Acta Physiologiae Plantarum (2018) 40:87
https://doi.org/10.1007/s11738-018-2663-2

REVIEW

Induction of agricultural weed seed germination by smoke and smoke-derived karrikin (KAR_1), with a particular reference to *Avena fatua* L.

Jan Kępczyński[1]

Received: 3 August 2017 / Revised: 6 April 2018 / Accepted: 6 April 2018 / Published online: 16 April 2018

Abstract
Plant-derived smoke, its water extract—the smoke water (SW), and karrikin (KAR_1) present in the smoke stimulate seed germination in plants from fire-prone and fire-free areas, including weeds and cultivated plants. There are also plants, the seeds of which can respond only to smoke, but not to KAR_1, and vice versa. Smoke and/or KAR_1 can be applied in horticulture, agriculture, and revegetation. This review describes effects of smoke and KAR_1 on weed seed germination and focuses mainly on the recent knowledge about the physiological role of these factors in dormancy release and germination of *Avena fatua* caryopses. The involvement of gibberellins, ethylene, and abscisic acid (ABA) in the response to smoke or KAR_1 is discussed. Effects of smoke or KAR_1 on the contents of reactive oxygen species (ROS), non-enzymatic antioxidants, and activity of the enzymes participating in ROS removal are presented. Cell cycle activity in the response to SW and KAR_1 is also considered. Effects of KAR_1 on thermodormancy release in *A. fatua* caryopses are highlighted, as well.

Keywords *Avena fatua* · Caryopsis · Dormancy · Floret · Gibberellin · Karrikin · Weed

Introduction

Viable seeds of numerous plant species are not capable of germinating immediately after harvest under conditions suitable for the germination process. Such seeds are termed primarily dormant. Primary dormancy is established during seed development and maturation on the mother plant. This type of dormancy is particularly common in wild plants. Dormancy is a very important phenomenon which prevents germination on mother plants, facilitates seed dispersal, ensures plant survival of natural catastrophes, and reduces intra-specific competition (Bewley et al. 2013). The phenomenon has turned out to be non-obligatory, its expression depending on environmental factors, e.g., temperature. Seeds can be fully dormant; such seeds are not able to germinate at any temperature (Hilhorst 2007). There are also seeds which are not capable of germination only within a certain temperature range, whereas they germinate at temperatures outside that range. Thus, the expression of dormancy in such seeds depends on temperature, and dormancy release is associated with widening the range of germination temperature. Under natural conditions, primarily dormant seeds are exposed to fluctuating environmental conditions, e.g., light, temperature, moisture, and the presence of gases, which leads to dormancy state cyclicity (Finkelstein et al. 2008). Primary dormancy can be removed also by cold stratification, dry storage, light, or chemicals (Bewley et al. 2013).

It is commonly accepted that the balance between abscisic acid (ABA) and gibberellins (GAs) and/or sensitivity to these hormones are responsible for regulation of the dormancy state and germination of seeds in response to environmental signals (Finkelstein et al. 2008; Rodríguez-Gacio et al. 2009). ABA is considered as the most important hormone responsible for the establishment of dormancy during seed development and for maintenance of dormancy during seed imbibition. In turn, GAs have been cited as factors involved in dormancy release and/or germination. Dormancy release has been shown as involving a decline in the ABA content and an increase of the GAs level (Bewley et al. 2013). Participation of ABA and GAs in the regulation

Communicated by S. Srivastava.

✉ Jan Kępczyński
jankepcz@wp.pl

[1] Department of Plant Physiology and Genetic Engineering, Faculty of Biology, University of Szczecin, Wąska 13, 71-415 Szczecin, Poland

of dormancy state has been demonstrated in experiments involving their application. Exogenous GA_3 is able to stimulate germination of dormant seeds of many plant species. In contrast, exogenous ABA increases the dormancy depth. Other hormones, e.g., ethylene, cytokinins, and brassinosteroids, have been reported to be involved in releasing seed dormancy and germination (for a review, see Gubler et al. 2005; Finkelstein et al. 2008; Hilhorst 2007). In addition, non-hormonal compounds such as reactive oxygen species (ROS) play an important role during the whole seed lifespan, from embryogenesis to germination (El-Maarouf-Bouteau and Bailly 2008). ROS can have a detrimental effect or can serve a key signaling function in dormancy release and germination. Germination can be possible only when endogenous concentration of ROS reaches a specific suitable level. Several ROS, such as the superoxide anion (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical (OH), are involved in regulation of dormancy release and germination of various seeds (El-Maarouf-Bouteau and Bailly 2008). Application of exogenous ROS or ROS-generating compounds can break dormancy in seeds of several plant species. An adequate level of ROS depends on their production and scavenging by the enzymatic system and non-enzymatic compounds. The enzymatic system includes superoxide dismutase (SOD), catalase (CAT), peroxidases, glutathione reductase (GR), monodehydroascorbate reductase (MDHAR), and non-enzymatic compounds such as reduced glutathione and ascorbate. ROS have been found to be able to cooperate with ABA, gibberellins, and ethylene in the control of dormancy release and seed germination (Diaz-Vivancos et al. 2013; Corbineau et al. 2014). Once dormancy has been released, the seeds can germinate if the conditions are suitable for the process. The process terminates with radicle protrusion through the external seed envelopes, which ends the sensu stricto germination (phase II) and begins growth (phase III). Radicle protrusion can be accomplished by elongation of the existing cells or may be preceded by cell division and new cell growth (Baíza et al. 1989; de Castro et al. 2000; Barrôco et al. 2005; Masubelele et al. 2005; Gendreau et al. 2008). The cell cycle requires β-tubulin, a microtubular cytoskeleton component. β-tubulin accumulation has been shown to precede, or to occur simultaneously with, DNA replication (Górnik et al. 1997; Śliwińska et al. 1999). De Castro et al. (2001) demonstrated dormant imbibed tomato seeds to remain at the G1 phase; dormancy release was accompanied by accumulation of β-tubulin and induction of DNA replication before radicle protrusion. In barley grains, expression of dormancy coupled with the cell cycle being blocked at the S phase (Gendreau et al. 2012).

Biological activity of the plant-derived smoke

Numerous data indicate that fires can stimulate seed germination of many plant species in the fynbos (South Africa), chaparral (Southern California), kwongan (Australia), and in Mediterranean areas (Light and Van Staden 2004). The stimulatory effect of fire may be associated with influence exerted by different physical and chemical factors. High temperature generated by fire may remove seed coat dormancy by producing coat structure scarification, or it can stimulate the embryo directly. Fire also produces plant-derived smoke which can, alone or in conjunction with heat, promote the dormancy release in seeds. De Lange and Boucher (1990) were the first to report that smoke induces seed germination. Subsequent research revealed that smoke breaks dormancy in seeds of several fynbos species which are dominant in the Cape Floristic region (South Africa). Seeds can be treated by direct exposure to smoke, by incubation of untreated seeds on sand, or by imbibition in aqueous smoke extract, the smoke water (SW). The latter is prepared by combusting plant material and bubbling the smoke through the water. A very important finding was that SW is as effective as smoke itself, and that biological activity was independent of plant material used for smoke generation. SW is highly active at very high dilutions and can be stored for long periods at room temperature without any loss to its biological activity (Brown and Van Staden 1997). Smoke or SW was found to stimulate seed germination of species from fire-prone areas. Seeds of 1200 species in more than 80 genera were demonstrated to positively respond to smoke (Dixon et al. 2009). Likewise, dormant and non-dormant seeds from fire-free environments, including agricultural weeds and even crop plants, such as lettuce, celery, tomato, and maize, turned out to be sensitive to smoke, as well. The high biological activity of SW had made it possible to use it in commercial products. African scientists developed a seed primer, marketed as the "Kirstenbosch Instant Smoke Plus Seed Primer", which incorporates aqueous smoke extracts and a mixture of other natural germination stimulators (Brown and Van Staden 1997). Australian studies developed a commercial product named the "Seed Starter, Australian Smoky Water". Smoke or SW treatment is widely used not only in plant propagation, but also in ecological restoration (Nelson et al. 2012).

Biological activity of KAR_1

For many years, scientists have been looking for chemical(s) responsible for the stimulatory effect of plant-derived smoke. Since such smoke contains ammonia,

ethylene, and nitric oxide, it was logical to expect one or more compounds in smoke to be responsible for breaking seed dormancy (Nelson et al. 2012). However, although the compounds mentioned can remove dormancy in seeds of many species responsive to smoke, there are also seeds which do not respond to these compounds, but are nevertheless sensitive to smoke. Thus, it was concluded that smoke produces a specific signal(s) inducing germination. However, as smoke contains several thousand compounds (Maga 1988), it was difficult to isolate the active component(s) from smoke. As it turned out, over 200 compounds extracted from SW did not affect seed germination. Although the role of smoke in germination of dormant and non-dormant seeds, and in seedling growth, has been extensively studied since 1990, it was only in 2004 that a germination-active compound, 3-methyl-2*H*-furo[2,3-*c*] pyran-2-one was identified in plant-derived smoke (Van Staden et al. 2004) and burnt cellulose (Flematti et al. 2004). Initially, this compound was termed butenolide; later on, to distinguish the butenolide present in smoke from other butenolides, it was named karrikinolide or karrikin-1 (KAR_1) (Flematti et al. 2009; Fig. 1), derived from the word "karrik" meaning smoke in the Australian Aboriginal language. KAR_1 has been demonstrated to be active at very low concentrations, in the range of 10^{-10}–10^{-7} M. It was found to be neither toxic nor genotoxic at 3×10^{-10}–10^{-4} M (Light et al. 2009), and thus, it is safe for animals and humans. KAR_1 is produced from D-xylose during fire, in small amounts (Flematti et al. 2015; Fig. 2). Due to a huge demand for the compound in research and on account of its potential application in agriculture, several methods of synthesis using D-xylose or other compounds as substrates have been developed (Fig. 2). KAR_1 was shown to stimulate seed germination in fire-prone environments (Flematti et al. 2004; Merritt et al. 2006), in hemi- and holo-parasitic seeds (Daws et al. 2007), and in several Australian *Asteraceae* species (Merritt et al. 2006). Likewise, seeds of some crop plants such as lettuce, tomato, okra, bean, maize, and rice responded positively to KAR_1 (Kulkarni et al. 2011). The compound is able to increase both the rate and percentage of seed germination; it also improves seedling growth. Moreover, and importantly, KAR_1 allows seeds to germinate at sub- and supra-optimal temperatures, and also at a low water potential (Light et al. 2009). It can be used as a priming agent, e.g., for tomato seeds. The studies on karrikin structure–activity relationship demonstrated that the methyl group at C-3 is important for biological activity: introduction of methyl at C-4 or C-7 reduces the activity, introduction at C-5 being tolerated well (Nelson et al.

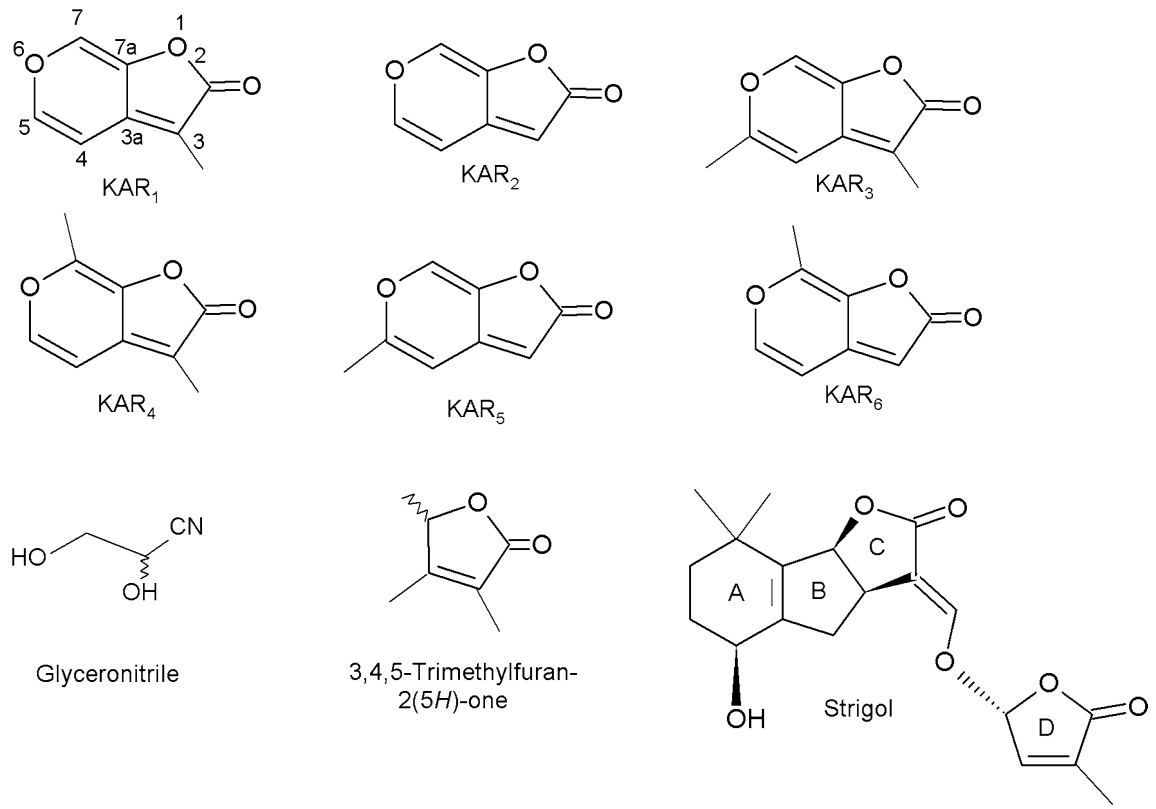

Fig. 1 Structure of karrikins, glyceronitrile, 3,4,5-trimethylfuran-2(5*H*)-one, and strigol

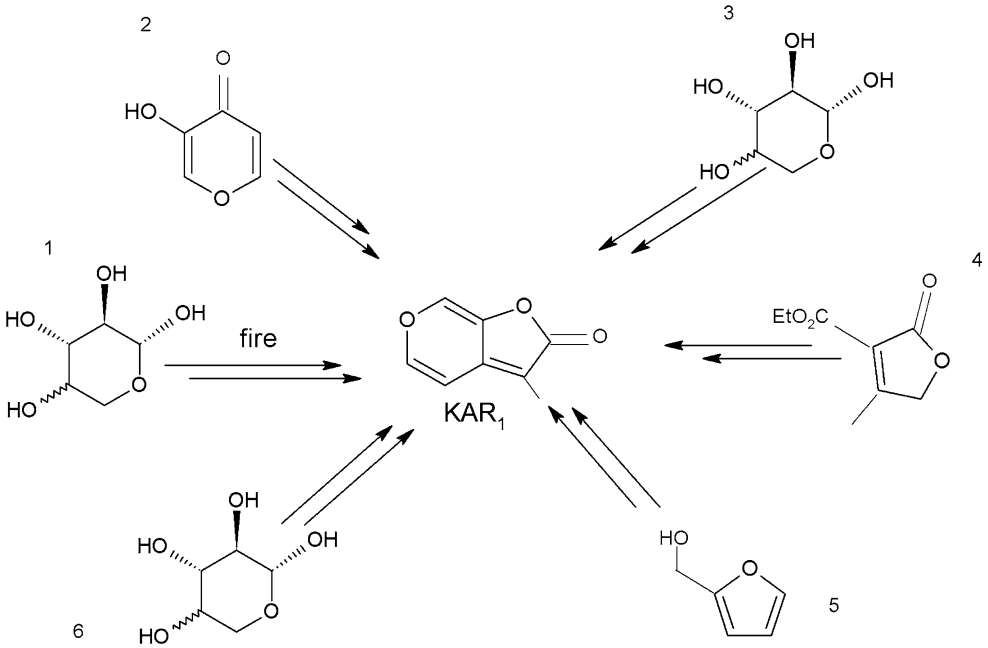

Fig. 2 Synthesis of KAR_1 from various substrates. Karrikin can be produced by burning sugars such as xylose, and thus, the pyran ring is probably derived from such pyranose sugar (Flematti et al. 2015). **1** D-xylose (Flematti et al. 2015), **2** pyromeconic acid (Flematti et al. 2005), **3** D-xylose (Goddard-Borger et al. 2007), **4** ethyl-4-methyl-2-oxo-2,5-dihydro-furan-3-carboxylate (Sun et al. 2008), **5** 2-furfurylmethanol (Nagase et al. 2008), and **6** D-xylose (Matsuo and Shindo 2011)

2012). Subsequent studies led to detection of five KAR_1 analogs, KAR_2–KAR_6, in smoke (Flematti et al. 2009; Fig. 1). Concentrations of these compounds were much lower than those of KAR_1. Thus, KAR_1 was considered to be the major factor responsible for the stimulatory effect of smoke on seed germination. The comparison of KAR_1 effects on seed germination with those exerted by other karrikins revealed different, plant species-specific responses to these compounds (Waters et al. 2014).

Responses of weed seeds to smoke and KAR_1

Seeds of many weed species may persist in agricultural soils for as long as several years, because they are dormant and, therefore, incapable of germinating after they fall on the ground. Weed seeds even from one harvest may be in different dormancy states; they can germinate during several years, making it difficult to control a weed population. Annual application of herbicides for crop protection is costly and may bring up herbicide resistance in some weeds. Moreover, multiple applications of herbicides pollute the environment. A more secure and promising strategy of weed population control is to break dormancy and stimulate germination of all the seeds in the soil seed bank (Dyer 1995). Following such treatment, the emerging seedlings can be removed, manually or mechanically, or destroyed by a single herbicide application. Thus, such a strategy could prove useful in depleting the agricultural soil weed seed bank. A better understanding of dormancy may result in developing new, or in improving the existing, strategies of weed control. Smoke and KAR_1 are effective agents in stimulating seed germination of a broad spectrum of both monocotyledonous and dicotyledonous weed species. Studies of effects of commercially available "Seed Starter" on seed germination in several arable weeds demonstrated smoke to strongly stimulate seeds of three monocot (*Alopecurus myosuroides, Avena sterilis,* and *Phalaris paradoxa*) and one dicot (*Malva neglecta*) species (Adkins and Peters 2001). In subsequent studies, responses of seeds of weed species from non-fire-prone environments to SW and KAR_1 were compared (Stevens et al. 2007; Daws et al. 2007; Kępczyński et al. 2010; Table 1). The percentage seed germination of seven plant species was increased by both SW and KAR_1. There were also species the seeds of which were not sensitive to smoke or KAR_1, but responded positively to KAR_1 or SW, respectively. The seed germination in response to smoke but not to KAR_1 (Table 1) is, perhaps, associated with response to cyanide, since the cyanohydrin glyceronitrile, a seed germination stimulant which releases cyanide after hydrolysis, was detected in smoke (Nelson et al. 2012; Fig. 1). The inhibitory effect of SW on germination of seeds which respond positively to KAR_1 was also noted. This effect of SW is probably related to toxic or germination-inhibiting compound(s) in smoke. The compound 3,4,5-timethylfuran-2(5*H*)-one, identified in smoke, was shown to have germination-inhibiting activity at 10–100 µM, and to counteract the stimulatory effect of KAR_1 in lettuce seeds (Light et al. 2010; Fig. 1). The very high activity of KAR_1, when applied at very low concentrations, makes it possible to control weeds in soil. The potential benefits of KAR_1 as a weed control agent were demonstrated by Stevens et al. (2007). They showed that a single application of KAR_1 to the soil surface at rates as low as 2–20 g/ha was sufficient to promote

Table 1 Responses of weed seeds to smoke water and KAR_1

Family	Species	Smoke water	KAR_1	References
Amaranthaceae	*Chenopodium album*	+	0	1
Asteraceae	*Chrysanthemum segetum*	+	+	1
	Senecio jacobinae	0	+	1
Boraginaceae	*Echium plantagineum*	+	+	2
Brassicaceae	*Brassica tournefortii*	+	+	2
	Capsella bursapastoris	+	0	1
	Raphanus raphanistrum	+	+	2
	Sinapis alba	0	+	1
	Sisymbrium orientale	-	+	2
Caryopbyllaceae	*Stellaria media*	+	+	1
Malvaceae	*Malva neglecta*	0	+	1
Papaveraceae	*Papaver rhoeas*	0	+	1
Poaceae	*Avena fatua*	-	+	1
	A. fatua	+	+	2
	A. fatua	+	+	3
	Bromus sterilis	−	0	1
	Hordeum leporinum	+	+	2
	Sorghum halepense	0	+	1

1—Daws et al. (2007), 2—Stevens et al. (2007), 3—Kępczyński et al. (2010)

0 No significant effect

+ Stimulation

− Inhibition

emergence of *Arctotheca calendula*, *Brassica tournefortii,* and *Raphanus raphanistrum.* Thus, the discovery of a high response of weed seeds to KAR_1 generates new opportunities for weed control in agricultural soils. Very important from the practical point of view were data reported by Long et al. (2010) who showed that fully imbibed, or fully imbibed, and re-dried, *Brassica tournefortii* seeds were less sensitive to KAR_1 than dry ones. Thus, it can be concluded that, under field conditions, KAR_1 should be applied prior to the rainfall.

Responses of *Avena fatua* primarily dormant florets and caryopses to smoke and KAR_1

Germination

Smoke and KAR_1

Avena fatua is an annual weed, very important economically, which infests most major cereals: wheat, barley, oats, rye, and flax, worldwide (Simpson 2007). Information on the role of smoke and/or KAR_1 with respect to caryopses and florets (caryopses with lemma and palea) of *A. fatua* is more extensive than information concerning seeds of other weeds. Except for some lines, freshly harvested florets or caryopses of *A. fatua* cannot germinate because of their dormancy. Several dormancy mechanisms existing in *A. fatua* florets may be associated with the hulls, pericarp, testa, and embryo (Simpson 2007). Up to ca. 40–50% caryopses from florets of *A. fatua* collected in 2007–2010 in Poland were able to germinate at 15 °C, for which reason they were regarded as partially dormant (Kępczyński et al. 2010, 2013; Cembrowska-Lech and Kępczyński 2017). At 20–35 °C, the caryopsis germination was almost completely or completely inhibited, dormancy being expressed at this temperature range. Thus, the primary dormancy of *A. fatua* caryopses can be regarded as relative, depending on the imbibition temperature.

Smoke was found to stimulate or inhibit, depending on the concentration and germination of caryopses (Adkins and Peters 2001). Not only caryopses, but also florets responded positively to SW (Kępczyński et al. 2006); however, caryopses proved more sensitive (Adkins and Peters 2001; Kępczyński et al. 2006). Adkins and Peters (2001) observed that freshly harvested florets were less sensitive to smoke than those partially after-ripened under room conditions for 8–12 weeks. A stimulatory effect of SW was observed when caryopses from different biotopes in Australia, Canada, England, United States of America (Adkins and Peters 2001), and Poland (Kępczyński et al. 2006) were used. *A. fatua* caryopses are also sensitive to KAR_1. The compound stimulated germination of partly dormant *A. fatua* from Australia, resulting in ca. 30% higher germination rate, compared to untreated seeds (Stevens et al. 2007). Both SW

and KAR_1 were found to be very effective in removing dormancy of caryopses at 20 and 25 °C, since all or almost all the caryopses were able to germinate, compared to ca. 10% of untreated caryopses (Kępczyński et al. 2010). At 30 °C, both SW and KAR_1 stimulated germination only slightly, KAR_1 being somewhat more effective. The continuous presence of KAR_1 is not necessary for improving germination of dormant caryopses at 20 °C, but just a 24 h preincubation was sufficient to germinate almost all the caryopses (Kępczyński et al. 2010). The KAR_1 sensitivity of caryopses decreased during prolonged preincubation in water. Dormant caryopses are more responsive to both SW and KAR_1 than dormant florets (Kępczyński et al. 2010; Cembrowska-Lech and Kępczyński 2017). Studies conducted by Stevens et al. (2007) showed KAR_1 to be capable of stimulating also the seedling emergence of *A. fatua* under field conditions.

Tricyclic butenolides

The discovery of KAR_1 and its high biological activity stimulated studies on synthesis of ca. 50 analogs (Nelson et al. 2012). In one of the studies (Krawczyk et al. 2017), tricyclic butenolides (KAR_1 with an additional aromatic ring or an aromatic ring without the methyl group at the C3 position of karrikin) were applied to dormant and non-dormant *A. fatua* caryopses. A low stimulatory effect on germination of dormant caryopses was shown only by those butenolides without the methyl group. Like KAR_1, they accelerated germination of non-dormant caryopses, but only when applied at concentrations much higher than KAR_1. All tricyclic butenolides increased also the root growth.

Gibberellins and abscisic acid

Effects of GA_3 and smoke or KAR_1 on *A. fatua* seeds from different areas were presented during the past years (Adkins and Peters 2001; Daws et al. 2007; Stevens et al. 2007). The combined action of GA_3 and SW was observed to enhance caryopsis germination (Kępczyński et al. 2006). Both florets and caryopses responded to GA_3, but the response to KAR_1 was much stronger (Kępczyński et al. 2013). A similar stimulatory effect was observed when concentrations of KAR_1 1000–10,000 times lower than those of GA_3 were applied. The caryopsis sensitivity to SW, KAR_1, and GA_3 was found to gradually increase over time of dry storage (Kępczyński et al. 2013; Cembrowska-Lech and Kępczyński 2017). The stimulatory effect of smoke on germination of dormant caryopses was completely antagonized by paclobutrazol, a gibberellin biosynthesis inhibitor. GA_3 reversed the paclobutrazol-induced inhibition when used alone or in combination with SW (Cembrowska-Lech and Kępczyński 2017). Paclobutrazol as well as ancymidol and flurprimidol, the other gibberellin biosynthesis inhibitors, inhibited also the effect of KAR_1; this inhibition was, too, reversed by GA_3 (Kępczyński et al. 2013). These results indicate that endogenous gibberellins play an important role in the caryopsis response to smoke and KAR_1. Both KAR_1 and GA_3 were also found to reduce the embryo's ABA content at the beginning of phase II of imbibition, i.e., 10 and 12 h, before coleorhizae emergence and radical protrusion, respectively (Cembrowska-Lech et al. 2015; Cembrowska-Lech and Kępczyński 2016). These data provide evidence that induction of dormancy release by KAR_1 and GA_3 involves control on the ABA level. Dormancy release by KAR_1 was accompanied by degradation of ABA to phaseic acid (Cembrowska-Lech and Kępczyński 2016).

Ethylene

Ethylene is known as a stimulant of germination of dormant and non-dormant seeds (Kępczyński and Kępczyńska 1997; Matilla and Matilla-Vazquez 2008; Corbineau et al. 2014). The use of ethylene biosynthesis inhibitors and their action in seeds of numerous species showed endogenous ethylene to participate in dormancy release and germination. It had been earlier shown that ethephon, an ethylene-liberating compound, did not affect germination of dormant *A. fatua* florets, but enhanced germination of partially afterripened (Adkins and Ross 1981). In another experiment, ethephon, ethylene, ACC, a precursor of its biosynthesis, almost did not affect the germination of dormant *A. fatua* caryopses (Kępczyński and Van Staden 2012), indicating that, unlike KAR_1, ethylene alone, was unable to remove dormancy. However, 2,5-norbornadiene (NBD), a competitive, reversible inhibitor of ethylene binding to its receptor, as well as 1-MCP, a non-reversible inhibitor, counteracted the stimulatory effect of KAR_1 on dormant caryopses, providing a strong indication that ethylene action is necessary for dormancy release and germination. Counteraction of NBD inhibitory effect by exogenous ethylene confirmed the importance of ethylene binding to its receptor for germination of dormant caryopses. α-Aminoisobutyric acid (AIB) and aminoethoxyvinylglycine (AVG), ethylene biosynthesis inhibitors, did not change KAR_1 effect to any significant degree (Kępczyński and Van Staden 2012). However, AVG was shown to potentiate the inhibitory effect of NBD and to lower the germination ability after the caryopses were transferred from the NBD atmosphere to air. These experiments with inhibitors suggest that a certain level of endogenous ethylene is necessary for its action, which is required to release dormancy and germination by KAR_1.

Coleorhiza and radicle protrusion

Caryopsis hydration is known to be essential for dormancy release and germination. Water uptake during imbibition

of dormant caryopses takes place during two phases only. SW, KAR_1, and GA_3 were observed to increase albeit very slightly, the water uptake rate during phase I (Cembrowska and Kępczyński 2016, 2017). Both KAR_1 and GA_3 increased the embryo size, the number of caryopses with coat rupture, and the coleorhiza length in phase II; they also stimulated coleorhiza emergence (Cembrowska-Lech and Kępczyński 2016). Finally, SW, KAR_1, and GA_3 induced coleorhiza protrusion by the radicle. Thus, only the treated caryopses could enter phase III which involves an increased water uptake, and with a progressive increase in the percentage of coleorhizae emergence and radicle protrusion. Caryopses with radicles protruding over the coleorhiza were regarded as germinated, which is a consequence of dormancy release.

ROS-antioxidant status

Hydrogen peroxide (H_2O_2) was observed to induce germination of dormant *A. fatua* caryopses (Cembrowska-Lech et al. 2015) and seeds of other species (El-Maarouf-Bouteau and Bailly 2008; Diaz-Vivancos et al. 2013). Likewise, inhibition of catalase activity by aminotriazole (AT) stimulated germination of dormant *A. fatua* caryopses, possibly via an increase in the H_2O_2 content (Cembrowska-Lech et al. 2015). Both the endogenous H_2O_2, the content of which was probably increased by AT, and the exogenous H_2O_2 reduced the ABA content in embryos from caryopses to a level similar to that produced by KAR_1 and GA_3. Thus, dormancy removal by H_2O_2, KAR_1, and GA_3 is associated, at least in part, with a reduction in the ABA content. Moreover, menadione (MN) and methylviologene (MV), O_2^--generating compounds, were also able to stimulate germination. It was, therefore, concluded that ROS are involved in the regulation of dormancy state in caryopses. H_2O_2 seems to be important for the response of caryopses to KAR_1 and GA_3, since DPI, an NADPH oxidase inhibitor catalyzing its apoplastic production, reduced effects of KAR_1 and GA_3 on germination. Both KAR_1 and GA_3 increased the contents of H_2O_2 and O_2^- and the activities SOD and CAT in embryos, indicating that ROS homeostasis is probably required for dormancy release and germination of caryopses. Moreover, KAR_1 and GA_3 control the AsA-GSH cycle (Cembrowska-Lech and Kępczyński 2016), suggesting their participation in germination of dormant caryopses. Both compounds increased the contents of ascorbate and dehydroascorbate, and reduced those of glutathione and oxidized glutathione. They also induced an additional activity of ascorbate peroxidase isoenzyme and glutathione reductase (Cembrowska-Lech and Kępczyński 2016). The ROS-antioxidant status is controlled by KAR_1 and GA_3 not only in embryos, but also in aleurone layers. In those layers, the two compounds were observed to progressively increase the H_2O_2 and O_2^- contents associated with decreasing activities of superoxide dismutase and catalase from the beginning of phase II, thus preparing the cells to death.

The cell cycle activity

It was earlier reported that DNA replication was inhibited during imbibition of *A. fatua* dormant caryopses (Elder and Osborne 1993). The flow cytometry analysis showed cells in the radicle tips of dormant, dry and imbibed caryopses to be mostly arrested at the G1 phase (Cembrowska-Lech and Kępczyński 2016, 2017). SW was found to reduce the percentage of nuclei at the G1 phase, the percentage being increased at the S and G2 phases (Cembrowska-Lech and Kępczyński 2017). The cell cycle activation occurred 4 and 6 h before coleorhiza emergence and radicle protrusion, respectively. Like SW, also KAR_1 and GA_3 activated the cell cycle (Cembrowska-Lech and Kępczyński 2016).The initiation of the process occurred 6 and 4 h before radicle protrusion when KAR_1 and GA_3, respectively, were applied. A densitometric analysis of β-tubulin demonstrated accumulation of the protein to precede or to coincide with DNA replication in the radicle tip of KAR_1- or GA_3-treated *A. fatua* caryopses, respectively (Cembrowska-Lech and Kępczyński 2016). All the data referred to above indicate that induction of dormancy release and germination of *A. fatua* caryopses by SW, KAR_1, and GA_3 involves activation of the cell cycle.

Response of *Avena fatua* secondary dormant caryopses to KAR_1

After a prolonged incubation under conditions not amenable for germination, seeds with some degree of primary dormancy, or non-dormant seeds, may enter the secondary dormancy (Kępczyński and Kępczyńska 2003; Hilhorst 2007). There are examples of secondary dormancy induction in seeds of several plant species by anaerobic conditions, darkness, light, water stress, and temperature sub-optimal or supra-optimal for germination (Kępczyński and Kępczyńska 2003). It was earlier demonstrated that secondary dormancy can be induced by anoxia in dormant or partially after-ripened *A. fatua* caryopses (Symons et al. 1987). Likewise, the temperature above 25 °C induced secondary dormancy, termed the thermodormancy. This state has been recently shown to be induced in dormant *A. fatua* caryopses by imbibition at 30 °C, a temperature supra-optimal for germination (Ruduś and Kępczyński 2017). Thermodormancy in caryopses was markedly or completely relieved at 10 °C by KAR_1 or GA_3, respectively. At 20 °C, the stimulatory effect of both compounds was similar and lower than that at 10 °C. A comparison of effects of KAR_1 and GA_3 on germination of primarily dormant and secondarily dormant caryopses indicates a decreased responsiveness of the latter. The response

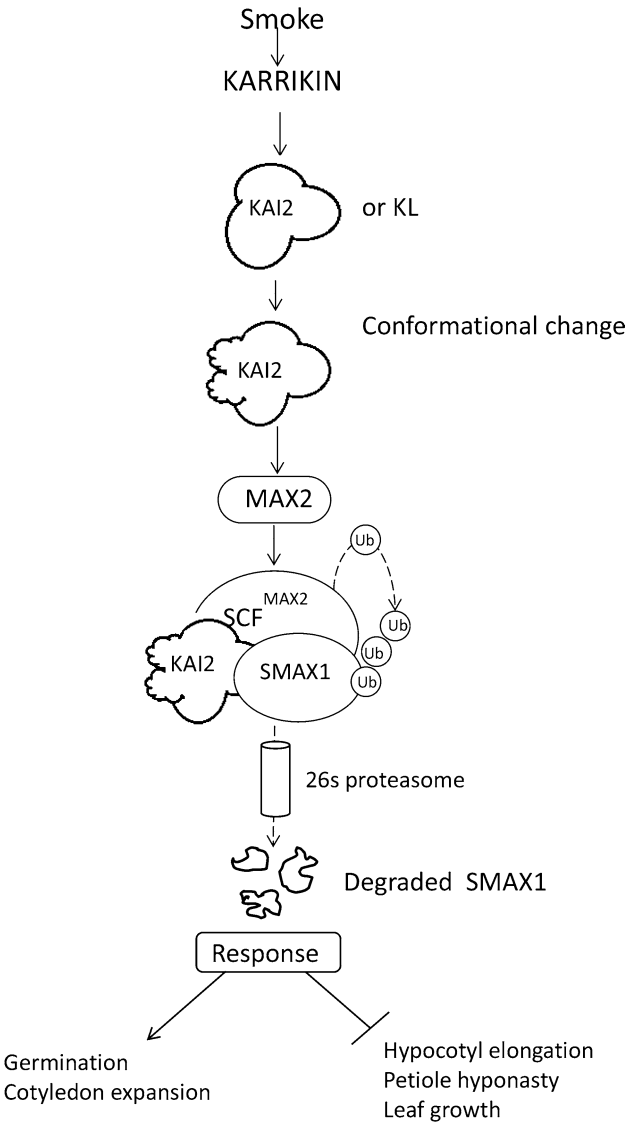

Fig. 3 Proposed mechanism of KAR_1 signaling in *Arabidopsis thaliana* (Nelson et al. 2012; Waters et al. 2014; Morffy et al. 2016). KAR or KL causes conformational change of KAI2. This conformational change enables interaction with MAX2 and the formation of SCF^{MAX2}–KAI2-SMAX1 complex. SMAX1 undergoes polyubiquitinylation and then is degraded by proteasome. Finally, the expression of KAR response genes is possible. *KAI2* (KARRIKIN INSENSITIVE2)-alpha/beta hydrolases, KAR receptor; *KL* putative KAI2 ligand; *MAX2* MORE AXILLARY GROWTH2, KAR response repressor; *SMAX1* SUPPRESSOR OF MAX2

of thermodormant caryopses to both KAR_1 and GA_3 was increased by putrescine (Ruduś and Kępczyński 2017).

Summing up and perspectives

Plant-derived smoke and smoke water were found to stimulate germination of several plant species form fire-prone and fire-free zones. The detection of KAR_1 in the smoke created a new opportunity for using it to promote germination of seeds sensitive to smoke as well as the non-responsive ones. It is very important to explore a mechanism of dormancy release and caryopsis germination in *A. fatua*, a very serious pest weed in agricultural ecosystem in numerous areas, including Poland. KAR_1 turned out to require endogenous gibberellins, ethylene action, and probably degradation of ABA for its stimulating effect to be expressed. The mechanism of KAR_1-induced dormancy release and caryopsis germination involves regulation of ROS-antioxidant status both in embryos and in aleurone layers and the cell cycle activity. However, the mechanism underlying regulation of KAR_1-induced dormancy release and germination in *A. fatua* caryopses is still poorly understood. So far, information on the mechanism of karrikin effects at the molecular level has been provided by research on *Arabidopsis thaliana* (Waters et al. 2014; Mindrebo et al. 2016; Morffy et al. 2016; Li et al. 2017; Fig. 3). A highly similar signaling mechanism of karrikins, found in smoke, and strigolactones, synthesized in plants, both containing 3-methyl-butenolide ring, has been recognized. Moreover, the signaling pathways of karrikins and strigolactones remind those of GA_s where the receptors bind to FBOX proteins in the SCF E3 ubiquitin–ligase complexes to regulate the degradation of transcriptional repressors (Mindrebo et al. 2016). It is also necessary to find out whether KAR_1 can control the expression of gibberellins biosynthesis and catabolism genes, as found in experiments involving *Arabidopsis* seeds (Nelson et al.2009), and also ethylene pathways. It would be also interesting to resolve whether KAR_1 is responsible for controlling the enzymes most likely weakening the coleorhiza, which allows the radicle to break outside.

Author contribution statement JK prepared review article on basis of last original articles.

Acknowledgements This paper is dedicated to the memory of my father, Professor Klemens Kępczyński, Ph.D., D.Sc., a botanist and phytosociologist. It is to my father that I owe inspiration to study physiology of caryopses of *Avena fatua*, a very interesting weed commonly found in Poland. I am indebted to Professor Teresa Radziejewska for linguistic assistance. Chapters related to *Avena fatua* were prepared mainly on basis original papers supported by the Ministry of Science and Higher Education Grant NN 310 726340.

References

Adkins SW, Peters NCB (2001) Smoke derived from burnt vegetation stimulates germination of arable weeds. Seed Sci Res 11:213–222. https://doi.org/10.1079/SSR200177

Adkins SW, Ross JD (1981) Studies in wild oat seed dormancy. Plant Physiol 67:358–362. https://doi.org/10.1104/pp.67.2.358

Baíza AM, Vázquez-Ramos M, Sánchez de Jiménez E (1989) DNA synthesis and cell division in embryonic maize tissues during germination. J Plant Physiol 135:416–421. https://doi.org/10.1016/S0176-1617(89)80097-5

Barrôco RM, Van Poucke K, Bergervoet JHW, De Veylder L, Groot SPC, Inze D, Engler G (2005) The role of the cell cycle machinery in resumption of postembryonic development. Plant Physiol 137:127–140. https://doi.org/10.1104/pp.104.049361

Bewley JD, Bradford KJ, Hilhorst HWM, Nonogaki H (2013) Seeds: physiology of development, germination and dormancy. Springer, New York. https://doi.org/10.1007/978-1-4614-4693-4

Brown NAC, Van Staden J (1997) Smoke as a germination cue: a review. Plant Growth Regul 22:115–124. https://doi.org/10.1023/A:1005852018644

Cembrowska-Lech D, Kępczyński J (2016) Gibberellin-like effects of KAR_1 on dormancy release of *Avena fatua* caryopses include participation of non-enzymatic antioxidants and cell cycle activation in embryos. Planta 243:531–548. https://doi.org/10.1007/s00425-015-2422-1

Cembrowska-Lech D, Kępczyński J (2017) Plant-derived smoke induced activity of amylases, DNA replication and β-tubulin accumulation before radicle protrusion of dormant *Avena fatua* L. caryopses. Acta Physiol Plant 39:39. https://doi.org/10.1007/s11738-016-2329-x

Cembrowska-Lech D, Koprowski M, Kępczyński J (2015) Germination induction of dormant *Avena fatua* caryopses by KAR_1 and GA_3 involving the control of reactive oxygen species (H_2O_2 and O_2^-) and enzymatic antioxidants (superoxide dismutase and catalase) both in the embryo and the aleurone layers. J Plant Physiol 176:169–179. https://doi.org/10.1016/j.jplph.2014.11.010

Corbineau F, Xia Q, Bailly C, El-Maarouf-Bouteau H (2014) Ethylene, a key factor in the regulation of seed dormancy. Front Plant Sci 5:539. https://doi.org/10.3389/fpls.2014.00539

Daws MI, Davies J, Pritchard HW, Brown NAC, Van Staden J (2007) Butenolide from plant-derived smoke enhances germination and seedling growth of arable weed species. Plant Growth Regul 51:73–82. https://doi.org/10.1007/s10725-006-9149-8

De Castro RD, van Lammeren AAM, Groot SPC, Bino RJ, Hilhorst HWM (2000) Cell division and subsequent radicle protrusion in tomato seeds are inhibited by osmotic stress but DNA synthesis and formation of microtubular cytoskeleton are not. Plant Physiol 122:327–335. https://doi.org/10.1104/pp.122.2.327

De Castro RD, Bino RJ, Jing HC, Kieft H, Hilhorst HWM (2001) Depth of dormancy in tomato (*Lycopersicon esculentum* Mill.) seeds is related to the progression of the cell cycle prior to the induction of dormancy. Seed Sci Res 11:45–54. https://doi.org/10.1079/SSR200059

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 56:700–703. https://doi.org/10.1016/S0254-6299(16)31009-2

Diaz-Vivancos P, Barba-Espín G, Hernández JA (2013) Elucidating hormonal/ROS networks during seed germination: insights and perspectives. Plant Cell Rep 32:1491–1502. https://doi.org/10.1007/s00299-013-1473-7

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 813:155–170. https://doi.org/10.17660/ActaHortic.2009.813.20

Dyer WE (1995) Exploiting weed seed dormancy and germination requirements through agronomic practices. Weed Sci 43:498–503. https://doi.org/10.1017/S0043174500081534

Elder RH, Osborne DJ (1993) Function of DNA synthesis and DNA repair in the survival of embryos during early germination and in dormancy. Seed Sci Res 3:43–53. https://doi.org/10.1017/S0960258500001550

El-Maarouf-Bouteau H, Bailly C (2008) Oxidative signalling in seed germination and dormancy. Plant Signal Behav 3:175–182. https://doi.org/10.4161/psb.3.3.5539

Finkelstein R, Reeves W, Ariizumi T, Steber C (2008) Molecular aspects of seed dormancy. Annu Rev Plant Biol 59:387–415. https://doi.org/10.1146/annurev.arplant.59.032607.092740

Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD (2004) A compound from smoke that promotes seed germination. Science 305:977. https://doi.org/10.1126/science.1099944

Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD (2005) Synthesis of the seed germination stimulant 3-methyl-*2H*-furo[2,3-*c*]pyran-2-one. Tetrahedron Lett 46:5719–5721. https://doi.org/10.1016/j.tetlet.2005.06.077

Flematti GR, Ghisalberti EL, Dixon KW, Trengove RD (2009) Identification of alkyl substituted *2H*-furo[2,3-*c*]pyran-2-ones as germination stimulants present in smoke. J Agric Food Chem 57:9475–9480. https://doi.org/10.1021/jf9028128

Flematti GR, Dixon KW, Smith SM (2015) What are karrikins and how were they 'discovered' by plants? BMC Biol 13:108. https://doi.org/10.1186/s12915-015-0219-0

Gendreau E, Romaniello S, Barad S, Leymarie J, Benech-Arnold R, Corbineau F (2008) Regulation of cell cycle activity in the embryo of barley seeds during germination as related to grain hydration. J Exp Bot 59:203–212. https://doi.org/10.1093/jxb/erm296

Gendreau E, Cayla T, Corbineau F (2012) S phase of the cell cycle: a key phase for the regulation of thermodormancy in barley grain. J Exp Bot 63:5535–5543. https://doi.org/10.1093/jxb/ers204

Goddard-Borger ED, Ghisalberti EL, Stick RV (2007) Synthesis of the germination stimulant 3-methyl-*2H*-furo[2,3-*c*]pyran-2-one and analogous compounds from carbohydrates. Eur J Org Chem 2007:3925–3934. https://doi.org/10.1002/ejoc.200700334

Górnik K, de Castro RD, Liu Y, Bino RJ, Groot SPC (1997) Inhibition of cell division during cabbage (*Brassica oleracea* L.) seed germination. Seed Sci Res 7:333–340. https://doi.org/10.1017/S0960258500003731

Gubler F, Millar AA, Jacobsen JV (2005) Dormancy release, ABA and pre-harvest sprouting. Curr Opin Plant Biol 8:183–187. https://doi.org/10.1016/j.pbi.2005.01.011

Hilhorst HWM (2007) Definitions and hypotheses of seed dormancy. In: Bradford KJ, Nonogaki H (eds) Seed development, dormancy and germination. Wiley-Blackwell, Hoboken, pp 50–71

Kępczyński J, Kępczyńska E (1997) Ethylene in seed dormancy and germination. Physiol Plant 101:720–726. https://doi.org/10.1111/j.1399-3054.1997.tb01056.x

Kępczyński J, Kępczyńska E (2003) Ethylene and secondary seed dormancy. In: Vendrell M, Klee H, Pech JC, Romojaro F (eds) Biology and biotechnology of the plant hormone ethylene III. IOS Press, Amsterdam, pp 209–215

Kępczyński J, Van Staden J (2012) Interaction of karrikinolide and ethylene in controlling germination of dormant *Avena fatua* L. caryopses. Plant Growth Regul 67:185–190. https://doi.org/10.1007/s10725-012-9675-5

Kępczyński J, Białecka B, Light ME, Van Staden J (2006) Regulation of *Avena fatua* seed germination by smoke solutions, gibberellin A_3 and ethylene. Plant Growth Regul 49:9–16. https://doi.org/10.1007/s10725-006-0008-4

Kępczyński 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. https://doi.org/10.1007/s10725-010-9493-6

Kępczyński J, Cembrowska-Lech D, Van Staden J (2013) Necessity of gibberellin for stimulatory effect of KAR_1 on germination of dormant *Avena fatua* L. caryopses. Acta Physiol Plant 35:379–387. https://doi.org/10.1007/s11738-012-1080-1

Krawczyk E, Koprowski M, Cembrowska-Lech D, Wójcik A, Kępczyński J (2017) Synthesis of tricyclic butenolides and comparison their effects with known smoke-butenolide, KAR_1. J Plant Physiol 215:91–99. https://doi.org/10.1016/j.jplph.2017.04.021

Kulkarni MG, Light ME, Van Staden J (2011) Plant-derived smoke: old technology with possibilities for economic applications in agriculture and horticulture. S Afr J Bot 77:972–979. https://doi.org/10.1016/j.sajb.2011.08.006

Li W, Nguyen KH, Chu HD, Ha CV, Watanabe Y, Osakabe Y, Leyva-Gonzalez MA, Sato M, Toyooka K, Voges L, Tanaka M, Mostofa MG, Seki M, Seo M, Yamaguchi S, Nelson DC, Tian C, Herrera-Estrella L, Tran LSP (2017) The karrikin receptor KAI2 promotes drought resistance in *Arabidopsis thaliana*. PLoS Genet. https://doi.org/10.1371/journal.pgen.1007076

Light ME, Van Staden J (2004) The potential of smoke in seed technology. S Afr J Bot 70:97–101. https://doi.org/10.1016/S0254-6299(15)30311-2

Light ME, Daws MI, Van Staden J (2009) Smoke-derived butenolide: towards understanding its biological effects. S Afr J Bot 75:1–7. https://doi.org/10.1016/j.sajb.2008.10.004

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 73:267–269. https://doi.org/10.1021/np900630w

Long RL, Williams K, Griffiths EM, Flematti GR, Merritt DJ, Stevens JC, Turner SR, Powles SB, Dixon KW (2010) Prior hydration of *Brassica tournefortii* seeds reduces the stimulatory effect of karrikinolide on germination and increases seed sensitivity to abscisic acid. Ann Bot 105:1063–1070. https://doi.org/10.1093/aob/mcq061

Maga JA (1988) Smoke in food processing. CRC Press, Boca Raton

Masubelele NH, Dewitte W, Menges M, Maughan S, Collins C, Huntley R, Nieuwland J, Scofield S, Murray JAH (2005) D-type cyclins activate division in the root apex to promote seed germination in *Arabidopsis*. PNAS 102:15694–15699. https://doi.org/10.1073/pnas.0507581102

Matilla AJ, Matilla-Vazquez MA (2008) Involvement of ethylene in seed physiology. Plant Sci 175:87–97. https://doi.org/10.1016/j.plantsci.2008.01.014

Matsuo K, Shindo M (2011) Efficient synthesis of karrikinolide via Cu(II)-catalyzed lactonization. Tetrahedron 67:971–975. https://doi.org/10.1016/j.tet.2010.11.108

Merritt DJ, Kristiansen M, Flematti GR, Turner SR, Ghisalberti EL, Trengove RD, Dixon KW (2006) Effects of a butenolide present in smoke on light-mediated germination of Australian Asteraceae. Seed Sci Res 16:29–35. https://doi.org/10.1079/SSR2005232

Mindrebo JT, Nartey CM, Seto Y, Burkart MD, Noel JP (2016) Unveiling the functional diversity of the alpha/beta hydrolase superfamily in the plant kingdom. Curr Opin Struct Biol 41:233–246. https://doi.org/10.1016/j.sbi.2016.08.005

Morffy N, Faure L, Nelson DC (2016) Smoke and hormone mirrors: action and evolution of karrikin and strigolactone signaling. Trends Genet 32:176–188. https://doi.org/10.1016/j.tig.2016.01.002

Nagase R, Katayama M, Mura H, Matsuo N, Tanabe Y (2008) Synthesis of the seed germination stimulant 3-methyl-*2H*-furo[2,3-*c*]pyran-2-ones utilizing direct and regioselective Ti-crossed aldol addition. Tetrahedron Lett 49:4509–4512. https://doi.org/10.1016/j.tetlet.2008.05.052

Nelson DC, Riseborough JA, Flematti GR, Stevens J, Ghisalberti EL, Dixon KW, Smith SM (2009) Karrikins discovered in smoke trigger *Arabidopsis* seed germination by a mechanism requiring gibberellic acid synthesis and light. Plant Physiol 149:863–873. https://doi.org/10.1104/pp.108.131516

Nelson DC, Flematti GR, Ghisalberti EL, Dixon KW, Smith SM (2012) Regulation of seed germination and seedling growth by chemical signals from burning vegetation. Annu Rev Plant Biol 63:107–130. https://doi.org/10.1146/annurev-arplant-042811-105545

Rodríguez-Gacio MC, Matilla-Vázquez MA, Matilla AJ (2009) Seed dormancy and ABA signaling. Plant Signal Behav 4:1035–1048. https://doi.org/10.4161/psb.4.11.9902

Ruduś R, Kępczyński J (2017) Exogenous putrescine increases the responsiveness of thermodormant *Avena fatua* L. caryopses to karrikinolide and gibberellic acid. Acta Physiol Plant 39:49. https://doi.org/10.1007/s11738-016-2344-y

Simpson GM (2007) Seed dormancy in grasses. Cambridge University Press, Cambridge

Śliwińska E, Jing H-C, Job C, Job D, Bergervoet JHW, Bino RJ, Groot SPC (1999) Effect of harvest time and soaking treatment on cell cycle activity in sugarbeet seeds. Seed Sci Res 9:91–99. https://doi.org/10.1017/S0960258599000100

Stevens JC, Merritt DJ, Flematti GR, Ghisalberti EL, Dixon KW (2007) Seed germination of agricultural weeds is promoted by the butenolide 3-methyl-*2H*-furo[2,3-*c*]pyran-2-one under laboratory and field conditions. Plant Soil 298:113–124. https://doi.org/10.1007/s11104-007-9344-z

Sun K, Chen Y, Wagerle T, Linnstaedt D, Currie M, Chmura P, Song Y, Xu M (2008) Synthesis of butenolides as seed germination stimulants. Tetrahedron Lett 49:2922–2925. https://doi.org/10.1016/j.tetlet.2008.03.024

Symons SJ, Simson GM, Adkins SW (1987) Secondary dormancy in *Avena fatua*: effect of temperature and after-ripening. Physiol Plant 70:419–426. https://doi.org/10.1111/j.1399-3054.1987.tb02838.x

Van Staden J, Jäger AK, Light ME, Burge BV (2004) Isolation of the major germination cue from plant-derived smoke. S Afr J Bot 70:654–659. https://doi.org/10.1016/S0254-6299(15)30206-4

Waters MT, Scaffidi A, Sun YK, Flematti GR, Smith SM (2014) The karrikin response system of *Arabidopsis*. Plant J 79:623–631. https://doi.org/10.1111/tpj.12430