

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	108
Fire and Plant Evolution	108
Plant Regeneration After Fire	109
DISCOVERY OF BIOACTIVE COMPOUNDS FROM PYROLYSED PLANT MATERIAL	109
Recognition That Products of Pyrolysis Stimulate Seed Germination	109
Discovery of Karrikins	110
Properties and Structure-Activity Relationships of Karrikins	111
Discovery and Properties of Cyanohydrins	113
Germination Inhibitors Removed and Released by Fire	113
PHYSIOLOGICAL ROLE OF KARRIKINS	114
Diversity of Plants Responding to Smoke and Karrikins	114
Similarity Between Karrikins and Strigolactones	114
Physiological Responses to Smoke and Karrikins	115
Seed Dormancy Gates Responses to Germination Stimulants in Smoke	115
Light and Hormone Interactions with Smoke-Stimulated Germination	117
KAR ₁ and Smoke Water Improve Suboptimal Germination and Seedling Vigor	119
MOLECULAR MODE OF ACTION OF KARRIKINS	119
Effects of Smoke and Karrikins on Gene Expression	119
Investigating the Karrikin Mode of Action Using an <i>Arabidopsis</i> <i>thaliana</i> Model	121
Genetic Screens for Karrikin-Response Mutants	122
Future Genetic Screens to Identify Karrikin- and Strigolactone-Response Mutants	123
THE FUTURE	124

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.

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

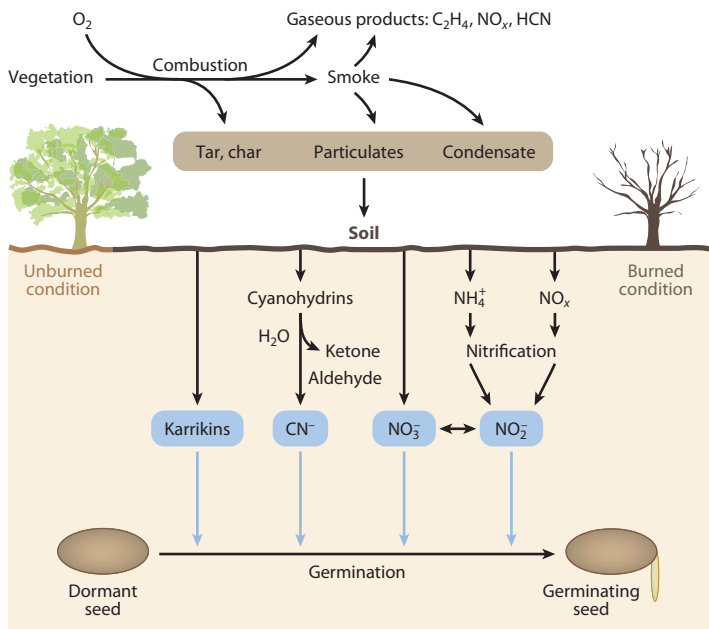


Figure 1

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.

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 2a**) (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 (*karrikin* being a traditional word for smoke in the language of the Aboriginal Noongar people of southwestern Australia) and abbreviated as KAR₁. 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 KAR₁ production by burning pure xylose, glucose, or cellulose, it has been proposed that KAR₁ is derived from a pyranose sugar (40).

Five KAR₁ analogs, KAR₂–KAR₆ (**Figure 2b–f**), which along with KAR₁ 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 KAR₁ were 39.8 μg (39.8 ppb, 265 nM); for KAR₂, 6.5 μg (6.5 ppb, 48 nM); for KAR₃, 5.2 μg (5.2 ppb, 32 nM); for KAR₄, 3.4 μg (3.4 ppb, 21 nM); for KAR₅, 1.0 μg (1.0 ppb, 7 nM); and for KAR₆, 1.3 μg (1.3 ppb, 9 nM). Because KAR₁ 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 KAR₁ 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 KAR₁ (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 KAR₁ (34, 47, 76, 80, 106). Nearly 50 analogs have been synthesized by substitutions at carbons 3, 4, 5, and

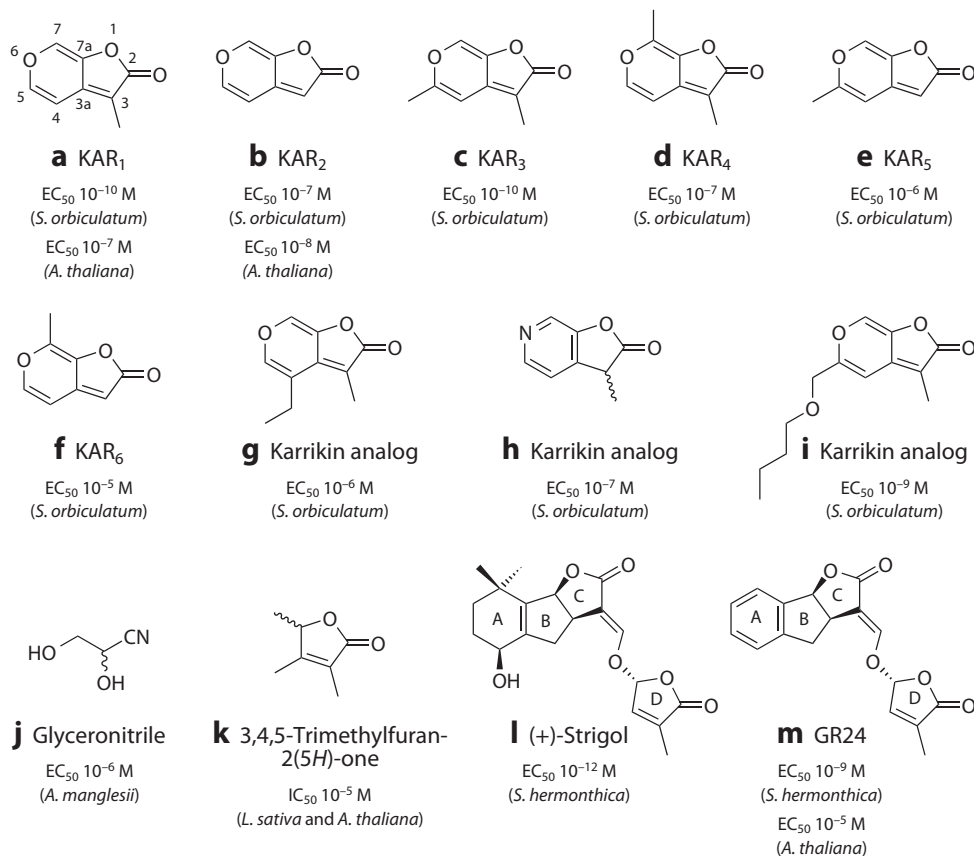


Figure 2

Structures and bioactivity [half maximal effective concentration (EC₅₀)] 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 (IC₅₀) 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% (KAR₁ 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 2d**) reduces activity. Addition of a methyl at C-5 (**Figure 2c**) is reasonably well tolerated, whereas modification at C-4 (**Figure 2g**) decreases germination activity. Replacement of the pyran oxygen with nitrogen (**Figure 2h**) does not eliminate the activity. The observation that C-5 is tolerant of simple variations (**Figure 2i**) 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 KAR₁ 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 KAR₁ or nitrate (27). *Anigozanthos manglesii* (red and green kangaroo paw; Haemodoraceae) also responds to SW but not to KAR₁ 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 KAR₁ 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 2j**) 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 KAR₁ (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 NO₂) 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 2j**) 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 non-dormant *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 μ g 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 2k**), was recently isolated from plant-derived smoke as an inhibitor of lettuce seed germination (68). This inhibitor was active at concentrations above 10 μ M, and applying it with 0.1- μ M KAR₁ markedly suppressed germination compared with KAR₁ 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 μ M inhibited germination, whereas the three analogs were inactive (83). Any direct interactions between KAR₁ 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 2l**) was the first SL identified (19, 20), but most studies utilize the simpler synthetic analog GR24 (**Figure 2m**) (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 KAR₁ (**Figure 2a**). 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 KAR₁ purified from SW stimulates germination of numerous parasitic weeds. However, Nelson et al. (82) showed that chemically synthesized KAR₁ has no effect on *Orobancha 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 KAR₁ 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 KAR₁, (b) karrikin response is distinct from other germination cues, and (c) KAR₁ 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 KAR₁ 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 KAR₁; in one experiment, KAR₁ at a concentration of less than 1 nM

improved seed germination from 4% to 40%, and 67-nM KAR₁ 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 KAR₁ (72, 105). A 2005 seed lot from the Perth metropolitan area had <10% germination in the presence of 0.67-μM KAR₁, 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 KAR₁ 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 KAR₁. 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 KAR₁-responsiveness in seed of four Brassicaceae species, providing a higher relative stimulation by KAR₁ 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 KAR₁ (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 candidans* (Haemodraceae), 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 KAR₁. One study (73) found that *B. tournefortii* germination was strongly promoted with as little as 3 min of exposure to 1-μM KAR₁. 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 KAR₁ 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/KAR₁ and light act independently to influence seed germination; SW/KAR₁ 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), KAR₁ can induce germination in darkness (77). KAR₁ also stimulates germination of negatively photoblastic species; for example, *Avena fatua* (Poaceae) germination is enhanced by KAR₁ (22, 105), but light treatments reduce the response to KAR₁ (63).

Assessment of the interaction between KAR₁, after-ripening treatments, germination

temperature, dormancy cycling, and light in eight Brassicaceae species showed that KAR₁ 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 KAR₁-responsive species is also stimulated by GA treatment. *Anthocercis littorea* (Solanaceae) germination has a strong requirement for karrikin treatment. However, high concentrations of GA₃ (2.89 mM) can promote its germination, as can KAR₁ (0.67 μM) (18). GA₃ and KAR₁ 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 GA₃ (289 μM) and KAR₁ (0.67 μM), all nine responded to GA₃ and seven responded to KAR₁ (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 GA₃ and GA₁ 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. candidans* was SW-responsive but not GA-responsive (113). Lower concentrations of GA₃ (30 μM) and KNO₃ (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 KAR₁ 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, GA₃, KNO₃, and alternating temperature treatments, GA₃ had the strongest correlation with KAR₁ treatment. However, GA₃ had distinct effects on seedling morphology not seen with KAR₁.

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 KAR₁ 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 KAR₁-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 KAR₁ response.

KAR₁ and Smoke Water Improve Suboptimal Germination and Seedling Vigor

Several studies have reported that smoke or KAR₁ treatment enhances suboptimal germination, seedling survival, and seedling vigor. KAR₁ 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 KAR₁ (55). Germination of *Kunzea ambigua* and *Kunzea capitata* (Myrtaceae) increased at low water potentials in response to smoke (109). SW/KAR₁ 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/KAR₁ 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 KAR₁ treatment. Maize seedlings developed more roots in response to SW/KAR₁, 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 pachyphlamys* (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 (*Alopecurus myosuroides*, *Sorghum halepense*), Brassicaceae (*Capsella bursapastoris*), Rubiaceae (*Galium aparine*), and Papaveraceae (*Papaver rhoeas*) had significantly increased seedling dry mass following KAR₁ treatment (22). *P. rhoeas* and *S. halepense* had increased germination in response to KAR₁, 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/KAR₁ 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 KAR₁ 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 ≤ 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 KAR₁, and smoke on the transcriptome of maize embryos during a pre-germination time course (1.5–24-h imbibition). The authors reported that SW and KAR₁ 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 KAR₁ 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 KAR₁ (4 nM), and additionally contained 13- μ M 3,4,5-trimethylfuran-2(5H)-one [which is known to inhibit germination at 10 μ M 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 KAR₁-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 KAR₁ throughout Soós et al.’s (101) experiment, leading to an investigation of the role of aquaporins in SW/karrikin response. An aquaporin inhibitor, AgNO₃, reduced maize seedling vigor, whereas KAR₁ enhanced vigor. The positive effect of karrikins on growth was not reduced by AgNO₃ treatment (101), in agreement with a prior experiment showing that KAR₁ improved tomato seed imbibition and seedling root growth that had been reduced by the aquaporin inhibitors HgCl₂ and ZnCl₂ (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 AgNO₃ 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 KAR₁, KAR₂, and KAR₃. Although it is not as sensitive as other Brassicaceae species (e.g., *B. tournefortii*), 1-μM KAR₁ 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, KAR₁ had little effect on germination, whereas primary dormant seed of the *Ler* (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 KAR₁ and GA as germination stimulants. *Arabidopsis* mutants defective in GA biosynthesis (*ga1-3* and *ga3ox1 ga3ox2*) showed no germination response to KAR₁ treatment (82). As KAR₁ 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, GA₄ levels in *Arabidopsis* seed were negligibly affected by KAR₁ 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 KAR₁ 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 KAR₁ 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 KAR₁ (81). Transcriptome analysis of KAR₁-treated seed imbibed in light showed a significant enrichment of light-regulated genes among KAR₁-induced transcripts. Although a light pulse was not required for all KAR₁ transcriptional responses, it was required or had a positive influence on KAR₁ response for at least some genes (81). Similarly, light treatment strongly enhanced induction of *GA3ox1* by KAR₁ in seeds (82). KAR₁ and KAR₂ 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). KAR₁ also reduced hypocotyl elongation under continuous red light in lettuce and *B. tournefortii*. In these species, KAR₁ inhibited growth of dark-grown hypocotyls, although this effect on growth was much stronger in the light (82). Thus, KAR₁ 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 KAR₁ during seed imbibition, and 54% of KAR₁-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, *hyb* 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 KAR₁ 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 KAR₁ with at least a 2-fold change in expression, and only 157 had a ≥ 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.

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

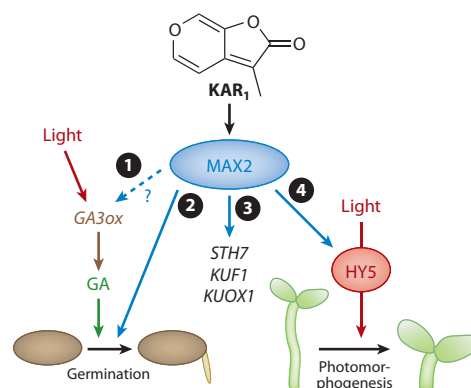


Figure 3

Diagram of currently known karrikin action in *Arabidopsis thaliana*. ① KAR₁ requires both gibberellin (GA) biosynthesis and light to stimulate *Arabidopsis* seed germination, and upregulates expression of *GA3-oxidase* (*GA3ox*) genes. *GA3ox1* induction by KAR₁ 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).

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- μ M KAR₁ (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 2k**), 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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Contents

There Ought to Be an Equation for That <i>Joseph A. Berry</i>	1
Photorespiration and the Evolution of C ₄ Photosynthesis <i>Rowan F. Sage, Tammy L. Sage, and Ferit Kocacinar</i>	19
The Evolution of Flavin-Binding Photoreceptors: An Ancient Chromophore Serving Trendy Blue-Light Sensors <i>Aba Losi and Wolfgang Gärtner</i>	49
The Shikimate Pathway and Aromatic Amino Acid Biosynthesis in Plants <i>Hiroshi Maeda and Natalia Dudareva</i>	73
Regulation of Seed Germination and Seedling Growth by Chemical Signals from Burning Vegetation <i>David C. Nelson, Gavin R. Flematti, Emilio L. Gbisalberti, Kingsley W. Dixon, and Steven M. Smith</i>	107
Iron Uptake, Translocation, and Regulation in Higher Plants <i>Takanori Kobayashi and Naoko K. Nishizawa</i>	131
Plant Nitrogen Assimilation and Use Efficiency <i>Guohua Xu, Xiaorong Fan, and Anthony J. Miller</i>	153
Vacuolar Transporters in Their Physiological Context <i>Enrico Martinoia, Stefan Meyer, Alexis De Angeli, and Réka Nagy</i>	183
Autophagy: Pathways for Self-Eating in Plant Cells <i>Yimo Liu and Diane C. Bassham</i>	215
Plasmodesmata Paradigm Shift: Regulation from Without Versus Within <i>Tessa M. Burch-Smith and Patricia C. Zambryski</i>	239
Small Molecules Present Large Opportunities in Plant Biology <i>Glenn R. Hicks and Natasha V. Raikhel</i>	261
Genome-Enabled Insights into Legume Biology <i>Nevin D. Young and Arvind K. Bharti</i>	283

Synthetic Chromosome Platforms in Plants <i>Robert T. Gaeta, Rick E. Masonbrink, Lakshminarasimban Krishnaswamy, Changzeng Zhao, and James A. Birchler</i>	307
Epigenetic Mechanisms Underlying Genomic Imprinting in Plants <i>Claudia Köhler, Philip Wolff, and Charles Spillane</i>	331
Cytokinin Signaling Networks <i>Ildoo Hwang, Jen Sheen, and Bruno Müller</i>	353
Growth Control and Cell Wall Signaling in Plants <i>Sebastian Wolf, Kian Hématy, and Herman Höfte</i>	381
Phosphoinositide Signaling <i>Wendy F. Boss and Yang Ju Im</i>	409
Plant Defense Against Herbivores: Chemical Aspects <i>Axel Mithöfer and Wilhelm Boland</i>	431
Plant Innate Immunity: Perception of Conserved Microbial Signatures <i>Benjamin Schwessinger and Pamela C. Ronald</i>	451
Early Embryogenesis in Flowering Plants: Setting Up the Basic Body Pattern <i>Steffen Lau, Daniel Slane, Ole Herud, Jixiang Kong, and Gerd Jürgens</i>	483
Seed Germination and Vigor <i>Loïc Rajjou, Manuel Duval, Karine Gallardo, Julie Catusse, Julia Bally, Claudette Job, and Dominique Job</i>	507
A New Development: Evolving Concepts in Leaf Ontogeny <i>Brad T. Townsley and Neelima R. Sinha</i>	535
Control of <i>Arabidopsis</i> Root Development <i>Jalean J. Petricka, Cara M. Winter, and Philip N. Benfey</i>	563
Mechanisms of Stomatal Development <i>Lynn Jo Pillitteri and Keiko U. Torii</i>	591
Plant Stem Cell Niches <i>Ernst Aichinger, Noortje Kornet, Thomas Friedrich, and Thomas Laux</i>	615
The Effects of Tropospheric Ozone on Net Primary Productivity and Implications for Climate Change <i>Elizabeth A. Ainsworth, Craig R. Yendrek, Stephen Sitch, William J. Collins, and Lisa D. Emberson</i>	637
Quantitative Imaging with Fluorescent Biosensors <i>Sakiko Okumoto, Alexander Jones, and Wolf B. Frommer</i>	663