Minireview

Smoke-derived butenolide: Towards understanding its biological effects

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Abstract

The smoke-derived butenolide, 3-methyl-2H-furo[2,3-c]pyran-2-one, is a simple organic compound that can increase both the level and rate of seed germination, widen the environmental range over which germination can occur and have a positive effect on seedling vigour. Consequently, butenolide has a wide range of potential applications spanning horticulture, weed control and ecological restoration. Here we review the isolation and identification of this compound from plant-derived smoke, its effects on plants and the current state of knowledge on possible mode of action, as well as impacts it may have in the natural environment.

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1. Introduction

1.1. Smoke as a germination cue

The major role of fire as a disturbance factor and driver of ecosystem properties and function is well recognised (Keeley and Fotheringham, 2000). Beyond the effects on biomass and mortality of established plants, the effect on seed germination has also been identified as a mechanism through which the effects of fire are modulated. This is particularly seen in species with seeds that have hard, water-impermeable seed coats (e.g. species of Fabaceae; Keeley and Fotheringham, 2000). In such seeds, heat from the fire ‘cracks’ the seed coat enabling subsequent water uptake and hence germination. However, not until more recently was it discovered that in addition to heat, smoke or aqueous extracts of smoke could function as germination stimulants in the threatened fynbos species Auboustinia capitata (De Lange and Boucher, 1990).

Since that landmark discovery, smoke has been shown to stimulate germination of numerous species from a range of fire-prone environments worldwide including Australian kwongan (Roche et al., 1997), Californian chaparral (Keeley and Fotheringham, 1998a), Western Cape fynbos (Brown et al., 2003) and the Mediterranean basin (Crosti et al., 2006). In a survey of 301 South African fynbos species, it was found that 49.8% of species, ranging from annual herbs to geophytes to trees, had a positive germination response to smoke (Brown et al., 2003). However, a smoke germination response is not limited to species from fire-prone environments as smoke stimulates germination in species such as lettuce (Drewes et al., 1995), red rice (Doherty and Cohn, 2000) and wild oats (Adkins and Peters, 2001).

Although the role of smoke in germination and its potential applicability have been previously reviewed (Brown and Van Staden, 1997; Van Staden et al., 2000; Light and Van Staden, 2004), the aim of this paper is to highlight recent findings related to the highly active germination component in smoke, namely, 3-methyl-2H-furo[2,3-c]pyran-2-one (Fig. 1-1).

1.2. Discovery of the active butenolide in smoke

Following the initial reports on smoke-stimulated seed germination in the early 1990s, various research groups worldwide attempted to isolate and characterize the chemical(s) present in plant-derived smoke responsible for the phenomenal
promotion of germination. Baldwin et al. (1994) identified 71 compounds in active fractions of smoke by GC-MS and atomic absorption (AA) spectrometry, and tested a total of 233 compounds using seeds of Nicotiana attenuata. None of these compounds, however, promoted germination. In this study, they also demonstrated that germination activity could be obtained from smoke produced from burned cellulose and it was estimated that less than 1 pg of the active chemical is needed per seed (Baldwin et al., 1994). Thus, the difficulty in isolating the active component(s) from aqueous smoke extracts was partly due to the large number of compounds present in the smoke extract, possibly up to several thousand (Maga, 1988), and partly due to the very low concentration of the active compound(s) relative to the other components present in the smoke. Prior to the isolation and identification of the active butenolide from smoke, it was known that the germination cue(s) were water soluble chemical(s) that were thermostable, long-lasting in solution, and highly active at very low concentrations (Baldwin et al., 1994; Van Staden et al., 2000).

Van Staden et al. (1995a) conducted chromatographic separation of two different smoke extracts, from burned fynbos material or burned Themeda triandra (climax grass). One major peak of germination activity, with a similar retention time from the non-polar fraction, was found in both extracts although, some other fractions also had limited positive effects on germination. Germination activity was tested using achenes of Syncarpha vestita (a fynbos species) and caryopses of T. triandra. Similar results were also observed using achenes of Grand Rapids lettuce. The lettuce seeds, which germinated within 24 h, proved to be more suitable for bioactivity-guided fractionation than the S. vestita achenes, which required 20 days or longer to show an optimal response (Drewes et al., 1995). The study by Van Staden et al. (1995a) also demonstrated that ethylene was probably not responsible for the observed germination activity, because it is unlikely that it would still be present in the active fractions following the various chromatographic procedures.

Subsequently, Van Staden et al. (1995b) identified seven compounds present in both Passerina vulgaris and T. triandra smoke extracts. Four of the compounds (available commercially) were tested in the Grand Rapids lettuce seed bioassay at concentrations from $10^{-4}$ to $10^{-15}$ M. However, none were found to be active. Jäger et al. (1996a) investigated a smoke extract from T. triandra and a liquid food-flavouring concentrate, using Grand Rapids lettuce seeds to detect germination activity. Chromatographic separation of these two extracts, using thin layer chromatography (TLC), semi-preparative high-performance liquid chromatography (HPLC), and analytical HPLC, indicated that the compound with germination activity was present in the same fractions. Although bioactivity-guided fractionation led to one major peak of activity, there was some chromatographic evidence indicating that there may be more than one active component in smoke that promotes seed germination (Baldwin et al., 1994; Van Staden et al., 1995a).

It was of interest that a wide range of sources of plant material produced smoke that stimulated the germination of T. triandra seeds (Baxter et al., 1995) and Grand Rapids lettuce seeds (Jäger et al., 1996a,b). Of particular importance was the finding that smoke from burned paper, or even an extract prepared from heated agar or cellulose, could stimulate the germination of Grand Rapids lettuce seeds (Jäger et al., 1996b). Results from these studies demonstrated that the germination-promoting compound(s) were produced from commonly occurring plant constituents. Furthermore, chromatographic purification of aqueous smoke extracts from fynbos material and T. triandra, as well as a commercial food-flavourant, supported the notion of a common active compound (Van Staden et al., 1995a,b; Jäger et al., 1996a).

Prior to the identification of the active compound in smoke, Keeley and Fotheringham (1998a,b) concluded that nitrogen oxides, and nitric oxide (NO) in smoke were most likely responsible for stimulating seed germination. However, a study by Light and Van Staden (2003) examined the effects of two NO-releasing compounds, N-tert-butyl-α-phenylnitrone (PBN) and sodium nitroprusside (SNP), on the germination of Grand Rapids lettuce seeds. In contrast to smoke application, neither PBN nor SNP stimulated the germination of Grand Rapids lettuce seeds in the dark. Additionally, the NO-specific scavenger 2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-oxyl-3-oxide potassium (c-PTIO) was unable to reduce the germination response observed with smoke solutions. These results suggested that NO was unlikely to be responsible for the enhanced germination of Grand Rapids lettuce seeds by smoke solutions (Light and Van Staden, 2003). Preston et al. (2004) also could not detect NO$^+$ in aqueous smoke solutions derived from burned cellulose or wood, although these solutions effectively promoted germination of Emmenanthe penduliflora and N. attenuata.

Continued efforts at isolating the compound by both the South African and Australian research groups culminated in the characterization of a highly active butenolide compound, 3-methyl-2H-furo[2,3-c]pyran-2-one, from plant-derived smoke (Van Staden et al., 2004) and burned cellulose (Flematti et al., 2004), respectively. The compound has become commonly referred to as 'butenolide' in several studies, although strictly speaking this name refers to the type/class of compound, and in a recent article, it has been referred to as 'karrikinolide' (KAR$_1$) (Commander et al., 2008). It was found that the compound promoted the germination of certain seeds over a wide range of concentrations, and at concentrations as low as $10^{-9}$ M for Grand Rapids lettuce seeds, and in the region of $10^{-7}$ M for Conostylis aculeata and Stylidiyum affine (Flematti et al., 2004). Similarly,
Van Staden et al. (2004) showed activity in Grand Rapids lettuce seeds from $10^{-4}$ M down to $10^{-9}$ M. Flematti et al. (2008) discusses in detail the various strategies carried out by the Australian research group leading up to the isolation of butenolide.

Further experiments by the South African research group showed that 3-methyl-2H-furo[2,3-c]pyran-2-one could also be formed during Maillard reactions between sugars and amino acids (Light et al., 2005). Heating proteins or amino acids with sugars at 180 °C for 30 min produced water-soluble extracts that promoted the germination of Grand Rapids lettuce seeds in the dark. Using HPLC fractionation, it was demonstrated that the active compound(s) formed during these reactions co-eluted with the active fraction from the smoke extract. Further analysis using GC-MS showed that the active constituent was identical to the active compound isolated from plant-derived smoke (Van Staden et al., 2004; Light et al., 2005). Thus, the study confirmed that germination promoting compounds, including 3-methyl-2H-furo[2,3-c]pyran-2-one, could indeed be formed by heating ubiquitously occurring organic compounds. In particular, extracts prepared from reactions between d-xylene or d-ribose (aldopentose sugars) with the amino acids arginine, asparagine, aspartic acid, glycine, serine, tyrosine or valine, gave the greatest germination response (Light et al., 2005).

The butenolide was tested by Verschaeeve et al. (2006) for possible mutagenic and genotoxic effects using the VITOTOX® test and the Ames assay. Importantly, the results indicated that the compound is not toxic nor genotoxic at the levels tested ($1 \times 10^{-4}$ to $3 \times 10^{-10}$ M), which raises the possibility of wide-scale usage of the compound as both a germination stimulant and in a field setting.

Following the identification of the active butenolide from smoke, there have been several groups who have successfully synthesised the active butenolide, as well as several analogues. The Australian research group, who originally reported the active compound, described the synthesis of 3-methyl-2H-furo[2,3-c]pyran-2-one from pyromeconic acid (Flematti et al., 2005) and the preparation and activity of several derivatives (Flematti et al., 2007). Goddard-Borger et al. (2007) described the synthesis of the compound, and several analogous compounds from d-xylene. Interestingly, in the study by Light et al. (2005) the butenolide was found to be a product formed during a heating reaction between d-xylene and glycine (Light et al., 2005). Two more recent publications by other research groups also describe the synthesis of the compound (Nagase et al., 2008; Sun et al., 2008).

2. What does butenolide do?

Since the identification of the butenolide in smoke, a significant number of species have been tested for a germination response. Species responding positively to butenolide include those from fire-prone environments (Flematti et al., 2004; Merritt et al., 2006), arable weeds (Daws et al., 2007; Stevens et al., 2007), hemi- and holo-parasitic weeds (Daws et al., 2008), several Australian Solanum spp. (Commander et al., 2008) and crop plants including lettuce, tomato, okra, bean, maize and rice (Van Staden et al., 2004; Jain and Van Staden, 2006; Kulkarni et al., 2006; Van Staden et al., 2006). Compared with crude smoke extracts, butenolide affects a broader range of species, is active across a wider concentration range and does not inhibit seed germination as observed with higher concentrations of crude smoke solutions (Flematti et al., 2004; Van Staden et al., 2004; Daws et al., 2007). For example, in a study of 18 arable weed species, Daws et al. (2007) found that, in terms of percentage germination, four species responded positively to smoke, whereas eight responded positively to butenolide. In addition, a further two species were negatively affected by smoke treatment, whereas no species were negatively affected by butenolide.

A negative effect of smoke on germination has been previously reported (e.g. Drewes et al., 1995; Adkins and Peters, 2001; Light et al., 2002; Daws et al., 2007) and this, coupled with smoke having a positive effect on a narrower range of species than butenolide, has been interpreted as smoke also containing compounds which may negatively affect germination (Light et al., 2002). Furthermore, smoke is a highly complex mix of several thousand compounds (Maga, 1988) with no two batches containing exactly the same balance or concentration of compounds. Consequently, the use of smoke in germination testing is complicated by the need to calibrate individual batches to identify optimum concentrations (Adkins and Peters, 2001; Boucher and Meets, 2004), a problem that is obviated by the use of the pure butenolide compound.

Butenolide not only enhances germination percentage and rate but can also widen the environmental window over which germination can occur. Thus, for a range of Australian ephemeral Asteraceae species, Merritt et al. (2006) reported that, like GA3, butenolide was able to partially or fully substitute for a light requirement for germination, as observed with Grand Rapids lettuce seeds (Drewes et al., 1995; Van Staden et al., 2004). Butenolide at $10^{-7}$ M can also affect the temperature range for germination/seedling development. For example, Jain et al. (2006) found that butenolide was able to overcome the deleterious effects of sub- and supra-optimal temperatures on germination and post-germination growth of tomato. Specifically, in the absence of butenolide, limited radicle emergence was observed at 10 and 40 °C and no onward growth occurred. However, $10^{-7}$ M butenolide enhanced germination (i.e. radicle emergence) at both 10 and 40 °C and normal onward growth was also observed. Jain and Van Staden (2006) also found that $10^{-7}$ M butenolide enhanced seedling quality from low quality tomato seedlots. It was reported that in a tomato seedlot where ca. 40% of seedlings exhibited arrested root growth, this value was reduced to ca. 12% in the presence of $10^{-7}$ M butenolide. Ghebrehiwot et al. (2008) reported that butenolide ($10^{-8}$ M) widens the water potential range over which germination of Eragrostis tef occurs, with no germination recorded at $-0.49$ MPa in the absence of butenolide compared to ca. 35% germination in the presence of butenolide. This type of effect is of great interest since unreliable rainfall is a major cause of crop establishment failure, and the use of butenolide may reduce seedling losses at elevated temperatures and under water stress.
Jain et al. (2008a) found that both HgCl$_2$ and ZnCl$_2$ reduced water uptake during germination and seedling growth in tomato thereby impacting negatively on germination and seedling growth. However, in the presence of butenolide ($10^{-7}$ M) the negative effects on water uptake and growth were largely overcome, raising the possibility of butenolide treatments being used for plant establishment in contaminated soils.

In addition to impacts on germination performance, butenolide applied as a germination treatment, has been reported to have positive impacts on seedling growth in arable weeds (Daws et al., 2007), Acacia species (Kulkarni et al., 2007a), E. tef (Ghebrehiwot et al., 2008) and the medicinal plant Dioscorea dregeana (Kulkarni et al., 2007b). A beneficial effect on seedling vigour has also been maintained in tomato seeds that were imbibed in $10^{-7}$ M butenolide and then re-dried and stored for 4 months (Jain and Van Staden, 2007), a technique that has potential for ‘priming’ seeds for enhanced seedling vigour. Post-germination, butenolide ($10^{-7}$ M) applied as a foliar application to seedlings (okra and tomato) has also been effective at enhancing plant growth (Kulkarni et al., 2007c). While effects on seed germination and seedling growth have been reported in a number of studies only one has followed the effects to monitor impacts on final yield. Kulkarni et al. (2008) investigated the effect of smoke-water and $10^{-9}$ M butenolide on growth and final yield of tomato under greenhouse conditions. Both smoke-water and butenolide-treated plants produced fruits earlier and in greater overall numbers than the control treatment. Additionally, in in vitro experiments, incorporation of butenolide (in lieu of the synthetic auxin 2,4-D) into the culture medium reduced the period of embryo development and improved the frequency of root formation in Baloskion tetraphyllum compared to a control treatment (Ma et al., 2006).

Consequently, butenolide has potential applications in agriculture, horticulture and re-vegetation of degraded landscapes. The stimulation of germination is a key application in areas spanning re-vegetation to weed control, although it should be noted that butenolide does not enhance germination of all species. A key goal in agriculture is the rapid establishment of a uniform crop cover and this aim can be hampered by sub-optimal conditions (e.g. with respect to temperature or water availability). Butenolide has, therefore, obvious potential for enhanced crop establishment in such conditions.

### 3. How does butenolide function?

There have been a wide range of studies aimed at understanding the mode of action of smoke in germination. These have typically been physiological in nature, investigating similarities between the effects of smoke and other plant growth regulators, particularly gibberellins. However, the identification of the active butenolide in smoke presents enhanced opportunities for elucidating the mode of action of this compound in the absence of artefacts and confounding influences caused by the additional compounds in smoke. Nonetheless, it is not likely to be an easy task to unravel the mechanisms involved.

The mode of action of smoke, and hence by implication butenolide, has been ascribed to an interaction with the gibberellin pathway in seeds. For example, smoke has a similar effect to GA$_3$ in substituting for red light ($640$ nm) in the stimulation of Grand Rapids lettuce germination (Drewes et al., 1995; Van Staden et al., 1995c). Similarly, butenolide has been reported to have similar effects on germination as GA$_3$ by both stimulating germination and substituting for light in the germination of Australian Asteraceae (Merritt et al., 2006) and stimulating germination in arable weeds (Daws et al., 2007). Daws et al. (2007) reported a significant relationship, across the study species, between the germination response to butenolide and GA$_3$. However, overall, butenolide was the most effective and did not result in the elongated internodes that are typically associated with GA$_3$. Consequently, butenolide is likely to be of greater value than GA$_3$ for germination testing on diverse species since the resulting seedlings are more likely to be morphologically ‘normal’. Other studies have also indicated that smoke affects endogenous GA synthesis and ABA content (Gardner et al., 2001; Krock et al., 2002; Schwachtje and Baldwin, 2004). However, the effect of butenolide on levels of endogenous plant hormones has not been fully investigated.

While there are clear similarities in the responses of seeds to butenolide/smoke and GA$_3$, there are few obvious similarities between the chemical structures of the two compounds. There are, however, structural similarities between butenolide and the strigolactones (Figs. 1-2), which stimulate germination in parasitic plant species such as Orobanche and Striga (Flematti et al., 2004). Recently, Daws et al. (2008) reported that butenolide can substitute for strigolactones in stimulating germination of parasitic weeds (including Striga and Orobanche) suggesting that butenolide may function in the same way as strigolactones. This proposition is further supported by structure-activity relationship studies of strigolactones, using synthetic analogues of strigol, which have shown that the lactone-enol ether (on the CD portion of the molecule, Fig. 1) is primarily responsible for the biological activity of these compounds (Mangnus and Zwanenburg, 1992; Wigchert and Zwanenburg, 1999).

Auxins play an important role in embryogenesis and seedling development and are important for ‘normal’ development in in vitro cultures by providing positional information for the coordination of correct cellular patterning from the globular stage onwards (Fischer-Iglesias and Neuhaus, 2001; Teale et al., 2006). Ma et al. (2006) found that butenolide may function in a similar way to auxins being able to substitute for 2,4-D (a synthetic auxin) in somatic embryogenesis of B. tetraphyllum. However, very little is known about what role auxins play during seed germination although a relationship between IAA, dormancy and pre-harvest sprouting of wheat has been reported (Ramaiah et al., 2003).

Butenolides that are structurally related to 3-methyl-2H-furo[2,3-c]pyran-2-one in smoke are produced by a range of microorganisms. For example, Fusarium sp. produces a ‘butenolide’ that functions as a mycotoxin with a mode of action resulting from an impact on the intracellular redox environment (Wang et al., 2006). Since oxidative stress has been proposed to have a signalling role in germination (Kraner et al., 2006) this area may also be worth pursuing in relation to the role of butenolide in germination.
Thus, studies suggest potential similarities between butenolide and gibberellins, auxin and strigolactones. However, it is perhaps not surprising that a single molecule can appear to have analogous properties to a range of plant growth regulating compounds (e.g. Jain et al., 2008b) since plants have signalling proteins that can function in several pathways. For example, a key integrating factor is BIG which is necessary for auxin transport, cytokinin, GA, ABA, ethylene and brassinosteroid signalling (Gil et al., 2001; Kanyuka et al., 2003).

To date, however, only one study has been published which aimed at elucidating the regulation of germination in the presence of butenolide at a molecular level, though additional studies are no doubt in progress. Jain et al. (2008c), using differential display during tomato seed germination, reported the up-regulation of genes encoding expansins in the presence of butenolide. Expansins genes are highly conserved and most have been proposed to be involved in cell expansion during tissue growth (Sampedro and Cosgrove, 2005). They are thought to function by disrupting the hydrogen bonds between cellulose and hemi-cellulose polymers thereby allowing cells to expand (McQueen-Mason and Cosgrove, 1994). Expansins have been reported previously in seed germination, playing a role in both endosperm cap weakening and embryo growth in tomato (Chen and Bradford, 2000). Germination is, in essence, cell expansion and/or elongation culminating in visible radicle emergence through the testa. If up-regulation of expansins by butenolide/smoke is widespread it also provides a mechanism to explain the enhancement in seed germination rate even in seedlots that germinate to 100% in the absence of butenolide. In addition, Chen et al. (2001) reported that germination associated expansins in tomato are under the control of GA, further reinforcing the likely cross-talk between butenolide and endogenous plant growth regulators.

4. What role does butenolide play in the natural environment?

Butenolide certainly holds potential for field-scale use in, for example, weed control and re-vegetation of degraded areas (Light and Van Staden, 2004; Stevens et al., 2007). However, for effective use in the natural environment, it is important to know (1) how long butenolide persists in the soil, (2) natural concentrations in fire-prone environments, and (3) potential impacts on soil microbes. However, these topics have received scant attention. The only study to date that has addressed any of these issues investigated the movement of butenolide down a soil (white silica sand) profile following simulated rainfall events ranging from 4 to 16 mm (Stevens et al. 2007). Stevens et al. (2007) found that application of butenolide, at a rate equivalent to 2 g/ha, resulted in butenolide, at germination active concentrations, moving down the profile to depths ranging from 8.5 to 18.3 cm (following simulated rainfall events of 4 and 16 mm, respectively). Thus, butenolide is mobile in soil and retains bioactivity, at least in the short-term. However, we still know neither the effects of soil type on the activity of butenolide nor the half-life of butenolide in soil. Such questions could partly be addressed using different soil types in the bioassay approach outlined by Stevens et al. (2007). However, it would also be of value to determine actual field level concentrations of butenolide in post-fire environments and the distribution of butenolide down soil profiles.

Merritt et al. (2006) investigated the sensitivity of seven Australian Asteraceae from non-fire-prone environments to butenolide and found that it was an effective germination stimulant that could also overcome the light requirement for germination. While Pierce et al. (1995) used the sensitivity of non-fire-prone Mesembryanthemaceae to smoke as a germination cue to question the ecological relevance of smoke as a germination cue, Merritt et al. (2006) hypothesised that this apparent paradox is caused by the release of butenolide by soil microbes during the decomposition of organic matter. They also speculated that the release of butenolide from soil surface layers following disturbance may be a mechanism to explain this apparent ecological anomaly and the large-scale emergence of these Asteraceae following soil disturbance. This premise remains to be tested, however, and a simpler or partial explanation for germination following disturbance in these species may be due to short-term exposure of these light-sensitive seeds to irradiance.

Another important aspect related to plant growth in natural or agricultural environments is the presence of arbuscular mycorrhizal (AM) fungi which form symbiotic relationships with plant roots. Such fungi supply the plant host with nutrients, such as phosphate and obtain photosynthates from the host plant. Plant roots secrete a ‘branching factor’, which stimulates branching of the fungal hyphae that penetrate plant roots. Recently the strigolactone 5-deoxy-strigol has been isolated from Lotus japonicus root exudates and identified as a branching factor (Akiyama et al., 2005). AM fungal spores can germinate in the absence of a host, but hyphae exhibit limited branching and development (Bécard and Piché, 1989). Strigol and the synthetic strigol analogue GR24 can also induce extensive hyphal branching in Gigaspora margarita (Akiyama et al., 2005) suggesting that parasitic plants find their hosts by detecting the same chemical signals that AM fungi use for host recognition (Akiyama et al., 2005). Since butenolide can also stimulate the germination of parasitic weeds (i.e. it can function as a strigolactone analogue) this raises the question of what impact butenolide may have on the growth and morphology of AM fungi if applied as a soil treatment. For example, would soil application of butenolide result in stimulation of hyphal growth and branching in the absence of suitable plant hosts? Such concerns are further reinforced by the mycotoxic effects of similar ‘butenolides’ produced by other microbes (Wang et al., 2006). While the natural occurrence of butenolide in fire-prone environments may appear to negate these arguments, there is evidence that fire reduces the levels of AM fungal colonization of roots (e.g. Hartnett et al., 2004), with the mechanisms causing these negative impacts unclear.

5. Conclusions

The active butenolide in smoke stimulates germination and seedling growth in a wide range of species including crops, weeds and species from both fire- and non-fire-prone
environments. The widespread nature of the response to smoke/butenolide and the analogy with endogenous growth regulators (e.g. gibberellins), or the effect of increasing the sensitivity of exogenous growth regulators, makes it tempting to speculate that the effectiveness of butenolide is a chance coming together of chemistry and biological pathways. Nonetheless, butenolide presents a number of opportunities for exploitation in, for example, horticulture by increasing seedling vigour through both seed-priming and foliar applications. It also has potential field-scale applications for weed control and stimulating germination in large-scale restoration projects. However, several knowledge gaps remain, not least the persistence of butenolide and its wider effects in the rhizosphere, before this potential can be fully realised. Finally, opportunities now present themselves to elucidate the mode of action of butenolide, whether ‘butenolides’ should be recognised as a new class of plant growth regulators and likely cross-talk with endogenous growth regulators using molecular biology techniques, such as microarrays and other developing technologies. As knowledge of the intricacies of seed germination and plant signalling become better understood, we will be able to piece together the fundamental mechanisms of this fascinating phenomenon.

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