butenolide, but to distinguish it from other butenolides it was more recently renamed karrikinolide (*karrik* being a traditional word for smoke in the language of the Aboriginal Noongar people of southwestern Australia) and abbreviated as KAR_1. It was found to be active at concentrations down to 10^{-10} M with several plant species (32). Its solubility and stability in water are consistent with knowledge of the bioactivity found in smoke (32). A separate group later isolated the same compound from burning plant material (117). On the basis of KAR_1 production by burning pure xylose, glucose, or cellulose, it has been proposed that KAR_1 is derived from a pyranose sugar (40).

Five KAR_1 analogs, KAR_2–KAR_6 (**Figure 2*b–f***), which along with KAR_1 are collectively known as karrikins, were later discovered in SW and confirmed with synthetic standards (36). It was also possible to determine the concentrations of the six karrikins, although both absolute and relative amounts vary depending on the plant material used and the method of smoke generation. By way of illustration, typical values for the amounts found in 1 liter of SW for KAR_1 were 39.8 μg (39.8 ppb, 265 nM); for KAR_2, 6.5 μg (6.5 ppb, 48 nM); for KAR_3, 5.2 μg (5.2 ppb, 32 nM); for KAR_4, 3.4 μg (3.4 ppb, 21 nM); for KAR_5, 1.0 μg (1.0 ppb, 7 nM); and for KAR_6, 1.3 μg (1.3 ppb, 9 nM). Because KAR_1 is the most abundant and usually the most active karrikin (**Figure 2**), it is responsible for most of the stimulatory activity.

It should be noted that although karrikins were identified in smoke, the majority of the KAR_1 is found in the residue (e.g., char and tar) that remains after burning filter paper or plant material (35). Given the water solubility and limited volatility of KAR_1 (32), we propose that it is not carried far in smoke, but instead condenses as the smoke cools and remains close to the site of a fire. We believe that local karrikin deposition—combined with the release of nutrients in ash, removal of litter containing allelopathic chemicals, and change in light quality—explains why the dormant seed bank is stimulated to germinate in post-fire sites but not in the immediately adjacent unburned sites during the subsequent rainy months (24, 89).

Properties and Structure-Activity Relationships of Karrikins

The karrikins identified so far are small molecules (seven to nine carbons), and the presence of a carboxylate ester and the bis-enol ether group of the pyran conveys a degree of polarity. The molecule is planar and has no chiral centers (37). In general, karrikins are stable under ambient temperatures and in aqueous solutions, with no loss of activity observed after several years of storage at 4°C in the absence of light (G.R. Flematti, unpublished data).

So far, five different synthetic routes have been developed to prepare KAR_1 (34, 47, 76, 80, 106). Nearly 50 analogs have been synthesized by substitutions at carbons 3, 4, 5, and

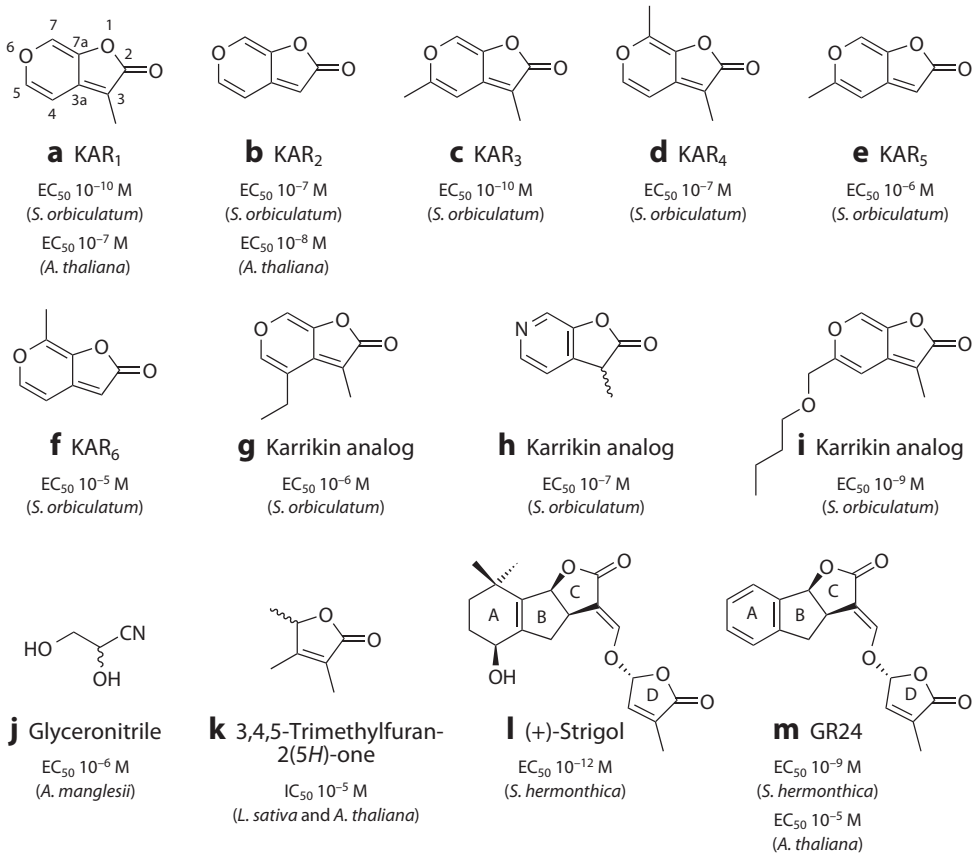

Figure 2

Structures and bioactivity [half maximal effective concentration (EC_{50})] of (*a–f*) karrikins, (*g–i*) karrikin analogs, (*j*) glyceronitrile, (*k*) 3,4,5-trimethylfuran-2(5*H*)-one, (*l*) (+)-strigol, and (*m*) GR24 in regulating seed germination. Karrikins were evaluated using *Solanum orbiculatum* (38, 41) and *Arabidopsis thaliana* (82). Glyceronitrile was tested with *Anigozanthos manglesii* (39). Inhibitory concentration (IC_{50}) for 3,4,5-trimethylfuran-2(5*H*)-one was determined with *Lactuca sativa* (Grand Rapids lettuce) (68) and *A. thaliana* (83). (+)-Strigol and GR24 were tested with *Striga hermonthica* (124) and *A. thaliana* (82).

7 (38, 41, 47, 106). The activity of the first generation of analogs was evaluated on three test species—*Solanum orbiculatum* (Solanaceae), *L. sativa*, and *E. penduliflora*—with broadly similar results for each (38). *S. orbiculatum* gave the largest response, from 10% germination (untreated) to >80% (KAR_1 treated), and *L. sativa* was also highly responsive. *E. penduliflora* was the least sensitive of the three, requiring higher concentrations of analogs to promote germination (38).

The results obtained from structure-activity studies can be summarized as follows: The methyl group at C-3 is important for germination activity, whereas introduction of a methyl at C-7 (**Figure 2*d***) reduces activity. Addition of a methyl at C-5 (**Figure 2*c***) is reasonably well tolerated, whereas modification at C-4 (**Figure 2*g***) decreases germination activity. Replacement of the pyran oxygen with nitrogen (**Figure 2*b***) does not eliminate the activity. The observation that C-5 is tolerant of simple variations (**Figure 2*i***) has led to the production of analogs that can be tagged at C-5 (95) for the purpose of investigating the karrikin mode of action.

Discovery and Properties of Cyanohydrins

Although KAR_1 is apparently the major germination stimulant present in smoke, it has been tested on only a relatively small number of the 1,200 known smoke-responsive species (15). *Tersonia cyathiflora* (Gyrostemonaceae) was recently identified as a species that is responsive to SW but not to KAR_1 or nitrate (27). *Anigozanthos manglesii* (red and green kangaroo paw; Haemodoraceae) also responds to SW but not to KAR_1 or nitrate (25, 93, 110, 112). These results suggest that a nonkarrikin bioactive agent is present in smoke.

Preliminary investigations revealed that this second class of stimulant is produced only in smoke derived from the combustion of plant material, and not in smoke derived from the burning of filter paper (cellulose). In addition, the compound was much more polar than KAR_1 and was not extractable from SW using dichloromethane. Bioassay-guided fractionation using *A. manglesii* as the test species led to the isolation of the cyanohydrin glyceronitrile (**Figure 2*j***) as the other active constituent in plant-derived smoke. A synthetic sample of glyceronitrile was found to significantly stimulate the germination of *A. manglesii* and 11 other smoke-responsive species, some of which also responded to KAR_1 (40). A number of cyanohydrin analogs—such as acetone cyanohydrin, glycolonitrile, and mandelonitrile—were also found to stimulate germination of *A. manglesii*. One property of cyanohydrins is that they can hydrolyze in aqueous solutions to liberate cyanide and an aldehyde or ketone; importantly, cyanide can stimulate seed germination in a number of plant species (11, 29, 51, 74, 84, 92, 98, 107), as can many aldehydes and ketones (42). It has been demonstrated that glyceronitrile promotes seed germination by spontaneously releasing cyanide when in contact with water, and that cyanide was the active stimulant (39).

The mode of action of cyanide in stimulating seed germination remains unclear but is thought to involve reactive oxygen species (85, 86) and ethylene (46, 84). It has also been proposed that cyanide acts at an initial step in dormancy alleviation and that nitrogen oxide is required at a later step (11). This is interesting given that nitrogen oxides (NO and NO_2) had been previously proposed as germination-active components of smoke (57) but were dismissed because they are not produced from cellulose combustion and are not persistent in the soil (90). The identification of cyanide as a natural germination stimulant suggests that a reevaluation of nitrogen oxides, which might be produced by oxidation of cyanide (21), should be undertaken.

In ecological terms, glyceronitrile (**Figure 2*j***) formed during wildfires must remain stable in soil until the next wet season, when it can release cyanide that then acts as a cue to stimulate seed germination. As glyceronitrile contains a glycol (1,2-dihydroxyl) functionality, it may be stabilized by forming cyclic borate complexes with borate minerals present in soil (39). This has previously been demonstrated with similar glycol-containing compounds (91). When dissolved in water, these cyclic borates release glyceronitrile and, subsequently, free cyanide.

Germination Inhibitors Removed and Released by Fire

Foliage and leaf litter can release compounds into the soil that suppress seed germination (4, 60). The removal of this inhibitor source by fire contributes to regeneration in some ecosystems (121). For some species, such as *N. attenuata*, it has been shown that the stimulatory effect of fire-derived signals combined with removal of inhibitory signals from litter is required for seed germination to occur (89). In contrast, the nondormant *Nicotiana trigonophylla* (Solanaceae), which grows in the same area as *N. attenuata*, is not affected by the inhibitory compounds. Thus, some species are able to detect both positive and negative signals in their environments to identify suitable conditions for germination. Some of the compounds in leaf litter that inhibit

N. attenuata germination have since been identified as vegetation-derived abscisic acid (ABA) as well as the terpenes 1,8-cineole, bornane-2,5-dione, camphor, and β-thujaplicin (64).

It is also apparent that pyrolysis of plant material generates new germination inhibitors. Undiluted solutions of SW are highly inhibitory to seed germination (14), but subsequent washing of seeds with water can remove the inhibitory effect (70). The usual practice to achieve optimal stimulation in germination assays is to dilute SW 100- or 1,000-fold, suggesting that inhibitory compounds in SW are active at higher concentrations than stimulatory compounds such as karrikins and cyanohydrins.

Ten compounds were identified in smoke that inhibited germination of *N. attenuata* at concentrations ranging from 0.5 mg ml^{-1} to 5 μg ml^{-1} (5). These included phenolic compounds, such as cresols and dihydroxybenzenes, as well as 2-furoic acid and naphthalene. A butenolide, 3,4,5-trimethylfuran-2(5*H*)-one (**Figure 2*k***), was recently isolated from plant-derived smoke as an inhibitor of lettuce seed germination (68). This inhibitor was active at concentrations above 10 μM, and applying it with 0.1-μM KAR_1 markedly suppressed germination compared with KAR_1 treatments alone. Testing the inhibitor and three simple butenolide analogs on *Arabidopsis thaliana* (Brassicaceae) showed that 3,4,5-trimethylfuran-2(5*H*)-one at 10 μM inhibited germination, whereas the three analogs were inactive (83). Any direct interactions between KAR_1 and this inhibitor in the control of germination remain to be established.

PHYSIOLOGICAL ROLE OF KARRIKINS

Diversity of Plants Responding to Smoke and Karrikins

Seeds of more than 1,200 plant species from 80 different genera respond to smoke. These include species from phylogenetically diverse groups, including gymnosperms, showing that smoke-responsiveness may be an ancient trait (15, 24). The action of smoke appears to be independent of plant phylogeny, life cycle, seed structure, ecosystem, and geography. It is a striking observation that many species that are not considered fire followers respond to smoke or karrikins, including *A. thaliana* and numerous crop species, such as tomato, maize, rice, and lettuce (28, 55, 66, 67, 82, 104, 119). It is currently unclear why these responses have been so broadly maintained. One idea is that karrikins or related compounds may not be specific to fire and smoke but could occur elsewhere in nature—for example, arising from the chemical or microbial breakdown of biomass or from plant metabolism (15).

Similarity Between Karrikins and Strigolactones

The newest members of the phytohormone family are the SLs, originally isolated from roots of cotton, maize, and millet by virtue of their ability to stimulate seed germination of parasitic plants, including *Striga* spp. (witchweeds; Orobanchaceae) (122) and *Orobanche* spp. (broomrapes; Orobanchaceae) (9). (+)-Strigol (**Figure 2*l***) was the first SL identified (19, 20), but most studies utilize the simpler synthetic analog GR24 (**Figure 2*m***) (75). SLs have been shown to play a role in the control of shoot branching (axillary bud outgrowth) in angiosperms (48, 115) and in the development of lateral roots and root hair (56). Furthermore, SLs exuded from roots serve as chemical signals for root colonization by arbuscular mycorrhizal (AM) fungi (1). It seems most likely that the exudation of SLs by host roots to stimulate AM associations has been exploited by parasitic plant seeds as a host-detection mechanism. Plants produce a wide range of SL structures, potentially providing the specificity required for a parasite to recognize and colonize its host (30, 123).

The essential molecular features for active SLs include a methylated butenolide (D-ring) and an enol ether moiety (15, 124), as found in KAR_1 (**Figure 2*a***). The fact that two classes of seed germination stimulants

contain similar functional groups raises the possibility of common modes of action. This has led to a number of investigations aimed at determining whether SLs can stimulate germination of smoke-responsive species and whether karrikins can stimulate germination of parasitic weed species; however, the results have been contradictory (15). For example, Daws et al. (22) showed that KAR_1 purified from SW stimulates germination of numerous parasitic weeds. However, Nelson et al. (82) showed that chemically synthesized KAR_1 has no effect on *Orobanche minor* seed germination; in the same study, although the synthetic SL GR24 promoted *A. thaliana* and *Brassica tournefortii* (Brassicaceae) seed germination, it required much higher concentrations than KAR_1 for similar effectiveness (82) (**Figure 2**). GR24 was also separately shown to be a potent inhibitor of light-dependent hypocotyl elongation in *A. thaliana*, but it did not promote cotyledon expansion as karrikins do (83). Furthermore, although SLs repress shoot branching and regulate AM fungal development, karrikins do not (2, 83). Thus, in multiple developmental processes, karrikins and SLs are not simply equivalent molecules.

Physiological Responses to Smoke and Karrikins

The majority of published investigations of smoke-induced germination have been categorical surveys of the capacity of various plant species to respond to smoke or its active constituents, and of the conditions under which they do so. This broad body of work is highly relevant for developing applications of smoke germination stimulants in land restoration, seed conservation, and weed control, and also illustrates the broad utilization of smoke-response mechanisms among angiosperms. However, as seed dormancy and germination are highly complex and heterogeneous processes even within a single genus, it is difficult to draw consistent conclusions from these types of studies regarding how smoke or karrikins may influence germination. To further complicate the matter, smoke, SW, and karrikins are not equivalent treatments. SW solutions vary between production batches, source material, and laboratories, and often require different levels of dilution for maximum effectiveness. (For this reason, we consider the use of individually synthesized active components of smoke to be the best practice, and advise caution in interpreting results from other experiments.) Despite these limitations, several themes have emerged: (*a*) Dormancy influences the degree of seed responsiveness to smoke or KAR_1, (*b*) karrikin response is distinct from other germination cues, and (*c*) KAR_1 can enhance suboptimal germination and seedling vigor.

Seed Dormancy Gates Responses to Germination Stimulants in Smoke

Physiological dormancy can be described most simply as a programmed state that restricts the set of environmental conditions under which a seed will germinate. These conditions include temperature, water potential, light spectra, light intensity, photoperiod, and chemical cues. In highly dormant seeds, the set of acceptable conditions for germination, if any, is stringent, whereas in nondormant seeds the set of conditions is relaxed. Typically, seed dormancy is high upon completion of seed maturation and release from the parent plant (known as primary dormancy) and then reduces with time (after-ripening). Seed storage conditions (e.g., soil moisture, temperature) fluctuate with seasonal changes and can influence the rate of dormancy loss. Seed dormancy may also be regained (secondary dormancy) in response to environmental cues, resulting in cycling of germination potential over the course of the year. This system permits seedling emergence at optimal times for establishment, survival, and reproductive success (6).

The efficacy of smoke or KAR_1 as seed germination stimulants depends upon the dormancy state of the tested seed. For example, the agricultural weed *B. tournefortii* can be extremely sensitive to KAR_1; in one experiment, KAR_1 at a concentration of less than 1 nM

improved seed germination from 4% to 40%, and 67-nM KAR_1 induced 90% germination. However, collections of *B. tournefortii* seed harvested from different regions or in different years showed a high degree of variability in their responsiveness to KAR_1 (72, 105). A 2005 seed lot from the Perth metropolitan area had <10% germination in the presence of 0.67-μM KAR_1, whereas a 2006 lot harvested from the same area exhibited nearly complete germination under similar conditions. After 6 months of storage at ambient laboratory conditions, however, >90% of the 2005 lot germinated with KAR_1 treatment while retaining <5% germination in water (105). This vividly illustrates that the capacity to respond to karrikins is highly dependent upon seed dormancy, even within a clearly sensitive species. In this instance, after-ripening during dry storage permitted a much stronger germination response to KAR_1. This also highlights a problem with declaring a species to be smoke/karrikin-responsive or not—a positive response may be observed only under a highly specific set of germination conditions or seed dormancy states, increasing the likelihood of false-negative conclusions. For this reason, it has been proposed that plant species should instead be classified as having an inherent, inducible, or undetected karrikin response (72).

Dormancy loss can occur for some species with sufficient storage time under laboratory conditions, as in the case of *B. tournefortii*, whereas others require burial in soil to achieve smoke-responsive competency. Soil storage for 1 year led to smoke-stimulated germination in *Dendromecon rigida* (Papaveraceae), *Dicentra chrysantha* (Fumariaceae), and *Trichostema lanatum* (Lamiaceae), whereas it enhanced smoke responses in *Romneya coulteri* (Papaveraceae) and *Phacelia minor* (Hydrophyllaceae) (59). Seeds buried in soil or stored under laboratory conditions exhibited increased SW-responsiveness in *Stylidium affine* (Stylidiaceae), *Stylidium crossocephalum*, and *A. manglesii* (111).

Annual cycling of dormancy in the soil seed bank has a corresponding influence on smoke/karrikin-responsiveness. A survey of 37 species representing 18 families revealed that the seasonal timing of smoke application to unburned soil plots had a significant effect on the germination responses of native perennials and native annuals, with autumn being the most effective time of treatment (94). *Actinotus leucocephalus* (Apiaceae) and *T. cyathiflora* seed buried in soil showed clear annual cycling of SW-responsiveness. Although *A. leucocephalus* seed stored under laboratory conditions showed a gradual rise in SW response over a 24-month period, seed buried in soil was strongly responsive to SW only after 12 and 24 months of burial and had significantly reduced germination after 6 and 18 months. *T. cyathiflora* seed had an obligate SW requirement and was unresponsive to SW when stored in laboratory conditions. Like *A. leucocephalus*, it exhibited a positive SW response at 12 and 24 months after burial (3). Seasonal dormancy cycling during 2 years of soil burial influenced both germination levels and KAR_1-responsiveness in seed of four Brassicaceae species, providing a higher relative stimulation by KAR_1 treatment during particular times of the year (72).

If a "just right" level of seed dormancy is a prerequisite for smoke- or karrikin-responsiveness, what environmental conditions in soil influence the transition into and out of this primed state? Temperature and moisture are two critical factors that affect dormancy, both as independent variables and in combination with each other. From the point of view of a seed, these two factors provide important cues about the time of year: Summers are typically hot and dry, whereas winters are cool and moist. Periods of 4–8 weeks of warm stratification increased germination in *Lomandra preisii* (Laxmanniaceae), but SW did so more strongly (78). Warm stratification for 8 weeks improved responses of the Brassicaceae species *B. tournefortii*, *Raphanus raphanistrum*, *Sisymbrium erysimoides*, and *Heliophila pusilla* to KAR_1 (72). After 1 month of cold stratification, *A. leucocephalus* became completely unresponsive to SW-induced germination, but

subsequent air-dry storage at temperatures alternating between 20°C and 50°C every 12 h gradually restored SW-responsiveness (3). High storage temperatures may indicate summer or proximity to the soil surface, where temperatures can sometimes exceed 65°C (114), whereas very high heat shock may signal the passage of a fire. A positive interaction between high storage temperatures (40°C–60°C) and smoke treatment on germination was found in *S. affine*, *A. manglesii*, and *A. leucocephalus* (110). A 1-h heat pulse of 70°C enhanced seed germination of *A. leucocephalus* and also had a minor positive interaction with SW treatment; however, in buried seed the efficacy of the heat shock was also sensitive to seasonal changes (3).

Seed moisture content (MC) during storage influences rates of after-ripening as well as the capacity to respond to SW. *Austrostipa elegantissima* (Poaceae), *Conostylis candicans* (Haemodoraceae), and *S. affine* seeds were equilibrated to a 5%–75% range of relative humidity (RH) and then tested for germination potential over the course of 36 months of storage. Seeds that had been equilibrated at higher RH after-ripened more quickly, but long-term storage after 75% RH equilibration was detrimental to seed viability. SW response was also influenced by after-ripening in these seeds. A positive SW response was apparent for only *A. elegantissima* seed with lower seed MC, as control germination of high-MC seed was very high after only 3 months of storage. At the high end of the dormancy spectrum, *S. affine* had an obligate SW requirement for germination and required 36 months of storage after 50% RH equilibration to achieve >60% germination with SW treatment, whereas seed stored at lower MC exhibited no germination in SW (113).

The immediate hydration state and recent imbibition history of a seed can also influence germination responses to KAR_1. One study (73) found that *B. tournefortii* germination was strongly promoted with as little as 3 min of exposure to 1-μM KAR_1. However, this response was inhibited in seeds with higher-hydration states and in seeds that had been fully imbibed and then redried to a low-hydration state. The seeds were also sensitive to extended exposure to very low KAR_1 concentrations (1 nM), but this was observed only in seeds with low water content (≤10% of dry weight) (73).

In summary, smoke and karrikins are not silver bullet treatments that broadly and readily overcome seed dormancy in a soil seed bank. Rather, they are signals taken in the context of the current environmental status to ensure successful post-fire seedling emergence during the appropriate season. As stated in one publication, "Some seeds may require a specific sequence of temperature and moisture cues to alleviate dormancy, and then a smoke cue as the final step for germination" (78).

Light and Hormone Interactions with Smoke-Stimulated Germination

Germination of positively photoblastic seed is promoted by light, which may signal a canopy opening or proximity to the soil surface, whereas negatively photoblastic seed germination is inhibited by light. Current evidence indicates that SW/KAR_1 and light act independently to influence seed germination; SW/KAR_1 stimulates seed germination, but light can either enhance or block this response, depending on the species. SW promotes dark germination of lettuce achenes, but light signaling is still important, as 20 min of phytochrome-deactivating far-red light has been shown to interfere with this effect (28, 118). *N. attenuata* seed is stimulated to germinate by SW but is unresponsive in the dark (96). However, in other light-requiring species, such as *Angianthus tomentosus* and *Podolepis canescens* (Asteraceae), KAR_1 can induce germination in darkness (77). KAR_1 also stimulates germination of negatively photoblastic species; for example, *Avena fatua* (Poaceae) germination is enhanced by KAR_1 (22, 105), but light treatments reduce the response to KAR_1 (63).

Assessment of the interaction between KAR_1, after-ripening treatments, germination

temperature, dormancy cycling, and light in eight Brassicaceae species showed that KAR_1 response occurs in both positively photoblastic (*S. erysimoides*, *Lepidum africanum*, *Carrichtera annua*, and *Sisymbrium orientale*) and negatively photoblastic (*B. tournefortii*, *R. raphanistrum*, and *H. pusilla*) species from the same family (72). Furthermore, the degree to which light influenced germination varied between seed lots of the same species and with seasonal seed dormancy changes during soil burial.

Gibberellin (GA) and ABA are key hormones with antagonistic roles in the control of seed germination: GA promotes seed germination, whereas ABA establishes and maintains dormancy (12, 31, 65). It is commonly observed that germination of KAR_1-responsive species is also stimulated by GA treatment. *Anthocercis littorea* (Solanaceae) germination has a strong requirement for karrikin treatment. However, high concentrations of GA_3 (2.89 mM) can promote its germination, as can KAR_1 (0.67 μM) (18). GA_3 and KAR_1 both induced germination of three Asteraceae species (*A. tomentosus*, *P. canescens*, and *Myriocephalus guerinae*) and enhanced dark germination at a range of temperatures (77). Of nine agricultural weeds tested for germination responses to GA_3 (289 μM) and KAR_1 (0.67 μM), all nine responded to GA_3 and seven responded to KAR_1 (105).

SW and GA act synergistically to promote germination in lettuce achenes and *N. attenuata* seed (96, 118). The positive effect of SW on lettuce germination in the dark was shown to be inhibited by GA biosynthesis inhibitors, and putative bioactive GA levels increased during germination, suggesting that de novo GA biosynthesis is a component of SW-induced germination (43). SW-stimulated germination in *N. attenuata* was also blocked by a GA biosynthesis inhibitor, but only when applied in the first 12 h of imbibition (96); surprisingly, this study also reported that GA_3 and GA_1 levels were lower in SW-treated *N. attenuata* seed than in dormant controls within 2 h of seed imbibition.

GA treatment is not equivalent to SW treatment, however. Fresh *A. elegantissima* seed was GA-responsive (2.89 mM) but not SW-responsive, whereas *C. candicans* was SW-responsive but not GA-responsive (113). Lower concentrations of GA_3 (30 μM) and KNO_3 (10 mM) were found to be less effective than SW in stimulating germination of soil-buried *A. leucocephalus* and *T. cyathiflora* seed (3). An assessment of KAR_1 effects on 18 weed species from non-fire-prone environments demonstrated that 8 species had a significant increase in seed germination, whereas 6 species had a reduced mean time to germination (22). Among smoke, GA_3, KNO_3, and alternating temperature treatments, GA_3 had the strongest correlation with KAR_1 treatment. However, GA_3 had distinct effects on seedling morphology not seen with KAR_1.

Although both SW/karrikins and GA are capable of stimulating seed germination in many species, there is largely only correlative evidence for a relationship between these signals. Synergistic interactions between SW/karrikins and GA may reflect the sum of two independent positive germination signals rather than a direct interaction. GA biosynthesis inhibitors are not ideal for evaluating this relationship, as they are not entirely specific and can also affect ABA catabolism (49). The development of *A. thaliana* as a model system to study karrikin signaling has allowed more direct hypothesis testing (82), but some additional clues for smoke-hormone interactions have emerged from other studies.

Compared with control seed equilibrated to 15% RH, *B. tournefortii* seed that was fully imbibed, or fully imbibed and redried to 15% RH, required longer exposure to KAR_1 treatment to achieve similar germination. Although both fully imbibed and redried seed had reduced levels of ABA, they were also much more sensitive to inhibition of KAR_1-induced germination by exogenous ABA. GA levels and sensitivity were consistent between the three seed hydration types (73). A comparison of inhibitory and stimulatory treatments on smoke-treated *N. attenuata* seed also found no correlation between ABA content and germination outcome (64). Thus, ABA sensitivity, and

not absolute abundance, may underlie the seed dormancy-gated capacity for KAR_1 response.

KAR_1 and Smoke Water Improve Suboptimal Germination and Seedling Vigor

Several studies have reported that smoke or KAR_1 treatment enhances suboptimal germination, seedling survival, and seedling vigor. KAR_1 improved the germination of tomato seed at temperatures outside the optimum range of 25°C–30°C, but at extreme temperatures of 10°C and 40°C, seeds that germinated failed to grow past radicle emergence unless treated with KAR_1 (55). Germination of *Kunzea ambigua* and *Kunzea capitata* (Myrtaceae) increased at low water potentials in response to smoke (109). SW/KAR_1 significantly increased the mass of tomato, okra, and maize seedlings (119). These three species as well as bean had higher vigor indices (seedling length × germination) following SW/KAR_1 treatment, and all four species showed significant increases in both root and shoot length. Strikingly, the length of the tomato seedling roots was reported to increase 10-fold following KAR_1 treatment. Maize seedlings developed more roots in response to SW/KAR_1, and 30-day-old plants were at a more advanced leaf stage and plant height in addition to having increased survival percentages (119). Smoke or SW increased seedling growth and survival of the South African species *Albuca pachychlamys* (Hyacinthaceae) and *Tulbaghia violacea* (Alliaceae) under laboratory conditions (103). In an assessment of 30 species from the Mediterranean Basin, 8 species from Ericaceae, Lamiaceae, and Primulaceae were found to be SW-responsive, and 6 species had enhanced seedling growth following SW treatment (79). Smoke application also led to improved seedling survival percentages of native perennial species in field plots treated in autumn (94).

Seedling vigor studies should ideally control for different germination times to distinguish between enhanced growth due to the treatment and longer periods of post-germination growth time. Several weed species from Poaceae (*Alopercus myosuroides*, *Sorghum halepense*), Brassicaceae (*Capsella bursapastoris*), Rubiaceae (*Galium aparine*), and Papaveraceae (*Papaver rhoeas*) had significantly increased seedling dry mass following KAR_1 treatment (22). *P. rhoeas* and *S. halepense* had increased germination in response to KAR_1, but only *G. aparine* had a reduced mean time to germination, suggesting that the measured increases in seedling growth cannot necessarily be attributed to the influence of karrikins on germination rates. It remains to be determined how karrikins may influence seedling vigor and promote suboptimal germination.

MOLECULAR MODE OF ACTION OF KARRIKINS

Effects of Smoke and Karrikins on Gene Expression

There have been several attempts to identify gene expression changes accompanying SW/KAR_1 treatment that could provide mechanistic insights for enhanced seed germination or seedling vigor. Differential display of complementary DNA (cDNA) from *Solanum esculentum* (tomato; Solanaceae) seed treated with KAR_1 for 72 h led to the tentative identification of four transcripts, including an expansin gene; however, no confirmation of differential expression was performed (54). Another investigation using fluorescent differential display of lettuce seed treated with SW in the dark found 11 cDNAs with altered expression at one or more time points during seed imbibition and early germination. Of the 11 transcripts, 8 featured transcriptional patterns in response to SW treatment that were similar to those in response to light, which also enhanced the rate of seed germination relative to the dark control. The 3 transcripts specifically upregulated by SW and not by light were classified as "ABA-related" on the basis of studies of homologous genes in other species. From this handful of genes, the authors concluded that smoke can induce genes linked to ABA action, that it creates patterns of gene expression

similar to those created in light, and that "smoke effects are manifested mainly through the induction of the cell division cycle, cell wall extension, and storage mobilization" (100).

A microarray-based experiment examined responses to SW in germinating *Zea mays* (maize; Poaceae) seed at the stages of ruptured testa (24 h) and emerged radicle (48 h) (102). This study employed only technical replicates derived from a single pooled RNA sample for the SW and control treatments, and therefore may not accurately represent biological variation in these responses. Nevertheless, the authors reported 1,842 genes at 24 h and 1,652 genes at 48 h, with significant, twofold or more changes in expression. An analysis of terms and promoter motifs from the Gene Ontology database led to the conclusion that stress- and ABA-responsive genes were upregulated by SW treatment. However, many of the "most significant Gene Ontology terms" had $\leq$5 instances, and the one with the most instances ("response to cold") had only 33. Given that these Gene Ontology term numbers came from an analysis of more than 721 or 887 upregulated genes (out of the 1,842 or 1,652 genes with significant changes in expression, respectively), this analytical approach and its conclusions are questionable. Although maize seedlings showed enhanced vigor with SW treatment, the authors did not investigate whether the SW-responsive genes were in fact regulated by stress or ABA, nor whether there was a change in seedling stress tolerance. Indeed, even if these conclusions were correct, without further experiments it would be difficult to assess whether SW treatment may be considered a stress in itself owing to the presence of inhibitory compounds (68), rather than a preparatory signal for stress during post-germinative growth (102).

In a subsequent, more rigorous investigation, Soós et al. (101) compared the effects of SW, 100-nM KAR_1, and smoke on the transcriptome of maize embryos during a pregermination time course (1.5–24-h imbibition). The authors reported that SW and KAR_1 treatment significantly increased the germination rate of maize kernels, but over a 10-day time course the maximal difference was only ~10%. This approach has the advantage that all samples are likely to be at the same germination stage, but also the disadvantage that the results may not reveal transcriptional changes underlying smoke-activated seed germination. SW and smoke treatments produced transcriptional changes that were comparable to each other as well as to those of the previous study (102). The response to KAR_1 treatment, however, was reported to be "completely different" (101). This may not be surprising, however, because the SW solution used in this study contained only a low concentration of KAR_1 (4 nM), and additionally contained 13-μM 3,4,5-trimethylfuran-2(*5H*)-one [which is known to inhibit germination at 10 μM in some species (68, 83)] as well as thousands of other compounds. Despite the dissimilarity in gene expression patterns, a Gene Ontology analysis of SW- and KAR_1-responsive transcripts suggested overrepresentation of genes involved in responses to stress, light, ABA, and brassinosteroids as well as genes related to phenylpropanoid and flavonoid metabolism.

The maize tonoplast intrinsic protein TIP3.1, an aquaporin, was upregulated by KAR_1 throughout Soós et al.'s (101) experiment, leading to an investigation of the role of aquaporins in SW/karrikin response. An aquaporin inhibitor, $AgNO_3$, reduced maize seedling vigor, whereas KAR_1 enhanced vigor. The positive effect of karrikins on growth was not reduced by $AgNO_3$ treatment (101), in agreement with a prior experiment showing that KAR_1 improved tomato seed imbibition and seedling root growth that had been reduced by the aquaporin inhibitors $HgCl_2$ and $ZnCl_2$ (53). These results could indicate that karrikin action involves aquaporins; however, these treatments are not highly specific for aquaporin inhibition, and the lack of inhibition of positive karrikin effects by $AgNO_3$ on maize vigor could alternatively demonstrate that karrikins promote growth independently of aquaporins. Although transcriptome studies may suggest hypotheses for the mode of karrikin action, genetic analyses are required to test them.

Investigating the Karrikin Mode of Action Using an *Arabidopsis thaliana* Model

A. thaliana is unparalleled among plants in its array of available mutants and genetic tools, making it a powerful system for detailed plant biology investigations—provided it is a relevant system for study. *A. thaliana* seed germination is stimulated by KAR_1, KAR_2, and KAR_3. Although it is not as sensitive as other Brassicaceae species (e.g., *B. tournefortii*), 1-μM KAR_1 promoted germination of primary dormant *Arabidopsis* seed from 20% to 95% over a 10-day period (82). As in other species, seed dormancy state in *Arabidopsis* is important for karrikin-responsiveness. In highly dormant and nondormant ecotypes, KAR_1 had little effect on germination, whereas primary dormant seed of the L*er* (Landsberg *erecta*) ecotype exhibits a clear response (82). The effects of suboptimal and supraoptimal temperatures, storage conditions, or seed MC on the dormancy and karrikin germination capacity of different ecotypes have not been investigated, leaving open the possibility that some of the less responsive ecotypes will show stronger karrikin-induced germination under particular conditions. Importantly, despite the lack of strong germination responses in some ecotypes—e.g., Col (Columbia) and Ws (Wassilewskija)—a clear and consistent effect of karrikins on seedling photomorphogenesis has been observed in those same ecotypes (81). Thus, it seems likely that seed dormancy quantitative trait loci in each *Arabidopsis* ecotype influence karrikin germination-promoting activity.

As described above, a number of studies have noted a correlation between KAR_1 and GA as germination stimulants. *Arabidopsis* mutants defective in GA biosynthesis (*ga1-3* and *ga3ox1 ga3ox2*) showed no germination response to KAR_1 treatment (82). As KAR_1 did not enhance sensitivity to exogenous GA in *ga1-3* seed, this suggested that karrikins might promote germination by increasing production of active GAs. Consistent with this hypothesis, during seed imbibition active karrikins induced expression of two genes, *GA3ox1* and *GA3ox2*, for the final step of GA biosynthesis. However, several lines of evidence indicate that the karrikin mode of action is not so simple. First, GA_4 levels in *Arabidopsis* seed were negligibly affected by KAR_1 treatment during the pre-germination period (82). Second, karrikin-treated seedlings have reduced hypocotyl elongation in the light, whereas GA treatment is known to increase hypocotyl elongation (81). Third, the majority of transcriptional responses to KAR_1 during imbibition of wild-type seed were also observed in *ga1-3* mutant seed (81). Thus, although GA biosynthesis is necessary for completion of karrikin-induced seed germination, it is not required for karrikin perception or signaling.

Arabidopsis seed typically requires light for germination. GA can overcome this light requirement and induce dark germination, but KAR_1 cannot (82). However, multiple examples of positive interactions between karrikins and light have been found in *Arabidopsis*. Seed treated with a low-fluence red-light pulse exhibited higher germination in the presence of KAR_1 (81). Transcriptome analysis of KAR_1-treated seed imbibed in light showed a significant enrichment of light-regulated genes among KAR_1-induced transcripts. Although a light pulse was not required for all KAR_1 transcriptional responses, it was required or had a positive influence on KAR_1 response for at least some genes (81). Similarly, light treatment strongly enhanced induction of *GA3ox1* by KAR_1 in seeds (82). KAR_1 and KAR_2 promoted seedling photomorphogenesis under continuous red light, reducing hypocotyl elongation and increasing cotyledon expansion; however, inhibition of hypocotyl elongation did not occur in the dark (82). KAR_1 also reduced hypocotyl elongation under continuous red light in lettuce and *B. tournefortii*. In these species, KAR_1 inhibited growth of dark-grown hypocotyls, although this effect on growth was much stronger in the light (82). Thus, KAR_1 acts as a germination stimulant and enhancer of seedling light responses independently of photoblastic response. The transcription factors *HY5* and *HYH*, which have major roles

in light signal transduction, were mildly upregulated (less than twofold) by KAR_1 during seed imbibition, and 54% of KAR_1-induced genes had previously been categorized by chromatin immunoprecipitation (ChIP)–chip analysis as putative HY5 targets. Mutant *hy5* seed maintained germination and early transcriptional marker responses to karrikins but had a significantly reduced seedling photomorphogenesis response to karrikins. In contrast, *hyh* seedlings maintained a normal response (81). This demonstrates that HY5 is not required for germination or for all transcriptional responses to karrikins, but does have a role in mediating developmental responses to this signal (**Figure 3**).

It is curious that despite a severalfold increase in seed germination by KAR_1 treatment, the *Arabidopsis* transcriptome study led to relatively few genes being identified as karrikin-regulated. After 24-h imbibition, only 33 transcripts were significantly affected by KAR_1 with at least a 2-fold change in expression, and only 157 had a $\geq$1.5-fold change in expression (81). Does this subtle transcriptional pattern imply that karrikin response is limited to a minor subset of seed tissues, such as the endosperm or the radicle tip? In this study, the authors proposed that enhanced light responses in seedlings treated with karrikins could be an adaptation for better growth in a post-fire environment—which is more prone to temperature extremes, changes in water availability, and high light intensities—but this hypothesis has yet to be evaluated in the field.

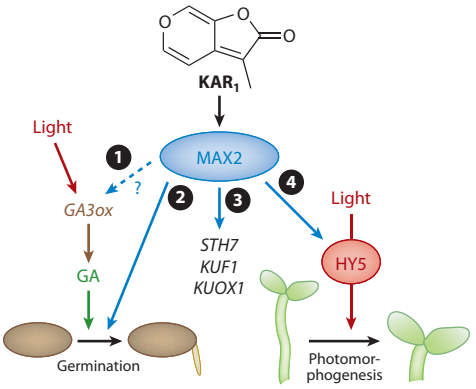

Figure 3

Diagram of currently known karrikin action in *Arabidopsis thaliana*. ① KAR_1 requires both gibberellin (GA) biosynthesis and light to stimulate *Arabidopsis* seed germination, and upregulates expression of *GA_3-oxidase* (*GA3ox*) genes. *GA3ox1* induction by KAR_1 is enhanced by light. However, GA levels are relatively unaffected within 48 h of seed imbibition (82). GA biosynthesis is not necessary for most transcriptional responses to karrikins during seed imbibition (81). The role of MAX2 in GA3ox response to karrikins has not been reported. ② MAX2 reduces *Arabidopsis* seed dormancy and is required for the stimulation of germination by karrikins. ③ Karrikins promote expression of genes such as *STH7*, *KUF1*, and *KUOX1*. These early transcriptional markers do not require GA biosynthesis, light, or HY5 for karrikin response (81), but do require MAX2 (83). ④ Karrikins also enhance photomorphogenesis of *Arabidopsis* seedlings (reducing hypocotyl elongation and increasing cotyledon expansion), but have no effect in the dark or in *max2* mutants (81, 83). This response is highly reduced in the *hy5* mutant (81). Karrikins mildly upregulate *HY5* expression (less than twofold) in a *MAX2*-dependent manner (83). Other transcriptional responses to karrikins partially or completely require light, and HY5 is likely to be involved, as it putatively targets 54% of karrikin-induced genes (81).

Genetic Screens for Karrikin-Response Mutants

With the establishment of several karrikin-induced phenotypes—enhanced seed germination and photomorphogenesis, transcriptional markers, and seedling vigor—genetic screens became possible. One may screen for karrikin-insensitivity or constitutive karrikin phenotypes with any of these responses. As always, the key to any successful genetic screen is specificity. It is important to distinguish karrikin-response mutants from other mutants that are nonspecifically affected in seed germination, seed dormancy, or hormone (e.g., ABA or GA) homeostasis and sensitivity. To accomplish this, it is necessary to assess multiple karrikin-related responses.

A genetic screen was performed in *Arabidopsis* for *kai* (*karrikin-insensitive*) mutants that did not show enhanced germination in the presence of 1-μM KAR_1 (83). The study focused on

two *kai1* mutants because they were karrikin-insensitive not only during seed germination, but also in their seedling photomorphogenesis and early transcriptional marker responses to karrikins. Thus, these mutants were likely to be specific to a karrikin signaling pathway. Proving the adage that a day in the library is worth a year in the lab, it was noted that the two *kai1* mutants exhibited several phenotypes that were consistent with mutations of the *MAX2* (*MORE AXILLARY GROWTH2*) gene in *Arabidopsis*. The authors quickly determined that each mutant contained a frameshift mutation of *MAX2* (83). This discovery was surprising as shoot branching responses to SL had previously been shown to require MAX2 (48, 115), and it now unequivocally linked karrikins to the SL family of plant hormones.

Comparison of *max2* with the SL-deficient mutants *max1*, *max3*, and *max4* demonstrated that *max2* had unique phenotypes of enhanced seed dormancy and reduced photomorphogenesis (**Figure 3**). The lack of these phenotypes in SL-deficient mutants implies that SLs are not normally involved in seed germination and seedling emergence, although the plant has the capacity to respond to SLs at these stages. During seed imbibition, karrikins and GR24 (SL analog) had similar MAX2-dependent effects on at least a few early transcriptional markers; however, it is currently unclear to what extent genome-wide transcriptional responses to karrikins and SLs overlap. Importantly, a clear distinction between karrikins and SLs was demonstrated: Neither KAR_1 nor KAR_2 had any effect on shoot branching in SL-deficient mutants in *Arabidopsis* or *Pisum sativum* (pea; Fabaceae) (83).

This evidence suggests that *Arabidopsis* has the capacity to respond to both karrikins and SLs during seed germination and early seedling emergence, but distinguishes between the two signals during cotyledon expansion and the control of shoot branching. How this may occur is an exciting subject for future investigation. MAX2 encodes an F-box protein that is highly conserved among land plants, suggesting that it may have a conserved role in mediating SL or karrikin responses (120). F-box proteins are adapter components of the SCF (Skp, Cullin, F-box containing) E3 ubiquitin ligase complex that target specific protein substrates for polyubiquitination and degradation. In a parallel to the GA signaling mechanism, one hypothesis proposed that MAX2 recognizes multiple karrikin- and SL-specific receptor-signaling repressor complexes that control distinct aspects of development (83).

Future Genetic Screens to Identify Karrikin- and Strigolactone-Response Mutants

Although the discovery of MAX2 as an essential regulator of karrikin response in *Arabidopsis* was an important step forward for the field, it is only the beginning of the journey. There are many additional genetic screens that can be envisioned to identify further components of karrikin- and SL-response pathways. For example, based upon the ability of plants to discriminate between karrikins and SLs, a screen for karrikin-insensitive mutants that retain SL responses seems plausible. This class of mutants could be affected in genes that act upstream of MAX2 or are receptors for these signals. Another approach would be to screen for suppressors of *max2*. As an F-box protein, MAX2 is anticipated to target a particular protein or set of proteins for degradation; thus, loss-of-function mutations in MAX2 substrates may produce a constitutive karrikin or SL response during seed, seedling, or branching stages. Of course, this approach is less likely to succeed if there are multiple, functionally redundant MAX2 targets.

It would be highly interesting to determine whether 3,4,5-trimethylfuran-2(5*H*)-one (**Figure 2*k***), the germination inhibitor recently found in smoke (68), directly blocks karrikin action (e.g., as a competitive inhibitor for a karrikin-binding site). This could be assessed by testing karrikin-insensitive mutants such as *max2* for germination inhibition, except that such mutants already exhibit highly reduced germination. Tests for *max2*-like

phenotypes, such as light-hyposensitive seedling morphology or repression of karrikin/SL reporter transcripts, in wild type treated with 3,4,5-trimethylfuran-2(5*H*)-one may provide alternative evidence for direct competition. If this inhibitor does specifically interfere with karrikin signal transduction, it would then be a useful tool for conducting genetic screens for constitutively activated karrikin/SL pathway mutants.

Reverse genetic approaches to studying karrikin function may also prove successful. A limited number of 121 genes were identified as karrikin-induced during wild-type *Arabidopsis* seed imbibition (82). Genes regulated by karrikins may prove to have roles in seed germination or hormone-responsiveness, or perhaps even direct involvement in karrikin response. Regardless, in the future we expect a variety of genetic approaches to be highly useful for clearing the smoke.

THE FUTURE

The discovery that both karrikins and SLs act through the F-box protein MAX2 raises intriguing questions about how the two classes of compounds are distinguished by plants and about which signaling system came first in evolutionary terms. The immediate challenges are to identify receptors for both karrikins and SLs, define their complete signal transduction pathways, and determine how they might carry out crosstalk with other signaling networks in plants. Then we can begin to understand the molecular basis for Orobanchaceae parasitic plants evolving to respond to SLs exuded from host roots while fire ephemerals became highly dependent upon karrikins for seed germination.

The identification of several plant-growth-regulating compounds in smoke provides us with a better understanding of the possible effects of fire on natural and managed ecosystems as well as powerful chemical tools to use in further investigations. This is of growing importance because increased human activity and demands for food are increasing the frequencies and distribution of fires (88). We need to better understand the consequences of burning crop residues as part of standard agricultural practice and of controlled burning to create firebreaks or manage fuel buildup. Furthermore, global warming and changes in precipitation are predicted to increase the frequency of wildfires (88).

Karrikins and cyanohydrins can potentially be used as management tools for land rehabilitation, plant conservation, and weed control. All of these applications would benefit from a clearer understanding of the environmental fate and modes of action of these growth regulators. For instance, we need to know how stable they are in the soil, whether they are metabolized by plants and microorganisms, and how they affect other organisms.

SUMMARY POINTS

1. Burning or heated plant material produces chemical stimulants of seed germination, including karrikins, cyanohydrins, and potentially nitrate and nitrogen oxides.
2. Many plant species in fire-prone environments (exemplified by Mediterranean ecosystems) are exquisitely adapted to respond to one or more of these signaling molecules by initiating seed germination.
3. Multiple species that are not considered to be fire-responsive also respond to karrikins, including *Arabidopsis thaliana*. This has opened up a new field of research using the genetic tools of *Arabidopsis* to investigate the karrikin mode of action.

4. Karrikins act through the same signal transduction pathway as the chemically related SL growth hormones. However, plants have the means to distinguish karrikins and SLs and to respond appropriately.

NOTE ADDED IN PROOF

At the proof stage of this review, a new manuscript was published (119a) describing two genes that enable karrikins and SLs to be distinguished in *Arabidopsis*. This validates the proposed genetic approaches (see Future Genetic Screens to Identify Karrikin- and Strigolactone-Response Mutants) and confirms the physiological evidence for discrimination of karrikins and SLs (see Genetic Screens for Karrikin-Response Mutants). It also suggests modes of action for karrikins and SLs involving specific α/β-fold-family proteins.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Akiyama K, Matsuzaki K, Hayashi H. 2005. Plant sesquiterpenes induce hyphal branching in arbuscular mycorrhizal fungi. *Nature* 435:824–27
2. Akiyama K, Ogasawara S, Ito S, Hayashi H. 2010. Structural requirements of strigolactones for hyphal branching in AM fungi. *Plant Cell Physiol.* 51:1104–17
3. Baker KS, Steadman KJ, Plummer JA, Merritt DJ, Dixon KW. 2005. The changing window of conditions that promotes germination of two fire ephemerals, *Actinotus leucocephalus* (Apiaceae) and *Tersonia cyathiflora* (Gyrostemonaceae). *Ann. Bot.* 96:1225–36
4. Baldwin IT, Morse L. 1994. Up in smoke: II. Germination of *Nicotiana attenuata* in response to smoke-derived cues and nutrients in burned and un-burned soils. *J. Chem. Ecol.* 20:2373–91
5. Baldwin IT, Staszak-Kozinski L, Davidson R. 1994. Up in smoke: I. Smoke-derived germination cues for postfire annual, *Nicotiana attenuata* Torr. Ex. Watson. *J. Chem. Ecol.* 20:2345–71
6. Baskin CC, Baskin JM. 1998. Types of seed dormancy. In *Seeds: Ecology, Biogeography and Evolution of Dormancy and Germination*, pp. 27–48. San Diego: Academic
7. Baudouin E. 2011. The language of nitric oxide signalling. *Plant Biol.* 13:233–42
8. Baxter BJM, Granger JE, van Staden J. 1995. Plant-derived smoke and seed germination: Is all smoke good smoke? That is the burning question. *S. Afr. J. Bot.* 61:275–77
9. Bergmann C, Wegmann K, Frischmuth K, Samson E, Kranz A, et al. 1993. Stimulation of Orobanche-Crenata seed-germination by (+)-strigol and structural analogs dependence on constitution and configuration of the germination stimulants. *J. Plant Physiol.* 142:338–42

10. Besson-Bard A, Pugin A, Wendehenne D. 2008. New insights into nitric oxide signaling in plants. *Annu. Rev. Plant Biol.* 59:21–39
11. Bethke PC, Libourel IGL, Reinohl V, Jones RL. 2006. Sodium nitroprusside, cyanide, nitrite and nitrate break Arabidopsis seed dormancy in a nitric oxide-dependent manner. *Planta* 223:805–12
12. Bewley JD. 1997. Seed germination and dormancy. *Plant Cell* 9:1055–66
13. Bradshaw SD, Dixon KW, Hopper SD, Lambers H, Turner SR. 2011. Little evidence for fire-adapted plant traits in Mediterranean climate regions. *Trends Plant Sci.* 16:69–76
14. Brown NAC, van Staden J. 1997. Smoke as a germination cue: a review. *Plant Growth Regul.* 22:115–24
15. Chiwocha SDS, Dixon KW, Flematti GR, Ghisalberti EL, Merritt DJ, et al. 2009. Karrikins: a new family of plant growth regulators in smoke. *Plant Sci.* 177:252–56
16. Chuvieco E, Giglio L, Justice C. 2008. Global characterization of fire activity: toward defining fire regimes from Earth observation data. *Glob. Change Biol.* 14:1488–502
17. Cohn MA, Chiles LA, Hughes JA, Boullion KJ. 1987. Seed dormancy in red rice VI. Monocarboxylic acids: a new class of pH-dependant germination stimulants. *Plant Physiol.* 84:716–19
18. Commander LE, Merritt DJ, Rokich DP, Dixon KW. 2009. Seed biology of Australian arid zone species: germination of 18 species used for rehabilitation. *J. Arid Environ.* 73:617–25
19. Cook CE, Whichard LP, Turner B, Wall ME, Egley GH. 1966. Germination of witchweed (*Striga lutea* Lour.): isolation and properties of a potent stimulant. *Science* 154:1189–90
20. Cook CE, Whichard LP, Wall ME, Egley GH, Coggon P, et al. 1972. Germination stimulants. II. The structure of strigol—a potent seed germination stimulant for witchweed (*Striga lutea* Lour.). *J. Am. Chem. Soc.* 94:6198–99
21. Dagaut P, Glarborg P, Alzueta MU. 2008. The oxidation of hydrogen cyanide and related chemistry. *Prog. Energ. Combust.* 34:1–46
22. Daws MI, Davies J, Pritchard HW, Brown NAC, van Staden J. 2007. Butenolide from plant-derived smoke enhances germination and seedling growth of arable weeds species. *Plant Growth Regul.* 51:73–82
23. De Lange JH, Boucher C. 1990. Autecological studies on *Audouinia capitata* (Bruniaceae). I. Plant-derived smoke as a seed germination cue. *S. Afr. J. Bot.* 56:700–3
24. Dixon KW, Merritt DJ, Flematti GR, Ghisalberti EL. 2009. Karrikinolide—a phytoreactive compound derived from smoke with applications in horticulture, ecological restoration and agriculture. *Acta Hortic.* 813:155–70
25. Dixon KW, Roche S, Pate JS. 1995. The promotive effect of smoke derived from burnt native vegetation on seed germination of Western Australian plants. *Oecologia* 101:185–92
26. Doherty LC, Cohn MA. 2000. Seed dormancy in red rice (*Oryza sativa*). XI. Commercial liquid smoke elicits germination. *Seed Sci. Res.* 10:415–21
27. Downes KS, Lamont BB, Light ME, van Staden J. 2010. The fire ephemeral *Tersonia cyathiflora* (Gyrostemonaceae) germinates in response to smoke but not the butenolide 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one. *Ann. Bot.* 106:381–84
28. Drewes FE, Smith MT, van Staden J. 1995. The effect of a plant derived smoke extract on the germination of light-sensitive lettuce seed. *Plant Growth Regul.* 16:205–9
29. Dziewanowska K, Niedzwiedz I, Chodelska I, Lewak S. 1979. Hydrogen-cyanide and cyanogenic compounds in seeds. I. Influence of hydrogen-cyanide on germination of apple embryos. *Physiol. Veg.* 17:297–303
30. Fernandez-Aparicio M, Yoneyama K, Rubiales D. 2011. The role of strigolactones in host specificity of *Orobanche* and *Phelipanche* seed germination. *Seed Sci. Res.* 21:55–61
31. Finkelstein R, Reeves W, Ariizumi T, Steber C. 2008. Molecular aspects of seed dormancy. *Annu. Rev. Plant Biol.* 59:387–415
32. Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD. 2004. A compound from smoke that promotes seed germination. *Science* 305:977
33. Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD. 2004. Molecular weight of a germination-enhancing compound in smoke. *Plant Soil* 263:1–4
34. Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD. 2005. Synthesis of the seed germination stimulant 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one. *Tetrahedron Lett.* 46:5719–21

35. Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD. 2008. Germination stimulant in smoke: isolation and identification. In *Bioactive Natural Products: Detection, Isolation and Structural Elucidation*, ed. SM Colegate, RJ Molyneux, pp. 531–54. Boca Raton, FL: CRC
36. Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD. 2009. Identification of alkyl substituted 2*H*-furo[2,3-*c*]pyran-2-ones as germination stimulants present in smoke. *J. Agric. Food Chem.* 57:9475–80
37. Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD, Skelton BW, White AH. 2005. Structural analysis of a potent seed germination stimulant. *Aust. J. Chem.* 58:505–6
38. Flematti GR, Goddard-Borger ED, Merritt DJ, Ghisalberti EL, Dixon KW, Trengove RD. 2007. Preparation of 2*H*-furo[2,3-*c*]pyran-2-one derivatives and evaluation of their germination-promoting activity. *J. Agric. Food Chem.* 55:2189–94
39. Flematti GR, Merritt DJ, Piggott MJ, Trengove RD, Smith SM, et al. 2011. Burning vegetation produces cyanohydrins that liberate cyanide and promote seed germination. *Nat. Commun.* 2:360
40. Flematti GR, Scaffidi A, Dixon KW, Smith SM, Ghisalberti EL. 2011. Production of the seed germination stimulant karrikinolide from combustion of simple carbohydrates. *J. Agric. Food Chem.* 59:1195–98
41. Flematti GR, Scaffidi A, Goddard-Borger ED, Heath CH, Nelson DC, et al. 2010. Structure-activity relationship of karrikin germination stimulants. *J. Agric. Food Chem.* 58:8612–17
42. Footitt S, Cohn MA. 2001. Developmental arrest: from sea urchins to seeds. *Seed Sci. Res.* 11:3–16
43. Gardner MJ, Dalling KJ, Light ME, Jäger AK, van Staden J. 2001. Does smoke substitute for red light in the germination of light-sensitive lettuce seeds by affecting gibberellin metabolism? *S. Afr. J. Bot.* 67:636–40
44. Giba Z, Grubisic D, Konjevic R. 2003. Nitrogen oxides as environmental sensors for seeds. *Seed Sci. Res.* 13:187–96
45. Glasspool IJ, Edwards D, Axe L. 2004. Charcoal in the Silurian as evidence for the earliest wildfire. *Geology* 32:381–83
46. Gniazdowska A, Krasuska U, Bogatek R. 2010. Dormancy removal in apple embryos by nitric oxide or cyanide involves modifications in ethylene biosynthetic pathway. *Planta* 232:1397–407
47. Goddard-Borger ED, Ghisalberti EL, Stick RV. 2007. Synthesis of the germination stimulant 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one and analogous compounds from carbohydrates. *Eur. J. Org. Chem.* 23:3925–34
48. Gomez-Polden V, Fermas S, Brewer PB, Puech-Pagès V, Dun EA, et al. 2008. Strigolactone inhibition of shoot branching. *Nature* 455:189–94
49. Grossmann K. 1990. Plant growth retardants as tools in physiological research. *Physiol. Plant.* 78:640–48
50. Gupta KJ, Fernie AR, Kaiser WM, van Dongen JT. 2011. On the origins of nitric oxide. *Trends Plant Sci.* 16:160–68
51. Hendricks SB, Taylorson RB. 1972. Promotion of seed germination by nitrates and cyanides. *Nature* 237:169–70
52. Jäger AK, Light ME, van Staden J. 1996. Effects of source of plant material and temperature on the production of smoke extracts that promote germination of light-sensitive lettuce seeds. *Environ. Exp. Bot.* 36:421–29
53. Jain N, Ascough GD, van Staden J. 2008. A smoke-derived butenolide alleviates $HgCl_2$ and $ZnCl_2$ inhibition of water uptake during germination and subsequent growth of tomato—possible involvement of aquaporins. *J. Plant Physiol.* 165:1422–27
54. Jain N, Soós V, Balazs E, van Staden J. 2008. Changes in cellular macromolecules (DNA, RNA and protein) during seed germination in tomato, following the use of a butenolide, isolated from plant-derived smoke. *Plant Growth Regul.* 54:105–13
55. Jain N, van Staden J. 2006. A smoke-derived butenolide improves early growth of tomato seedlings. *Plant Growth Regul.* 50:139–48
56. Kapulnik Y, Delaux PM, Resnick N, Mayzlish-Gati E, Wininger S, et al. 2011. Strigolactones affect lateral root formation and root-hair elongation in *Arabidopsis*. *Planta* 233:209–16
57. Keeley JE, Fotheringham CJ. 1997. Trace gas emissions and smoke-induced seed germination. *Science* 276:1248–50
58. Keeley JE, Fotheringham CJ. 1998. Mechanism of smoke-induced seed germination in a post-fire chaparral annual. *J. Ecol.* 86:27–36

59. Keeley JE, Fotheringham CJ. 1998. Smoke-induced seed germination in California chaparral. *Ecology* 79:2320–36
60. Keeley JE, Morton BA, Pedrosa A, Trotter P. 1985. Role of allelopathy, heat and charred wood in the germination of chaparral herbs and suffrutescents. *J. Ecol.* 73:445–58
61. Keeley JE, Pausas JG, Rundel PW, Bond WJ, Bradstock RA. 2011. Fire as an evolutionary pressure shaping plant traits. *Trends Plant Sci.* 16:406–11
62. Keeley SC, Pizzorno M. 1986. Charred wood stimulated germination of two fire-following herbs of the California chaparral and the role of hemicellulose. *Am. J. Bot.* 73:1289–97
63. Kepczynski J, Cembrowska D, van Staden J. 2010. Releasing primary dormancy in *Avena fatua* L. caryopses by smoke-derived butenolide. *Plant Growth Regul.* 62:85–91
64. Krock B, Schmidt S, Hertweck C, Baldwin IT. 2002. Vegetation-derived abscisic acid and four terpenes enforce dormancy in seeds of the post-fire annual, *Nicotiana attenuata*. *Seed Sci. Res.* 12:239–52
65. Kucera B, Cohn MA, Leubner-Metzger G. 2005. Plant hormone interactions during seed dormancy release and germination. *Seed Sci. Res.* 15:281–307
66. Kulkarni MG, Ascough GD, van Staden J. 2008. Smoke-water and a smoke-isolated butenolide improve growth and yield of tomatoes under greenhouse conditions. *HortTechnology* 18:449–54
67. Kulkarni MG, Sparg SG, Light ME, van Staden J. 2006. Stimulation of rice (*Oryza sativa* L.) seedling vigour by smoke-water and butenolide. *J. Agron. Crop Sci.* 192:395–98
68. Light ME, Burger BV, Staerk D, Kohout L, van Staden J. 2010. Butenolides from plant-derived smoke: natural plant growth regulators with antagonistic actions on seed germination. *J. Nat. Prod.* 73:267–69
69. Light ME, Daws MI, van Staden J. 2009. Smoke-derived butenolide: towards understanding its biological effects. *S. Afr. J. Bot.* 75:1–7
70. Light ME, Gardner MJ, Jäger AK, van Staden J. 2002. Dual regulation of seed germination by smoke solutions. *Plant Growth Regul.* 37:135–41
71. Light ME, van Staden J. 2003. The nitric oxide specific scavenger carboxy-PTIO does not inhibit smoke stimulated germination of Grand Rapids lettuce seeds. *S. Afr. J. Bot.* 69:217–19
72. Long RL, Stevens JC, Griffiths EM, Adamek M, Gorecki MJ, et al. 2011. Seeds of Brassicaceae weeds have an inherent or inducible response to the germination stimulant karrikinolide. *Ann. Bot.* 108:933–44
73. Long RL, Williams K, Griffiths EM, Flematti GR, Merritt DJ, et al. 2010. Prior hydration of *Brassica tournefortii* seeds reduces the stimulatory effect of karrikinolide on germination and increases seed sensitivity to abscisic acid. *Ann. Bot.* 105:1063–70
74. Major W, Roberts EH. 1968. Dormancy in cereal seeds I. The effects of oxygen and respiratory inhibitors. *J. Exp. Bot.* 19:77–89
75. Mangnus EM, Dommerholt FJ, Dejong RLP, Zwanenburg B. 1992. Improved synthesis of strigol analog GR24 and evaluation of the biological activity of its diastereomers. *J. Agric. Food Chem.* 40:1230–35
76. Matsuo K, Shindo M. 2011. Efficient synthesis of karrikinolide via Cu(II)-catalyzed lactonization. *Tetrahedron* 67:971–75
77. Merritt DJ, Kristiansen MV, Flematti GR, Turner SR, Ghisalberti EL, et al. 2006. Effects of a butenolide present in smoke on light-mediated germination of Australian Asteraceae. *Seed Sci. Res.* 16:29–35
78. Merritt DJ, Turner SR, Clarke S, Dixon KW. 2007. Seed dormancy and germination stimulation syndromes for Australian temperate species. *Aust. J. Bot.* 55:336–44
79. Moreira B, Tormo J, Estrelles E, Pausas JG. 2010. Disentangling the role of heat and smoke as germination cues in Mediterranean Basin flora. *Ann. Bot.* 105:627–35
80. Nagase R, Katayama M, Mura H, Matsuo N, Tanabe Y. 2008. Synthesis of the seed germination stimulant 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one utilising direct and regioselective Ti-crossed aldol addition. *Tetrahedron Lett.* 49:4509–12
81. Nelson DC, Flematti GR, Riseborough JA, Ghisalberti EL, Dixon KW, Smith SM. 2010. Karrikins enhance light responses during germination and seedling development in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 107:7095–100
82. Nelson DC, Riseborough JA, Flematti GR, Stevens J, Ghisalberti EL, et al. 2009. Karrikins discovered in smoke trigger Arabidopsis seed germination by a mechanism requiring gibberellic acid synthesis and light. *Plant Physiol.* 149:863–73

83. Nelson DC, Scaffidi A, Dun EA, Waters MT, Flematti GR, et al. 2011. F-box protein MAX2 has dual roles in karrikin and strigolactone signaling in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA* 108:8897–902
84. Oracz K, El-Maarouf-Bouteau H, Bogatek R, Corbineau F, Bailly C. 2008. Release of sunflower seed dormancy by cyanide: cross-talk with ethylene signalling pathway. *J. Exp. Bot.* 59:2241–51
85. Oracz K, El-Maarouf-Bouteau H, Farrant JM, Cooper K, Belghazi M, et al. 2007. ROS production and protein oxidation as a novel mechanism for seed dormancy alleviation. *Plant J.* 50:452–65
86. Oracz K, El-Maarouf-Bouteau H, Kranner I, Bogatek R, Corbineau F, Bailly C. 2009. The mechanisms involved in seed dormancy alleviation by hydrogen cyanide unravel the role of reactive oxygen species as key factors of cellular signalling during germination. *Plant Physiol.* 150:494–505
87. Pausas JG, Keeley JE. 2009. A burning story: the role of fire in the history of life. *Bioscience* 59:593–601
88. Pechony O, Shindell DT. 2010. Driving forces of global wildfires over the past millennium and the forthcoming century. *Proc. Natl. Acad. Sci. USA* 107:19167–70
89. Preston CA, Baldwin IT. 1999. Positive and negative signals regulate germination in the post-fire annual, *Nicotiana attenuata*. *Ecology* 80:481–94
90. Preston CA, Becker R, Baldwin IT. 2004. Is 'NO' news good news? Nitrogen oxides are not components of smoke that elicits germination in two smoke-stimulated species, *Nicotiana attenuata* and *Emmenanthe penduliflora*. *Seed Sci. Res.* 14:73–79
91. Ricardo A, Carrigan MA, Olcott AN, Benner SA. 2004. Borate minerals stabilize ribose. *Science* 303:196
92. Roberts E. 1969. Seed dormancy and oxidation processes. *Symp. Soc. Exp. Biol.* 23:161–92
93. Roche S, Dixon KW, Pate JS. 1997. Seed ageing and smoke: partner cues in the amelioration of seed dormancy in selected Australian native species. *Aust. J. Bot.* 45:783–815
94. Roche S, Dixon KW, Pate JS. 1998. For everything a season: smoke-induced seed germination and seedling recruitment in a Western Australian *Banksia* woodland. *Aust. J. Ecol.* 23:111–20
95. Scaffidi A, Flematti GR, Nelson DC, Dixon KW, Smith SM, Ghisalberti EL. 2011. The synthesis and biological evaluation of labelled karrikinolides for the elucidation of the mode of action of the seed germination stimulant. *Tetrahedron* 67:152–57
96. Schwachtje J, Baldwin IT. 2004. Smoke exposure alters endogenous gibberellin and abscisic acid pools and gibberellin sensitivity while eliciting germination in the post-fire annual, *Nicotiana attenuata*. *Seed Sci. Res.* 14:51–60
97. Scott AC, Glasspool IJ. 2006. The diversification of Paleozoic fire systems and fluctuations in atmospheric oxygen concentration. *Proc. Natl. Acad. Sci. USA* 103:10861–65
98. Siegien I, Bogatek R. 2006. Cyanide action in plants—from toxic to regulatory. *Acta Physiol. Plant.* 28:483–97
99. Smith CJ, Perfetti TA, Garg R, Hansch C. 2003. IARC carcinogens reported in cigarette mainstream smoke and their calculated log *P* values. *Food Chem. Toxicol.* 41:807–17
100. Soós V, Juhasz A, Light ME, van Staden J, Balazs E. 2009. Smoke-water-induced changes of expression pattern in Grand Rapids lettuce achenes. *Seed Sci. Res.* 19:37–49
101. Soós V, Sebestyen E, Juhasz A, Light ME, Kohout L, et al. 2010. Transcriptome analysis of germinating maize kernels exposed to smoke-water and the active compound KAR. *BMC Plant Biol.* 10:236
102. Soós V, Sebestyen E, Juhasz A, Pinter J, Light ME, et al. 2009. Stress-related genes define essential steps in the response of maize seedlings to smoke-water. *Funct. Integr. Genomics* 9:231–42
103. Sparg SG, Kulkarni MG, Light ME, van Staden J. 2005. Improving seedling vigour of indigenous medicinal plants with smoke. *Bioresour. Technol.* 96:1323–30
104. Sparg SG, Kulkarni MG, van Staden J. 2006. Aerosol smoke and smoke-water stimulation of seedling vigor of a commercial maize cultivar. *Crop. Sci.* 46:1336–40
105. Stevens JC, Merritt DJ, Flematti GR, Ghisalberti EL, Dixon KW. 2007. Seed germination of agricultural weeds is promoted by the butenolide 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one under laboratory and field conditions. *Plant Soil* 298:113–24
106. Sun K, Chen Y, Wagerle T, Linnstaedt D, Currie M, et al. 2008. Synthesis of butenolides as germination stimulants. *Tetrahedron Lett.* 49:2922–25
107. Taylorson RB, Hendricks SB. 1973. Promotion of seed germination by cyanide. *Plant Physiol.* 52:23–27

108. Thanos CA, Rundel PW. 1995. Fire-followers in chaparral: nitrogenous compounds trigger seed germination. *J. Ecol.* 83:207–16
109. Thomas PB, Morris EC, Auld TD, Haigh AM. 2010. The interaction of temperature, water availability and fire cues regulates seed germination in a fire-prone landscape. *Oecologia* 162:293–302
110. Tieu A, Dixon KW, Meney KA, Sivasithamparam K. 2001. The interaction of heat and smoke in the release of seed dormancy in seven species from southwestern Western Australia. *Ann. Bot.* 88:259–65
111. Tieu A, Dixon KW, Meney KA, Sivasithamparam K. 2001. Interaction of soil burial and smoke on germination patterns in seeds of selected Australian native plants. *Seed Sci. Res.* 11:69–76
112. Tieu A, Dixon KW, Meney KA, Sivasithamparam K, Barrett RL. 2001. Spatial and developmental variation in seed dormancy characteristics in the fire-responsive species *Anigozanthos manglesii* (Haemodoraceae) from Western Australia. *Ann. Bot.* 88:19–26
113. Turner SR, Merritt DJ, Renton MS, Dixon KW. 2009. Seed moisture content affects afterripening and smoke responsiveness in three sympatric Australian native species from fire-prone environments. *Austral Ecol.* 34:866–77
114. Turner SR, Merritt DJ, Ridley EC, Commander LE, Baskin JM, et al. 2006. Ecophysiology of seed dormancy in the Australian endemic species *Acanthocarpus preissii* (Dasypogonaceae). *Ann. Bot.* 98:1137–44
115. Umehara M, Hanada A, Yoshida S, Akiyama K, Arite T, et al. 2008. Inhibition of shoot branching by new terpenoid plant hormones. *Nature* 455:195–200
116. van Staden J, Drewes FE, Brown NAC. 1995. Some chromatographic characteristics of germination stimulants in plant-derived smoke extracts. *Plant Growth Regul.* 17:241–49
117. van Staden J, Jäger AK, Light ME, Burger BV. 2004. Isolation of the major germination cue from plant-derived smoke. *S. Afr. J. Bot.* 70:654–59
118. van Staden J, Jäger AK, Strydom A. 1995. Interaction between a plant-derived smoke extract, light and phytohormones on the germination of light-sensitive lettuce seeds. *Plant Growth Regul.* 17:213–18
119. van Staden J, Sparg SG, Kulkarni MG, Light ME. 2006. Post-germination effects of the smoke-derived compound 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, and its potential as a preconditioning agent. *Field Crops Res.* 98:98–105
119a. Waters MT, Nelson DC, Scaffidi A, Flematti GR, Sun YM, et al. 2012. Specialisation within the DWARF14 protein family confers distinct responses to karrikins and strigolactones in *Arabidopsis*. *Development.* In press
120. Waters MT, Smith SM, Nelson DC. 2011. Smoke signals and seed dormancy: where next for MAX2? *Plant Sig. Behav.* 6:1418–22
121. Wicklow DT. 1977. Germination response in *Emmenanthe penduliflora* (Hydrophyllaceae). *Ecology* 58:201–5
122. Wigchert SCM, Zwanenburg B. 1999. A critical account on the inception of *Striga* seed germination. *J. Agric. Food Chem.* 47:1320–25
123. Yoneyama K, Awad AA, Xie X, Yoneyama K, Takeuchi Y. 2010. Strigolactones as germination stimulants for root parasitic plants. *Plant Cell Physiol.* 51:1095–103
124. Zwanenburg B, Mwakaboko AS, Reizelman A, Anilkumar G, Sethumadhavan D. 2009. Structure and function of natural and synthetic signalling molecules in parasitic weed germination. *Pest Manag. Sci.* 65:478–91

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