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Smoke water and smoke-derived karrikinolide, KAR_1, improved germination in some medicinal and aromatic plants

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Seed dormancy challenges medicinal and aromatic plant (MAP) cultivation, often requiring lengthy cold stratification or application of germination stimulants such as gibberellic acid (GA_3). We evaluated if karrikinolide (KAR_1), smoke water (SW) and food-grade liquid smoke treatments would break dormancy of five MAPs—*Alchemilla xanthochlora* (yellow-green lady's mantle), *Lavandula angustifolia* (true lavender), *Rhodiola rosea* (golden root), *Verbena officinalis* (common vervain), and *Veronica officinalis* (heath speedwell). We hypothesised that KAR_1 and SW treatments would break dormancy in species originating in Mediterranean-type ecosystems (lavender, common vervain), offering comparable efficacy to GA_3. KAR_1 treatments at 1 µM and 10 µM significantly increased final germination percentage (FGP) in lavender, with cultivar 'Rapido' reaching 58.5% and 65.5%, and cultivar 'Carla' achieving 34% and 31.5%, compared to GA_3 which produced 67% and 49.5% FGP respectively. In heath speedwell, KAR_1 treatments from 0.001 to 10 µM consistently had high FGP (94.5–100%), comparable to GA_3 treatment at 94.5%, indicating near-complete dormancy alleviation. However, only heath speedwell achieved germination speeds similar to GA_3 with KAR_1. SW treatments were variable: while *Veronica officinalis* responded positively at 1:10 and 1:1000 dilutions, *Lavandula angustifolia* showed no response at these concentrations, and undiluted SW inhibited germination for all species. No detectable response to KAR_1 was observed for yellow-green lady's mantle and golden root. Despite not significantly increasing total germination of common vervain, KAR_1 increased germination speed, while SW treatments were less pronounced. While GA_3 was consistently effective in breaking dormancy—except for common vervain—KAR_1 and SW treatments are viable alternatives for lavender and heath speedwell. Optimisation of KAR_1 concentrations and SW formulations may enhance efficacy. SW could offer a novel, sustainable method, potentially compatible with organic production, for improving germination and thus cultivation of these MAP species.

Keywords Medicinal and aromatic plants, Germination, Seed dormancy, Karrikin, KAR_1, Smoke water

The plant kingdom has historically been humanity's primary source of therapeutic compounds, and an essential source of cosmetic, nutritional, and agricultural products[1,2]. In recent years, there has been a rising demand for therapeutics of natural origin[3,4]. This trend is driven by growing consumer interest in a healthier lifestyle, sustainability, environmental impact, and scepticism toward synthetic, laboratory-produced products[5–7]. Beyond that, there is a growing demand in agriculture for pesticides and herbicides of natural origin, which are more compatible with organic production and less harmful to the environment[8,9]. These market demands are driving the increased production and cultivation of medicinal and aromatic plant (MAP) products[10] that need to be standardised and produced in large quantities and of high quality[11].

Most plant species rely on seed germination for their cultivation[12]. The specific germination requirements remain uncharacterised for many MAP species and other newly cultivated plants[13,14]. Some species are difficult to germinate due to seed dormancy, which can be broadly classified as endogenous or exogenous[15,16]. In

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endogenous dormancy, which includes physiological and morphological dormancy, characteristics of the embryo itself impede germination, while exogenous dormancy is caused by external structures, such as seed coats, endosperm, or fruit walls, which may create physical or chemical barriers to germination[17,18]. Seed dormancy severely restricts growers in nurseries and on farms in establishing these plants and creating conditions for rapid and uniform emergence. Due to the high value of many MAPs and that they are mostly rarely studied niche crops, further research is required to characterise and optimise their cultivation in general, and their germination in particular. A common strategy for overcoming endogenous dormancy is cold stratification for several months or treatment with GA_3, as hormone balance underlies the maintenance of or release from dormancy[12]. However, GA_3 is not permitted in organic agriculture[19]. One strategy that has garnered increasing attention in recent years is the use of plant-derived smoke chemicals.

The effectiveness of fire-derived chemical stimulants in seed germination has been demonstrated through research on smoke and smoke water (SW). Exposure to SW or smoke alone stimulated germination in species such as *Audouinia capitata* in South Africa's fynbos, with the aqueous phase being particularly active[20]. In 2004, karrikinolide (KAR_1), one of the active compounds in smoke, was identified from cellulose-derived smoke extracts[21,22]. KAR_1 stimulated germination at concentrations as low as 1 ppb[22,23]. Subsequent studies identified five additional karrikin analogues, collectively termed karrikins (KARs). Not all smoke-responsive species react to KAR_1, suggesting the presence of other germination stimulants in smoke. Cyanohydrin analogues such as glycolonitrile and mandelonitrile have also been shown to stimulate germination. These compounds release cyanide upon hydrolysis in water, and cyanide is understood to be the active agent promoting germination[24–26].

The karrikin response in plants depends on the receptor KARRIKIN-INSENSITIVE2 (KAI2), a paralog of the strigolactone (SL) receptor DWARF14 (D14), identified in karrikin-unresponsive mutants in *Arabidopsis thaliana*[27]. Along with KAI2, the MORE AXILIARY GROWTH2 (MAX2) signalling protein is crucial for KAR-mediated responses and is also involved in SL signalling[28]. Despite structural similarities between KARs and SLs, their perception pathways are distinct and elicit different responses, although both regulate similar processes in plant development such as seed germination[29–31], root architecture[32–36], and mycorrhizal symbiosis[33,37–40]. However, KARs and SLs cannot be substituted for each other to enhance germination[41–43].

The KAI2 signalling pathway is evolutionarily ancient, with KAI2 homologs detected in charophyte algae and confirmed functional across vascular plants, as shown by the ability of *Selaginella* KAI2 to rescue *Arabidopsis* kai2 mutants but not d14 mutants[44,45]. In contrast, D14 orthologs appear only in seed plants, suggesting that D14 arose later by gene duplication from KAI2, leading to differentiated ligand perception and signalling pathways[46,47]. This evolutionary exaptation may have allowed some plants to co-opt the KAI2 pathway for detecting fire-derived KARs, conferring a competitive advantage in post-fire environments[47].

It was initially thought that smoke-responsiveness was a trait for plant species specifically adapted to fire-prone environments, the so-called "fire-followers". But this trait appears to be independent of ecosystem and geography[31]. The universality of the response is supported by the smoke-derived chemical responsiveness of plants from diverse habitats where fires are not known to be prevalent, including *Arabidopsis* (Nelson et al. 2009a), tomato[48,49], capsicum pepper[50], coriander[51], maize[52], rice[53], lettuce[54], rapeseed[55], melon[56], carrot[57]. These observations further support the molecular biological findings described above. This opens up the possibility of screening the germination responses of diverse crops of commercial interest with KARs and aqueous smoke extracts.

This study investigated the germination responses of five dormant MAPs: *Alchemilla xanthochlora* Rothm. (yellow-green lady's mantle), *Lavandula angustifolia* Mill. (true lavender), *Rhodiola rosea* L. (golden root), *Verbena officinalis* L. (common vervain), and *Veronica officinalis* L. (common/heath speedwell) in this context. These species are suspected to be physiologically dormant as they are known to be responsive to cold stratification. Germination of most of these species is known to be stimulated by GA_3 treatment. The exceptions are common vervain, which was unresponsive[58], and heath speedwell, for which no references could be found.

Yellow-green lady's mantle, a member of the Rosaceae family, is a perennial medicinal plant native to temperate Europe[59]. In recent years, *Alchemilla xanthochlora* has been used in the food industry in products such as sweets, herbal teas, and iced teas. The seeds are dormant. A 0.2% GA_3 solution was effective at maximising germination[60]. 100% germination was obtained after stratification at 0–4 °C for 50 days[61]. Lavender is an aromatic perennial plant in the Lamiaceae family, valued for its essential oils used in cosmetics and aromatherapy[62], as well as for its use as an ornamental plant[63,64]. While lavender is native to and was originally cultivated in the Mediterranean region, it is now grown in many parts of the world[65]. GA_3 can either replace or be used in combination with cold stratification to increase total germination[66]. Golden root is a medicinal Arctic plant in the Crassulaceae family[67,68]. Harvesting is destructive and typically occurs after five to six years of cultivation[69]. As commercial demand for the roots continues to rise, it has become important to properly manage its cultivation[70]. Cold stratification or treatment with GA_3 are also necessary for golden root to germinate properly (Aiello et al. 2004). The common vervain, a member of the Verbenaceae family, is a perennial species that is regarded as an archaeophyte of Mediterranean origin in the temperate regions of Central Europe[71]. It is generally replanted every three to four years with rooted seedlings. However, producing these seedlings can be challenging because the seeds often have low and irregular germination rates, due to their seasonal dormancy[58]. Cold stratification or alternating temperatures improve germination rates[72], but GA_3 is ineffective[58]. Heath speedwell is an herbaceous perennial medicinal plant in the Plantaginaceae family[73]. It grows naturally in a wide range of habitats across Europe and Western Asia. It is one of the principal ingredients of the Ricola herbal candy[74]. Very little information is available on the seed germination of the species, however, cold stratification at 2–4 °C for at least 1 month is generally advised[75].

The objective of this research was to evaluate the germination responses of the five selected species to treatments with KAR_1, self-produced SW, and a food-grade liquid smoke product as alternatives to long cold stratification or the application of germination stimulants such as gibberellic acid (GA_3). This research will

serve as a basis for devising species-specific protocols that can be implemented at the (plant) producer level to improve the germination of the selected species. Ideally, the protocols should be simple, practical, time-efficient, and compatible with organic production guidelines. We hypothesised that species of fire-prone Mediterranean origins, namely lavender and common vervain, would exhibit the greatest positive germination responses to KAR_1 and smoke water treatments.

Materials and methods

Seed sources

Untreated dried commercial seeds used for the germination studies were acquired from mediSeeds, Switzerland, for *Alchemilla xanthochlora* Rothm. cultivar "Aper" (Lot number: BiACW2022), *Verbena officinalis* L. (Lot number: ACW,Mo2020) and *Rhodiola rosea* L. cultivar "Besse", iteipmai, France, for *Lavandula angustifolia* Mill. cultivars 'Carla' (Lot number: 09C/01; harvested in 2009) and 'Rapido' (Lot number: 23 V/01; harvested in 2023), and Jelitto, Germany, for *Veronica officinalis* L. (Lot number: VA15480115030AA0AA0g). The seeds were kept in their original packaging and stored in darkness, at room temperature until experimentation. The seed viabilities provided by the seed suppliers were 59% for *Alchemilla xanthochlora*, 73% *Verbena officinalis*, 50% for cultivar 'Carla' and 73% for 'Rapido', 77% for *Rhodiola rosea*, and unknown for *Veronica officinalis*.

Preparation of formulations

KAR_1

1 mg KAR_1 was purchased from Cayman Chemical Company (Item No. 29935). Once delivered, the sample was stored at −20 °C until further use. For the trials, a stock solution of 10 µM was prepared by dissolving the 1 mg KAR_1 in preheated 666 ml deionised H_2O to increase the solubility. Prior to dissolution, the solvent was heated to 37 °C, while covered, in an incubator. Once the desired temperature was reached, a small volume was used to fill the sample glass vial containing KAR_1 and continuously mixed. After mixing, the solution was transferred to 500 ml of the prepared deionised H_2O in an Erlenmeyer flask and stirred with a magnetic stirrer. The remaining ~ 166 ml was used to repeatedly rinse and dissolve any remaining solid KAR_1 in the sample vial and subsequently added to the bulk solution that was stirring contemporaneously. The final 10 µM stock solution was divided among separate 50 ml polypropylene tubes and stored at −80 °C until experimentation. Immediately before germination experiments, the required number of tubes were removed from the freezer and immerged in water at room temperature until completely defrosted. A dilution series was prepared, 0.001, 0.01, 0.1, 1, and 10 µM KAR_1, made up with deionised water. The KAR_1 stock solution was water-based, to reduce the potential effects of other solvents on the germination response, and therefore, the number of confounding variables. The pH of the KAR_1 stock solution was not measured.

Smoke-saturated water

Smoke water (SW) was produced by connecting the nozzle of a bee smoker with piping to a 2 L Büchner flask filled with 500 ml deionised water, the outlet of which is connected to a vacuum pump (Fig. 1). To do so, 100 g of commercial mixed natural cereal straw (Nagerstroh Relax, Schweizer) was progressively burned in the bee smoker, which was continuously pumped until thick smoke formed. Smoke was pumped through to the flask and, with the aid of the vacuum pump, bubbled through the water. This was continued for 25–30 min, occasionally interrupted to add more straw from the 100 g of straw used as the input material of this batch to the bee smoker, until yellowish-brown water was obtained. This solution was filtered with a coarse sieve to remove any particulate matter and then poured into glass bottles and stored at 4 °C until experimentation. A dilution

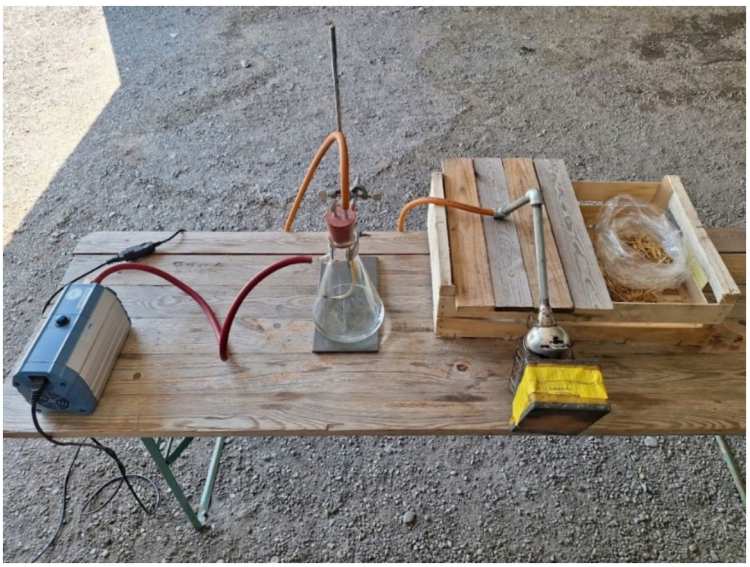

Fig. 1. Equipment used to produce smoke-saturated water.

series of 1:1, 1:10, 1:100 or 1:1000 was prepared from the stock solution on the same day that germination experiments were set up, 30 May 2024.

Food-grade liquid smoke

Two food-grade liquid smoke products, Stubb's Hickory Liquid Smoke and Stubb's Mesquite Liquid Smoke, were used for seed treatments. The listed ingredients for both products included: water, smoke flavour (Hickory or Mesquite), tamari sauce (water, soybeans, salt), cane sugar, vinegar, caramel colour, salt, < 0.5% garlic and onion. A mixture consisting of 50% of each product was used from which a dilution series of 1:1, 1:10, 1:100 or 1:1000 was prepared from the 50 :50 mixture on the same day that germination experiments were set up, 28 June 2024.

GA_3

Gibberellic acid (GA_3) was used as a positive control. 9% GA_3 tablets from Schneiter Agro AG were used to make a 2000 ppm stock solution. Since pure GA_3 would require 2000 mg GA_3 per litre of water, the required mass of 9% GA_3 was calculated to be 22.2 g per litre of water. As described for the preparation of other solutions, deionised water was used to make up the stock solution. Based on prescriptions by the seed supplier, mediSeeds, the seeds of *A. xanthochlora* and *Verbena officinalis* should be soaked in 2000 ppm for 2 h. This methodology was applied to common vervain in a separate publication[58]. Prescriptions for *L. angustifolia* of 500 ppm for 24 h, and for *R. rosea* of 100 ppm for 24 h, were provided by iteipmai and Agroscope, respectively. The appropriate GA_3 concentration and soaking time for *Veronica officinalis* was empirically determined to be 1000 ppm for 24 h.

Germination experiments

All treatments were applied to four replicates of 50 seeds. Each replicate was placed on a sheet of 87 mm (Grade 193, Ahlstrom) filter paper moistened with 5 ml deionised water, the negative control, or each dilution series of the respective formulations in a 9 cm diameter Petri dish sealed with a strip of Parafilm. All Petri dishes were placed in a Conviron G1000 seed germinator set at 25 °C (16 h)/18 °C (8 h) day-night cycles. Each of the four replicates was placed on different shelves in the germinator on 30 May 2024. Germinated seeds were counted daily. A seed was considered as germinated when the radicle was at least twice the length of the seed itself. Each germination experiment lasted for a period of 28 days.

UHPLC-MS/MS analyses of KAR_1 in smoke water

The method reported by[76] was adapted to the analyses of KAR_1 in smoke water and conducted in an Agroscope analytical laboratory. The analysis was performed on an Infinity 1290 UPLC system (Agilent Technologie, Santa Clara, US) connected to an Agilent 6460-C Triple Quadrupole LC–MS with an electrospray using Agilent Jet Stream technology and operating under MassHunter Software (Agilent Technologie, Santa Clara, US). Chromatographic separation was performed at 40 °C on a Pinnacle DB AQ C18 column (50 × 2.1 mm, 1.9 µm; Restek, Bellefonte, US), using 0.1% formic acid in water as solvent A and 0.1% of formic acid in methanol as solvent B. The flow rate was 0.3 mL min^{-1} and the linear gradient for solvent B was as follow: 0 min 5%, 3 min 20%, 8 min 20%, 8.5 min 100%, 9.5 min 100% and 10 min 5%. The column was equilibrated for 2 min with 5% of B between injections. For the analysis 1 ml of SW sample was injected without dilution. Detection was performed by Multiple Reaction Monitoring (MRM). Electrospray positive ionisation mode (ESI +) was applied with the following parameters in the source: gas temperature at 300 °C: gas flow at 5 l/min; nebuliser at 45 psi; Sheat gas heater at 250 °C; Sheat gas flow at 11 L min^{-1}, capillary voltage at 3500 V; Nozzle Voltage 500 V. The fragmentor voltage and collision energy were optimised separately with standards and were found to be 100 V and 20 V for KAR_1, respectively. The transition 151 m/z → 123 m/z was used for quantification and the 151 m/z → 67 m/z for confirmation. The calibration was done using the standard addition method. Known concentrations (0, 5, 20 and 40 nmol L^{-1}) of KAR_1 standard was spiked in each SW sample and after analysis the concentration of non-spiked sample was calculated using the so determined regression equation. The sample was diluted when the calibration curve had an $R^2 < 0.995$, to bring it within the optimal concentration range for accurate linearity.

Parameters analysed

Daily germination counts were compiled and organised for processing germination parameters with using R software version 4.4.1 and the SeedCalc package version 1.0.0[77]. The parameters selected were: final germination percentage (FGP); time to reach 50% germination (T50); mean germination rate (MGR); and germination speed index (GSI) (Table 1).

Statistical analyses

A mixed-effects linear model was used to analyse the effects of different treatments on the selected parameters with treatment included as a fixed factor and shelf position in the germinator as a random factor. These variables were analysed across multiple treatments, including the negative control (water) and positive control (GA_3). An analysis of variance (ANOVA) was conducted for each response parameter, based on the mixed-effects model. Treatments with no observable germination were omitted from the ANOVA. Following the ANOVA, post-hoc pairwise comparisons were performed using estimated marginal means (EMMs) and adjusted for multiple comparisons using Tukey's Honest Significant Difference (HSD) method. The non-adjusted results were also included in the final analysis. Pairwise comparisons were made post-hoc, but only the differences between the negative control and the positive control were reported, as these were considered the most important reference points for evaluating treatment effects. Statistical significance was evaluated at p-values of < 0.05 and < 0.01. Significant differences between treatments and the controls were denoted in the results, with (*) indicating $p < 0.05$, (**) indicating $p < 0.01$ with reference to the negative control. For the positive control, (†) indicated

Function	Measure	Formula	Reference
FGP	Final germination percentage (%)	$FGP = \left(\frac{n}{N}\right) \times 100$ n = number of seeds germinated N = total number of seeds	78
T50	Time to reach 50% germination (days)	$T_{50} = \frac{ti + \left\{\left[\frac{N}{\frac{100}{50}}\right] - ni\right\}(tj - ti)}{(nj - ni)}$ N = final number of seeds germinated; *ni* and *nj* = total number of seeds germinated in adjacent counts in time *ti* and *tj*, respectively, when $ni < \frac{N+1}{2} < nj$	79
MGR	Mean germination rate (% per day)	$MGR = \frac{1}{MGT*} \times 100$	80
GSI	Germination speed index	$GSI = \sum_{i=1}^{k} (n_i/t_i)$ *n* = number of seeds germinated on each day of daily counting up to the last count *t* = number of days after the beginning of the test in each count	81

Table 1. Functions contained in the SeedCalc package[77] for calculation of germination parameters using cumulative daily seed count data. * $MGT = \sum_{i=1}^{k} n_i t_i / \sum_{i=1}^{k} n_i$.

Treatment		FGP (%)	T50 (days)	MGR (%/day)	GSI
H_2O		57.50 ± 11.12	10.98 ± 0.82 ††	8.39 ± 0.31	2.74 ± 0.63
GA_3		80.50 ± 4.43	15.10 ± 1.14 **	6.41 ± 0.34	2.79 ± 0.17
KAR_1 (µM)	0.001	52.00 ± 9.52 ††	9.07 ± 1.24 ††	9.59 ± 0.99 ††	2.73 ± 0.47
	0.01	50.00 ± 3.65 ††	9.39 ± 0.74 ††	8.70 ± 0.70 ††	2.50 ± 0.19
	0.1	52.00 ± 19.18 ††	8.57 ± 1.17 ††	9.93 ± 1.34 ††	2.82 ± 0.86
	1	60.50 ± 8.23	8.40 ± 1.45 ††	10.18 ± 1.32 ††	3.38 ± 0.45
	10	67.00 ± 4.16	8.56 ± 1.58 ††	9.84 ± 1.51 ††	3.73 ± 0.57
Smoke water (dilution)	1:1000	59.00 ± 11.02	10.40 ± 1.36 ††	8.60 ± 0.92	2.89 ± 0.69
	1:100	55.50 ± 8.39 †	8.89 ± 0.80 ††	9.58 ± 0.88 ††	2.98 ± 0.59
	1:10	52.00 ± 11.43 ††	14.00 ± 1.15 *	6.70 ± 0.81	1.89 ± 0.38
	1:1	0	-	-	-
Food-grade liquid smoke (dilution)	1:1000	59.50 ± 3.79	11.45 ± 0.78 ††	7.73 ± 0.51	2.54 ± 0.27
	1:100	58.00 ± 2.31	11.28 ± 1.17 ††	7.97 ± 0.44	2.50 ± 0.15
	1:10	0	-	-	-
	1:1	0	-	-	-

Table 2. Final germination percentage (FGP), time required for 50% of total germination (T50), mean germination rate (MGR), and germination speed index (GSI), mean of *Alchemilla xanthochlora* seeds subjected to different treatments of KAR_1, smoke water and food-grade liquid smoke. Values are means ± standard deviations (n = 4). Treatments with no germination are indicated by an FGP of 0; for these treatments, other parameters are not applicable and are therefore omitted (–). Significant differences in p-values after adjustment using Tukey's Honest Significant Difference (HSD) method are denoted with (*) where $p < 0.05$ and (**) where $p < 0.01$ with reference to the negative control (water). For the positive control (GA_3), (†) indicates $p < 0.05$ and (††) $p < 0.01$.

$p < 0.05$ and (††) indicated $p < 0.01$. All data processing and calculations were performed using R software version 4.4.1.

Results

Alchemilla xanthochlora

None of the experimental treatments significantly improved FGP. GA_3 treatment numerically increased the FGP (80.5%) compared with the negative control (57.5%), albeit insignificantly ($p = 0.056$). Although, the germination of seeds treated with GA_3 was significantly delayed (T50 = 15.1 days) compared with the negative control (T50 = 10.98 days), all KAR_1 treatments (T50 = 8.4–9.39 days), and the 100-fold (T50 = 8.89 days) and 1000-fold SW treatments (T50 = 10.4 days). However, there were no significant differences between the KAR_1, SW and food-grade liquid smoke treatments compared to the negative control for any of the other germination parameters, apart from the tenfold diluted SW which had a T50 (14 days) value more similar to the GA_3 treatment (Table 2). At this point, a germination response to KAR_1 and SW for *A. xanthochlora* remains undetected.

Lavandula angustifolia

Among the KAR_1 treatments, both 1 µM and 10 µM concentrations significantly increased FGP compared to the negative control in both cultivars, with 'Carla' exhibiting 34% at 1 µM and 31.5% at 10 µM, while 'Rapido' reached 58.5% at 1 µM and 65.5% at 10 µM. In contrast, lower KAR_1 concentrations (0.001 µM, 0.01 µM, and 0.1 µM) resulted in modest increases in FGP for 'Carla' (18.5%, 22%, and 23.5%, respectively), none of which were significant compared to the control. However, in 'Rapido', the 0.1 µM KAR_1 treatment significantly improved FGP to 43.5% compared to the negative control (16.5%). GA_3 consistently produced the highest FGP, with 'Carla' reaching 49.5% and 'Rapido' achieving 67%, both significantly greater than the negative control.

Germination timing varied between the two cultivars. In 'Carla', T50 values ranged from 10.15 days (0.1 µM KAR1) to 13.96 days (10 µM KAR_1), with no significant differences from the control. Similarly, 'Rapido' exhibited no significant differences in T50 among KAR_1 treatments. Smoke water and food-grade liquid smoke treatments did not significantly reduce T50 values in either cultivar. The GA_3 treatment demonstrated the highest GSI in both cases, reaching 3.25 in 'Carla' and 8.88 in 'Rapido'. The 1 µM KAR_1 treatment achieved a moderate GSI in both cultivars (1.47 in 'Carla' and 3.79 in 'Rapido'), while the 10 µM KAR_1 treatment showed further improvement in 'Rapido' (4.24). Other KAR_1 treatments, as well as smoke water and food-grade liquid smoke treatments, exhibited either no or negligible increases in GSI compared to the control. In summary, GA_3 was the most effective treatment for improving both germination percentage and germination speed in both lavender cultivars. However, the 1 µM and 10 µM KAR_1 treatments demonstrated comparable FGPs in 'Rapido' and showed positive tendencies toward increasing FGP in 'Carla'.

(Table 3, * MERGEFORMAT Fig. 2, * MERGEFORMAT Fig. 3).

Rhodiola rosea

No significant effects were observed for any of the treatments on the germination of golden root seeds, except for GA_3, which yielded significantly higher FGP (50.5%) compared to the negative control (14.5%). The only other parameter which was significantly improved with GA_3 was GSI (5.81), compared to the negative control (1.35) (Table 4).

Verbena officinalis

The FGP for GA_3 treatment (51%) was even lower than the negative control (65%) and significantly lower than all KAR_1 treatments (71.5—80.5%). For this reason, the results for this species were interpreted only with reference to the negative control. Although the FGP values of the KAR_1 treatments (71.5—80.5%) were all greater than the negative control (65%), none of these differences was statistically significant. However, for a species that is supposedly impacted by seed dormancy, untreated seeds still achieved 65% total germination under these experimental conditions. In terms of germination timing, significant differences in T50 were found for all KAR_1 treatments (5.73—6.55 days) and SW treatments (6.7–7.32 days) compared to the negative control (9.26 days).

Treatment		FGP (%)		T50 (days)		MGR (%/day)		GSI	
Cultivar		Carla	Rapido	Carla	Rapido	Carla	Rapido	Carla	Rapido
H_2O		13.50 ± 4.73 ††	16.50 ± 9.15 ††	9.65 ± 2.53	8.50 ± 0.35 ††	8.75 ± 1.94	9.63 ± 1.16 ††	0.68 ± 0.25 ††	0.98 ± 0.63 ††
GA_3		49.50 ± 3.00 **	67.00 ± 4.76 **	7.47 ± 1.13	3.29 ± 0.30 **	11.24 ± 1.09	24.91 ± 2.16 **	3.25 ± 0.24 **	8.88 ± 0.26 **
KAR_1 (µM)	0.001	18.50 ± 6.40 ††	19.50 ± 11.36 ††	13.31 ± 4.41	6.94 ± 1.51 †	7.38 ± 1.72 †	10.26 ± 2.46 ††	0.79 ± 0.30 ††	1.23 ± 0.45 ††
	0.01	22.00 ± 4.32 ††	31.50 ± 3.42 ††	12.83 ± 1.93	8.04 ± 0.44 ††	7.30 ± 0.73 †	11.09 ± 1.22 ††	0.98 ± 0.25 ††	1.93 ± 0.22 ††
	0.1	23.50 ± 4.43 ††	43.50 ± 12.69 ** ††	10.15 ± 1.97	7.77 ± 0.64 ††	8.61 ± 0.85	10.25 ± 0.81 ††	1.20 ± 0.28 ††	2.56 ± 0.78 ** ††
	1	34.00 ± 9.93 ** †	58.50 ± 6.81 **	13.04 ± 2.80	7.89 ± 0.60 ††	7.21 ± 0.83 †	11.31 ± 1.08 ††	1.47 ± 0.42 ** ††	3.79 ± 0.36 ** ††
	10	31.50 ± 7.72 ** ††	65.50 ± 5.26 **	13.96 ± 1.04	7.87 ± 0.51 ††	6.78 ± 0.73 ††	10.53 ± 0.54 ††	1.20 ± 0.32 ††	4.24 ± 0.36 ** ††
Smoke water (dilution)	1:1000	19.00 ± 5.77 ††	14.50 ± 12.26 ††	11.19 ± 4.29	7.19 ± 2.88 †	7.94 ± 1.40	13.50 ± 5.20 ††	0.94 ± 0.29 ††	0.91 ± 0.67 ††
	1:100	13.00 ± 4.76 ††	30.50 ± 6.61 ††	13.67 ± 5.09	8.00 ± 0.58 ††	6.85 ± 1.99 ††	10.14 ± 1.46 ††	0.52 ± 0.21 ††	1.90 ± 0.45 ††
	1:10	18.50 ± 4.12 ††	30.50 ± 11.00 ††	13.81 ± 0.94	8.67 ± 1.52 ††	6.90 ± 0.18 ††	10.15 ± 1.13 ††	0.76 ± 0.15 ††	1.68 ± 0.58 ††
	1:1	0	0	-	-	-	-	-	-
Food-grade liquid smoke (dilution)	1:1000	10.00 ± 1.63 ††	17.50 ± 8.54 ††	12.50 ± 2.42	8.46 ± 2.31 ††	7.19 ± 1.12 ††	8.90 ± 1.36 ††	0.40 ± 0.12 ††	1.03 ± 0.69 ††
	1:100	18.00 ± 1.63 ††	20.50 ± 5.74 ††	12.81 ± 1.28	8.54 ± 1.72 ††	6.96 ± 0.60 ††	9.68 ± 2.03 ††	0.71 ± 0.08 ††	1.27 ± 0.55 ††
	1:10	12.50 ± 7.55 ††	1.00 ± 2.00 ††	19.84 ± 6.80 ** ††	4.25 ± 8.50 ** ††	5.61 ± 2.63 ††	1.32 ± 2.63††	0.31 ± 0.16 ††	0.03 ± 0.05 ††
	1:1	0	0	-	-	-	-	-	-

Table 3. Final germination percentage (FGP), time required for 50% of total germination (T50), mean germination rate (MGR), and germination speed index (GSI) of *Lavandula angustifolia* 'Carla' seeds subjected to different treatments of KAR_1, smoke water and food-grade liquid smoke. Values are means ± standard deviations (n = 4). Treatments with no germination are indicated by an FGP of 0; for these treatments, other parameters are not applicable and are therefore omitted (–). Significant differences in p-values after adjustment using Tukey's Honest Significant Difference (HSD) method are denoted with (*) indicating $p < 0.05$ and (**) indicating $p < 0.01$ with reference to the negative control (water). For the positive control (GA_3), (†) indicates $p < 0.05$ and (††) $p < 0.01$.

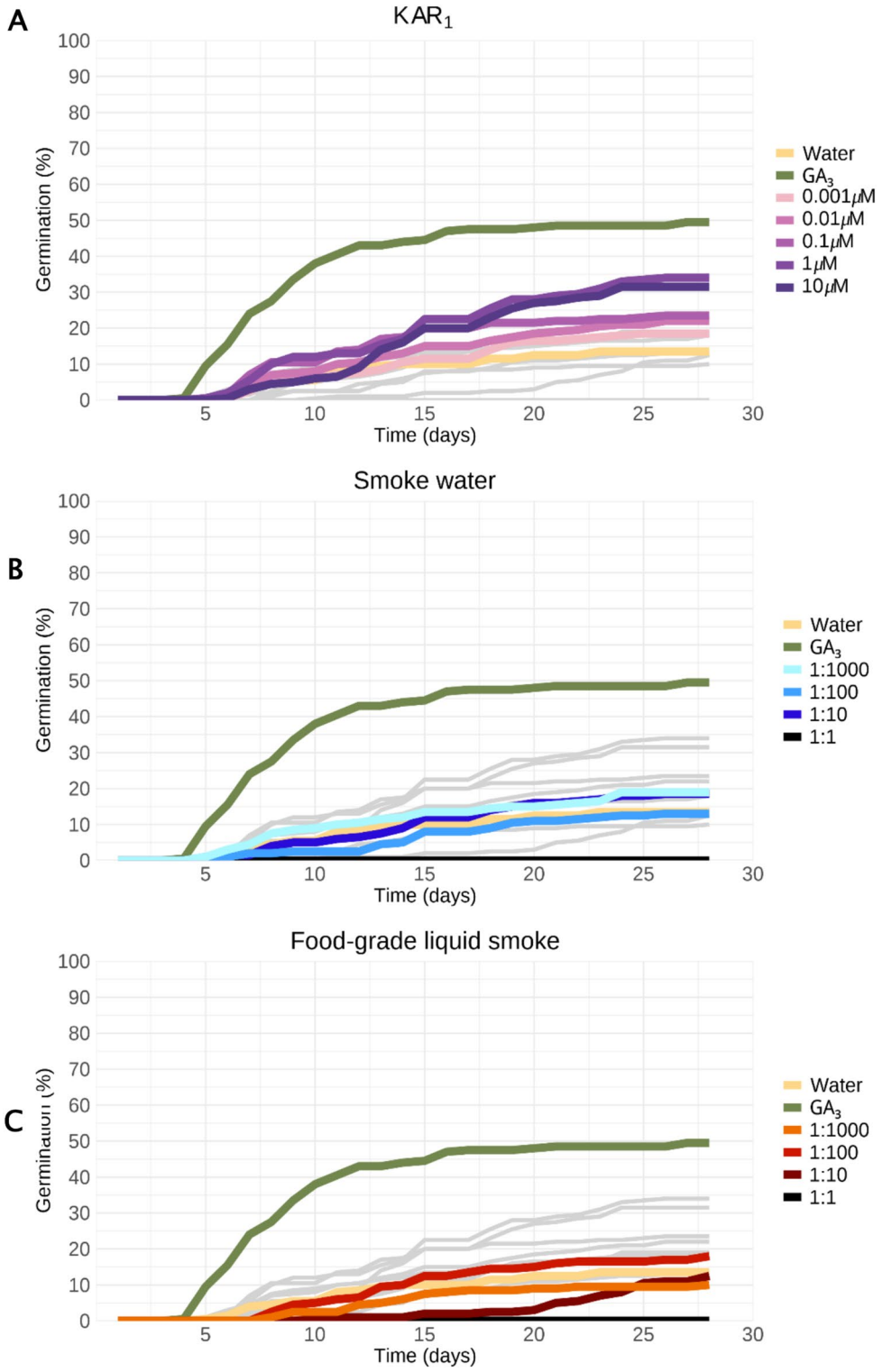

Fig. 2. Effects of different treatments on the cumulative germination percentage over time of *Lavandula angustifolia* 'Carla' : (**A**) KAR_1, (**B**) smoke water, (**C**) food-grade liquid smoke. Grey lines in each plot represent the treatments displayed in the other two plots. Data points represent mean germination percentages (n = 4).

However, no significant effects were observed with the food-grade liquid smoke. The MGR had a similar pattern of results to T50, except that the 1000-fold dilution of SW lost its significance. The greatest MGR was observed for 10 µM KAR_1 (13.13%/day). Among the KAR_1 treatments an increasing trend can be observed in the MGR as the KAR_1 concentration progressively increased. This trend was also reflected in T50, which progressively decreased with increasing KAR_1 concentrations. GSI showed significantly higher values for all KAR_1 (5.05–6.06)

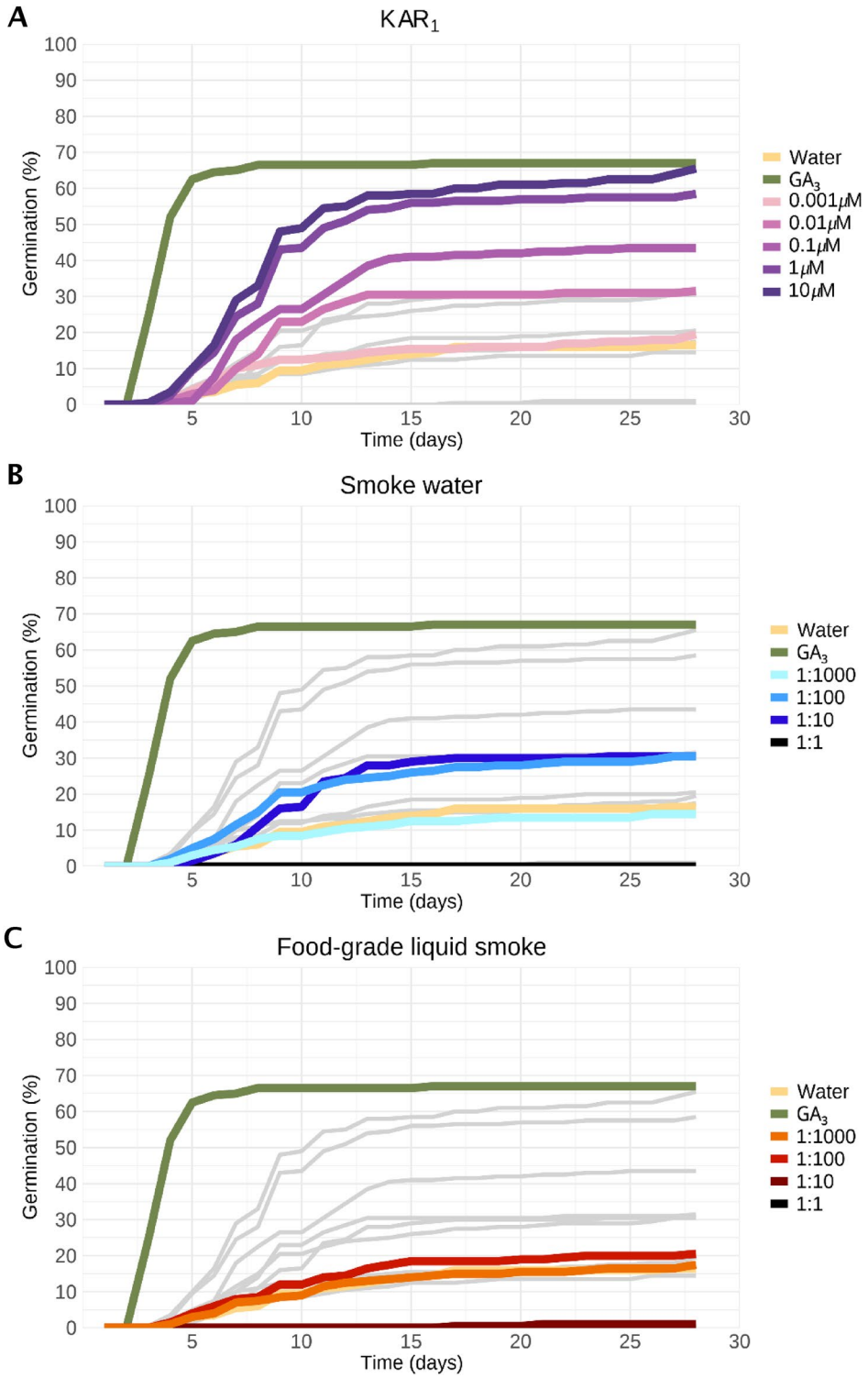

Fig. 3. Effects of different treatments on the cumulative germination percentage over time of *Lavandula angustifolia* 'Rapido': (**A**) KAR_1, (**B**) smoke water, (**C**) food-grade liquid smoke. Grey lines in each plot represent the treatments displayed in the other two plots. Data points represent mean germination percentages (n = 4).

treatments except for 0.01 µM KAR_1 (4.93) compared the negative control (3.54), but not for any SW treatments (4.2–4.67). As noted, the FGP values were statistically insignificant for all treatments, which meant that in GSI, the weights of the time to germination contribute greatly to shifting the GSI to levels of significance. This means that when the germination percentage and time are considered jointly, KAR_1 is the most effective treatment for improving germination. Accordingly, the most effective treatment for common vervain is between 0.1–10 µM

Treatment		FGP (%)	T50 (days)	MGR (%/day)	GSI
H_2O		14.50 ± 5.51 ††	4.84 ± 0.54	15.86 ± 2.56	1.35 ± 0.53 ††
GA_3		50.50 ± 11.70 **	3.68 ± 0.11	21.58 ± 1.39	5.81 ± 1.11 **
KAR_1 (µM)	0.001	18.50 ± 3.00 ††	4.73 ± 0.56	16.42 ± 2.37	1.75 ± 0.31 ††
	0.01	13.00 ± 5.29 ††	4.34 ± 0.53	17.95 ± 3.32	1.30 ± 0.48 ††
	0.1	17.00 ± 9.31 ††	5.54 ± 1.06	10.58 ± 2.08 ††	1.35 ± 0.84 ††
	1	13.50 ± 2.52 ††	5.81 ± 0.80	11.88 ± 2.62 ††	1.05 ± 0.31 ††
	10	10.00 ± 4.90 ††	6.34 ± 1.66	11.79 ± 4.24 ††	0.74 ± 0.52 ††
Smoke water (dilution)	1:1000	20.50 ± 4.12 ††	4.68 ± 0.57	13.62 ± 2.90 †	1.80 ± 0.24 ††
	1:100	11.00 ± 2.58 ††	6.98 ± 3.43	11.51 ± 4.52 ††	0.79 ± 0.19 ††
	1:10	12.50 ± 6.81 ††	8.62 ± 2.03 * ††	10.15 ± 2.38 ††	0.70 ± 0.47 ††
	1:1	0	-	-	-
Food-grade liquid smoke (dilution)	1:1000	7.00 ± 1.15 ††	6.00 ± 1.22	9.75 ± 1.85 ††	0.45 ± 0.10 ††
	1:100	10.00 ± 6.00 ††	5.36 ± 0.85	16.27 ± 1.82	0.83 ± 0.44 ††
	1:10	0	-	-	-
	1:1	0	-	-	-

Table 4. Final germination percentage (FGP), time required for 50% of total germination (T50), mean germination rate (MGR), and germination speed index (GSI) of *Rhodiola rosea* seeds subjected to different treatments of KAR_1, smoke water and food-grade liquid smoke. Values are means ± standard deviations (n = 4). Treatments with no germination are indicated by an FGP of 0; for these treatments, other parameters are not applicable and are therefore omitted (–). Significant differences in p-values after adjustment using Tukey's Honest Significant Difference (HSD) method are denoted with (*) indicating $p < 0.05$ and (**) indicating $p < 0.01$ with reference to the negative control (water). For the positive control (GA_3), (†) indicates $p < 0.05$ and (††) $p < 0.01$.

KAR_1, however, it should be noted that the 1 µM KAR_1 treatment had a much larger standard deviation than the other treatments. No significant effects were observed for any of the parameters with food-grade liquid smoke treatments. Therefore, under these experimental conditions, common vervain exhibits an acceleration of germination, demonstrated by the improvements in T50, MGR and GSI with KAR_1 and some SW treatments (Table 5).

Veronica officinalis

KAR_1 treatments across a wide range (0.001–10 µM) significantly increased FGP (94.5–100%) compared to the negative control (48.5%). There were no significant differences between the KAR_1 treatments themselves. Apart from the undiluted SW, all the other SW treatments, 1:10–1:1000 dilutions, significantly improved FGP and were comparable to GA_3 treatment. The exception was the 1:1000 treatment (71.5%), which was significantly lower than the GA_3 treatment (94.5%). The tenfold dilution yielded a maximum germination response and can therefore be considered a safe minimum dilution for seed treatment. However, the 1000-fold dilution produced a response midway between the maximal SW response and the negative control. Further research could investigate precisely where between the 100- and 1000-fold dilutions the maximum response is maintained and where the change within this range becomes linear. The food-grade liquid smoke product showed statistically significant effects on FGP for the 100-fold and 1000-fold dilutions, similar to the effect of GA_3. However, the tenfold and undiluted treatments were not conducive to germination. KAR_1 concentrations of 0.01–10 µM, SW dilutions of 1:100, and food-grade liquid smoke dilutions of 1:100 and 1:1000 achieved similar T50 results to GA_3. GSI showed significantly faster and more complete germination for all treatments compared to the negative control, except for both undiluted SW and food-grade liquid smoke. For the SW treatments, the T50, MGR, and GSI parameters tend to have the best results with the 100-fold dilution. The highest GSI value was obtained with 1 µM KAR_1, which consistently had slightly better or comparable effects to GA_3 across all the calculated parameters (Table 6, Fig. 4).

UHPLC-MS/MS analyses of KAR_1 in smoke water

The KAR_1 concentration in the self-produced smoke-saturated water used for seed treatments was quantified as 5.5 ± 0.23 nmol/L. This concentration falls within a range known to influence germination processes[22], supporting the validity of the smoke water as an effective treatment medium in this study.

Discussion

Germination responses of species

Responses to the SW and KAR_1 treatments varied greatly, depending on the species studied. *Alchemilla xanthochlora* and *Rhodiola rosea* showed no response to the SW and KAR_1 treatments. In the case of these two species, which are present in temperate and alpine habitats, respectively, and in the case of *R. rosea*, also in Arctic regions. Although fires are prevalent in boreal regions such as Alaska, Canada, Scandinavia, and Siberia[82], there

Treatment		FGP (%)	T50 (days)	MGR (%/day)	GSI
H_2O		65.00 ± 6.22	9.26 ± 0.72	9.03 ± 0.62	3.54 ± 0.38
GA_3		51.00 ± 11.14	8.88 ± 0.48	9.26 ± 0.56	2.72 ± 0.41
KAR_1 (µM)	0.001	72.50 ± 2.52 †	6.39 ± 0.08 ** ††	11.91 ± 0.48 ** ††	5.05 ± 0.27 * ††
	0.01	71.50 ± 5.26 †	6.55 ± 0.30 ** ††	11.98 ± 0.89 ** ††	4.93 ± 0.24 ††
	0.1	76.50 ± 3.42 ††	6.04 ± 0.22 ** ††	12.77 ± 1.18 ** ††	5.59 ± 0.43 ** ††
	1	80.50 ± 13.99 ††	5.90 ± 0.23 ** ††	12.96 ± 0.71 ** ††	6.06 ± 1.17 ** ††
	10	73.00 ± 5.29 ††	5.73 ± 0.29 ** ††	13.13 ± 1.58 ** ††	5.59 ± 0.41 ** ††
Smoke water (dilution)	1:1000	72.00 ± 6.32 †	7.32 ± 0.42 ** ††	10.65 ± 0.44	4.57 ± 0.25 ††
	1:100	69.50 ± 11.82	6.70 ± 0.62 ** ††	11.75 ± 0.67 ** ††	4.67 ± 0.77 ††
	1:10	63.00 ± 10.13	6.72 ± 0.53 ** ††	11.28 ± 0.63 *	4.20 ± 0.79 †
	1:1	0	-	-	-
Food-grade liquid smoke (dilution)	1:1000	59.00 ± 7.39	8.15 ± 0.48	9.97 ± 1.09	3.24 ± 0.51
	1:100	63.00 ± 4.76	8.71 ± 0.64	8.89 ± 0.47	3.20 ± 0.17
	1:10	3.5 ± 7 ** †	6.47 ± 12.94** ††	0.95 ± 1.89** ††	0.07 ± 0.13** †
	1:1	0	-	-	-

Table 5. Final germination percentage (FGP), time required for 50% of total germination (T50), mean germination rate (MGR), and germination speed index (GSI) of *Verbena officinalis* seeds subjected to different treatments of KAR_1, smoke water and food-grade liquid smoke. Values are means ± standard deviations (n = 4). Treatments with no germination are indicated by an FGP of 0; for these treatments, other parameters are not applicable and are therefore omitted (–). Significant differences in p-values after adjustment using Tukey's Honest Significant Difference (HSD) method are denoted with (*) indicating $p < 0.05$ and (**) indicating $p < 0.01$ with reference to the negative control (water). For the positive control (GA_3), (†) indicates $p < 0.05$ and (††) $p < 0.01$.

Treatment		FGP (%)	T50 (days)	MGR (%/day)	GSI
H_2O		48.50 ± 9.85 ††	10.57 ± 0.30 ††	9.01 ± 0.25 ††	2.23 ± 0.40 ††
GA_3		94.50 ± 11.00 **	8.42 ± 0.13 **	11.10 ± 0.25 **	5.31 ± 0.65 **
KAR_1 (µM)	0.001	94.50 ± 9.71 **	10.10 ± 0.14 ††	9.72 ± 0.26 ††	4.71 ± 0.47 **
	0.01	99.00 ± 2.00 **	9.63 ± 0.33 ††	10.06 ± 0.18	5.11 ± 0.14 **
	0.1	96.00 ± 5.66 **	8.89 ± 0.31 **	10.43 ± 0.55 **	5.25 ± 0.47 **
	1	100.00 ± 0.00 **	8.51 ± 0.35 **	11.10 ± 0.38 **	5.70 ± 0.17 **
	10	98.50 ± 3.00 **	9.04 ± 0.51 **	10.32 ± 0.63 *	5.31 ± 0.41 **
Smoke water (dilution)	1:1000	71.50 ± 2.52 ** ††	9.72 ± 0.66 ††	9.79 ± 0.57 †	3.73 ± 0.23 ** ††
	1:100	92.50 ± 8.70 **	9.25 ± 0.46 **	10.19 ± 0.18 *	4.90 ± 0.49 **
	1:10	99.00 ± 1.15 **	10.46 ± 0.51 ††	8.95 ± 0.79 ††	4.55 ± 0.48 **
	1:1	0	-	-	-
Food-grade liquid smoke (dilution)	1:1000	90.50 ± 3.42 **	9.16 ± 0.44 **	10.13 ± 0.46	4.67 ± 0.04 **
	1:100	95.00 ± 4.76 **	9.33 ± 0.23 **	9.69 ± 0.45 ††	4.77 ± 0.33 **
	1:10	1.00 ± 2.00 **	4.38 ± 8.75** ††	1.39 ± 2.78** ††	0.03 ± 0.06**
	1:1	0	-	-	-

Table 6. Final germination percentage (FGP), time required for 50% of total germination (T50), mean germination rate (MGR), and germination speed index (GSI) of *Veronica officinalis* seeds subjected to different treatments of KAR_1, smoke water and food-grade liquid smoke. Values are means ± standard deviations (n = 4). Treatments with no germination are indicated by an FGP of 0; for these treatments, other parameters are not applicable and are therefore omitted (–). Significant differences in p-values after adjustment using Tukey's Honest Significant Difference (HSD) method are denoted with (*) indicating $p < 0.05$ and (**) indicating $p < 0.01$ with reference to the negative control (water). For the positive control (GA_3), (†) indicates $p < 0.05$ and (††) $p < 0.01$.

is no evidence to date of germination responses of species from this ecoregion to fire. Nevertheless, many plant species from regions not prone to fire show responses to smoke-derived chemical stimulation[43,48–54,56,57,83]. It may be that other cues such as seed storage time, moisture, temperature or light could be prerequisites for

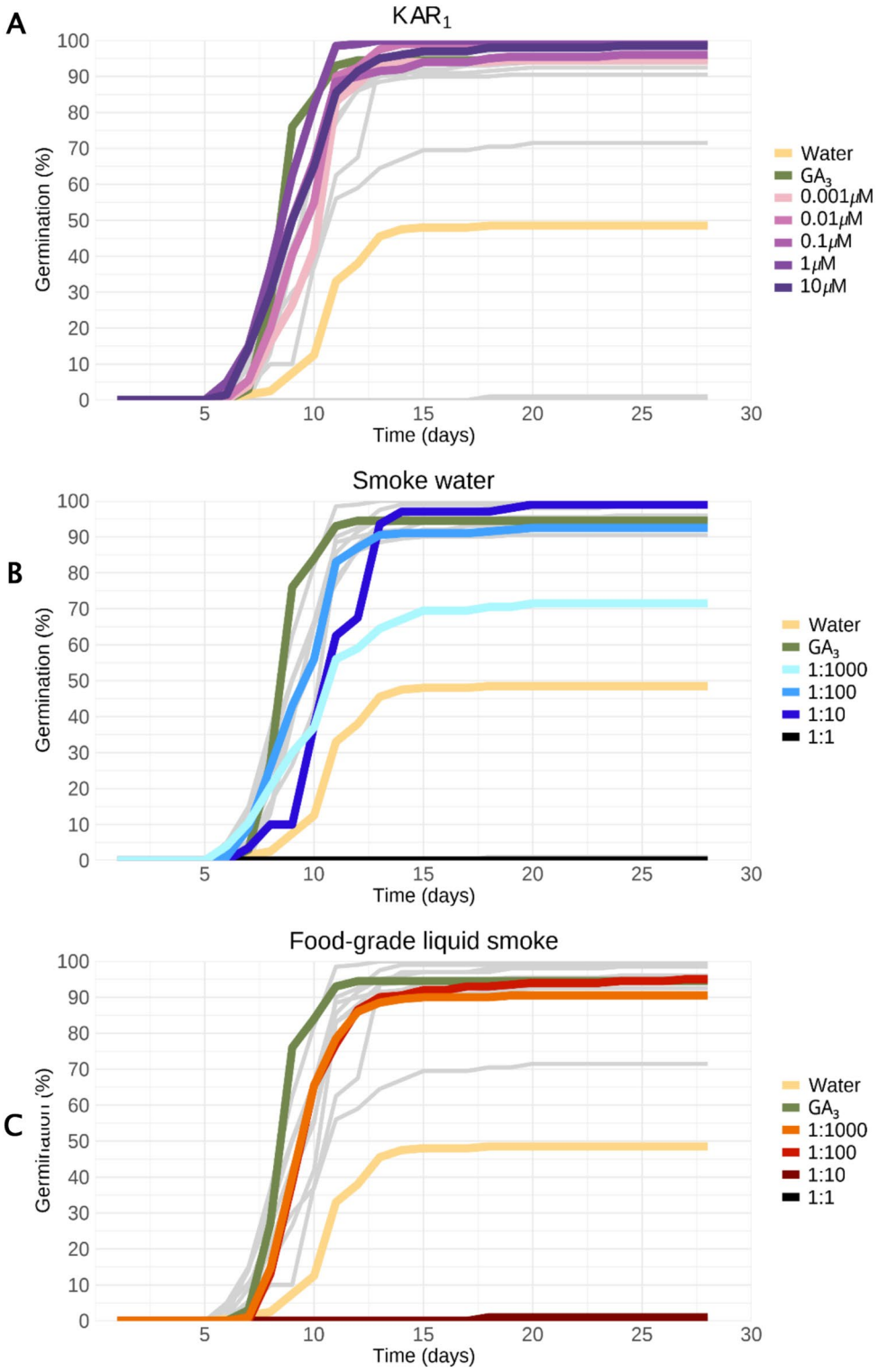

Fig. 4. Effects of different treatments on the cumulative germination percentage over time of *Veronica officinalis*: (**A**) KAR_1, (**B**) smoke water, (**C**) food-grade liquid smoke. Grey lines in each plot represent the treatments displayed in the other two plots. Data points represent mean germination percentages (n = 4).

increasing seed sensitivity to KAR_1 or SW treatments. Therefore, at this stage we can only declare an undetected response for both species.

Alchemilla xanthochlora and *Rhodiola rosea,* like the other species studied here, are little researched and nothing is known about their dormancy types, which are likely to differ. *Alchemilla xanthochlora* already has a good natural germination rate with a promotion of 40% with GA_3 (80.5% *vs* 57.5%), unlike *Rhodiola rosea* for

which GA_3 increased the germination rate by a factor of 2.5 (50.5% *vs* 14.5%). While it appears that the KAR-mediated response generally requires a functional GA biosynthesis and signalling pathway, this can be bypassed under specific conditions[84,85]. There is correlative evidence for the relationship between the germination responses to KAR and GA signals[31], but this is likely due to the KAR-KL and GA signalling pathways being tightly integrated, and the relative flux determining whether a seed germinates or not[86].

The results with *Verbena officinalis* are difficult to interpret. The batch of seeds used without treatment showed an unusually high germination rate of 65%, compared with less than 25% in previous work[58]. In stark contrast, other authors have reported germination of greater than 80% (Muñoz López de Bustamante 1987). The conflicting findings in this species requires further investigation. Aromatic and medicinal plants, except a few species such as *Lavandula angustifolia*, are generally grown on small areas, which does little to encourage plant breeding. It is therefore difficult to trace the seeds purchased, especially in the absence of selected varieties. As a result, apart from *Lavandula angustifolia*, it has only been possible to work with a single seed accession per species. Genetic variability within a species, which can also affect the depth of dormancy, combined with differences in seed production and conservation conditions, required that these tests be repeated on several seed lots of different origins.

Lavandula angustifolia and *Veronica officinalis*, responded well to treatment with KAR_1 but showed different responses to SW. The two varieties of *Lavandula angustifolia* responded well to the highest concentrations of KAR_1, the effect being much more marked with the youngest batch of seeds ('Rapido'). Carla's less pronounced response to KAR_1 can be attributed to a varietal effect, as well as to the older age of Carla's seeds (harvested in 2009, compared to 2023 for Rapido). The lower seed vigour observed in Carla (50% with GA_3, compared to 67% for Rapido) likely accounts for Carla's weaker response to KAR_1.

The effect was similar to that of GA_3 commonly used for this species, with the exception of the slower germination rate. Germination rate also increased with increasing KAR_1 concentration. As the stock solution prepared for these experiments was 10 µM, it was not possible to test the response to higher concentrations of KAR_1. This would perhaps be beneficial for seed lots with lower germination vigour, as was the case with the 'Carla' variety. On the other hand, SW had no significant effect on germination in this species. But other work on a related species, *Lavandula stoechas*, has shown a positive response to SW (Çatav et al. 2014). This could be explained by a species effect but also by a different quality of SW. In the study by Çatav et al. (2014), SW was prepared by burning dry needles of *Pinus brutia* in metal containers for 30 min at 193 °C, followed by rinsing in distilled water for 10 min. A change in the chemical profile of SW could provide a different response to germination.

Veronica officinalis was the only species in our study to respond positively to both KAR_1 and SW. It was also the only species to respond positively to the 'Food-grade liquid smoke' product. Unlike *Lavandula angustifolia, Veronica officinalis* was very sensitive to the lowest concentrations of KAR_1 (1 nM). Although the GA_3 concentration was not optimised here (1000 ppm ≈ 2.89 mM), KAR_1 was just as effective as GA_3 at a concentration approximately three million times lower. The results even suggested that KAR_1 was effective at a concentration below 1 nM. The concentration of KAR_1 in the effective SW diluted 1:100 is in fact only 0.055 nM, although the action of the SW cannot be reduced to its KAR_1 content alone. For *Veronica officinalis*, as for the four other species studied, undiluted SW prevented any germination. Between the undiluted SW and the 1:10 dilution, the concentration of toxic or inhibitory compounds probably reached a threshold inhibiting the germination process in *Veronica officinalis*. Further research could investigate precisely where between the 100- and 1000-fold dilutions the maximum response is maintained and where the change within this range becomes linear. These are complex processes that often interact with other external parameters. Guerin et al. (2013) also demonstrated the positive effect of SW on a neighbouring species, *Veronica parnkalliana*, endemic to Australia. They found that a combination of heat and SW was able to break dormancy after spring and summer, but not after autumn or winter, indicating an annual cycle of dormancy in this species, with the seeds being much more sensitive to smoke-derived stimulation in the warmer months.

Contextual limitations and ecological factors

The results of this study are only valid under these specific experimental germination conditions. The actual field conditions experienced by these seeds likely varies significantly from the stable and optimal conditions of the germinator used in these experiments. Temperature plays a critical role, as the set of species under investigation in this study tend to have their dormancy lifted by cold stratification i.e. winter conditions, with germination generally following in spring. Therefore, soil temperatures are not only important during winter for breaking dormancy, but also crucial during spring when exceedingly cool temperatures may slow down germination processes. Future research should perform these same experimental treatments across a broader temperature range that is more in line with the expected field conditions. Ideally, laboratory studies should be combined with field studies, so that the germination response is studied in the appropriate ecological context. One of the strongest determinants of germination timing is seasonal conditions, which widely vary both geographically and temporally[87]. There are also significant effects from the maternal plant on germination phenology[88,89], so, understanding the role of reproductive phenology and the seed maturation conditions, we can better contextualise the germination response. These factors all contribute to the high variability in seed dormancy itself[17]. Seeds from one geographical region may exhibit significantly increased germination after KAR_1 treatment, while seeds from different regions or years may exhibit a varied response[90,91]. So, even in cases where KAR-responsiveness is known and confirmed, this may only be the case under a strict specific set of germination conditions, or depths or types of dormancies. Thus, the complex nature of dormancy can result in an increased probability of false-negative conclusions[31]. Furthermore, there tends to be prerequisites to increase the receptivity of seeds for smoke-stimulated dormancy alleviation, such as soil temperature and moisture, and seed moisture content[92].

Therefore, smoke and KARs should not be seen as miraculous, golden solutions for dormancy alleviation, but rather as one cue along a broad cascade of sequential or interdependent cues[31].

Complexities and considerations in using smoke water and karrikins

When studying the influences of smoke, SW or KARs on seed germination, it is important to keep in mind that these treatments are not identical[31]. SW is a highly complex solution containing hundreds if not thousands of compounds[93]. Smoke may contain germination stimulants, such as KARs, but also potential inhibitors, adjuvants and even neutral compounds that have no notable effect on germination[94]. The relative concentrations of all these compounds and their interactions in the SW solution will determine the final effect on germination. The quality between different batches of SW produced can vary considerably. The material used for combustion, the equipment, the producers themselves (human-to-human variability), the order in which batches are produced (e.g. later batches will likely have more residual burnt material in the vessels, depending on the method of production, than earlier batches), all introduce variation into the final products. So, we must be cautious when comparing the results between different studies, as the SWs used are not equivalent. Furthermore, while experimental research can demonstrate that smoke or SW may have an effect on germination, we must also be cautious about drawing mechanistic conclusions based on these results. Rather pure active compounds, e.g. KAR_1, should be used for mechanistic elucidations[31]. While we should exercise caution when comparing the KAR_1 concentrations of pure KAR_1 with those of SW formulations, quantifying the KAR_1 content of the SW produced in this study is immensely valuable. This is because KAR_1 is considered the most abundant karrikin in smoke[31]. The SW used in this study was calculated to have a KAR_1 concentration of 5.5 ± 0.23 nmol/L. This places the undiluted SW, which inhibits germination, between the two lowest KAR_1 concentrations tested (0.001 µM and 0.01 µM). Once this SW is diluted at least tenfold to 0.6 nmol/L KAR_1 concentration, it becomes tolerable for seed germination. Future research could expand on this by applying the same UHPLC-MS/MS method to quantify other karrikins and by testing SW derived from different plant materials and production methods. Compared to study we used as the basis for KAR_1 quantification, which reported KAR_1 concentrations in the range of 10–100 nmol/l among various batches of SW[76], the concentration of KAR_1 in the SW used in this study was quite low. Therefore, significant optimisation of the SW production process could be achieved. Increasing the concentration of KAR_1 in SW, may yield positive germination effects with SW treatment in species now demonstrated to be KAR_1-responsive, such as *L. angustifolia*.

Interface between the induction of stress responses and secondary metabolite production

Beyond dormancy breakage and increasing germination rates, smoke and KAR treatments also offer promising solutions to improve germination under stressful conditions. Priming rapeseed, *Brassica napus*, seeds with KAR_1 and SW increased the upper limit of the germination temperature range of 10 °C – 35 °C up to 40 °C and reduced the damaging effect of heat stress[55]. Another study on wheat, *Triticum aestivum*, demonstrated increased tolerance to salinity stress with KAR_1 treatment on both seeds and seedlings[95]. KAR_1 and SW have also been shown to improve seedling vigour. The percentage germination, number of leaves, shoot height and root development were all increased in maize after KAR_1 treatment[96]. The research laid out in this study could be expanded in the future to assess seedling growth parameters and whether stress tolerance is conferred to these MAPs. Monitoring seedling growth is of great importance because the ultimate objective is to improve the cultivation of these species. Germination improvement is merely the first step in this direction.

Determining optimal growing conditions for MAPs is complex because the biosynthesis of secondary metabolites, which are highly desired by the herbal industry, can be induced under stress conditions[97]. Abiotic stresses such as UV radiation[98–101] and drought[102–107] are known to be implicated in secondary metabolite production, as well as biotic stresses[97]. Therefore, in favourable environments, plants would direct their metabolic activities toward increasing the biomass of their photosynthetic organs and prioritising reproduction, rather than producing secondary metabolites that are usually needed to manage stress[108]. So, there is a trade-off between biomass production and secondary metabolite-associated quality in MAPs cultivation. The signalling pathways activated by KARs appear to upregulate the expression of stress-responsive genes[86,109–111]. One could therefore hypothesise that secondary metabolite production would be concomitantly upregulated given the link between stress responses and secondary metabolite production. Micropropagated bananas treated with SW showed increased total phenolic content in leaves, while KAR_1 increased the total phenolic content in both leaves and roots[112]. In another study, KAR_1 and SW were applied to hairy root cultures of red sage, *Salvia miltiorrhiza*, resulting in increased contents of salvianolic acid and rosmarinic acid[113]. Further studies on the same species, *S. miltiorrhiza*, found increased biosynthesis of flavonoids and terpenoids with KAR_1 and SW treatments[114]. These promising findings should encourage the exploration of enhancing secondary metabolite production in MAPs by treating seeds and seedlings with KARs or SW.

Ecological and evolutionary reasons for interspecies differences

The reason for the broad phylogenetic representation of this germination response remains the subject of much speculation. Some possibilities are that perhaps karrikins are produced by other means, such as chemical or microbial breakdown of biomass or following soil disturbance[115], or that karrikins are endogenously produced[41]. One of most widely held theories is that KARs resemble an as yet unknown butenolide-like plant hormone essential for regulating seed dormancy and plant development[31,116,117]. Multiple lines of evidence suggest that KAR_1 is not directly perceived by KAI2, leading researchers to conclude that KARs are likely metabolised prior to KAI2 perception[118].

Practical and regulatory considerations

Regarding the practical application of SW in agricultural settings, the main problem is that many germination inhibitors are present[94]. This would then require further processing or diluting of SW[119], whose production for large-scale use has not yet been standardised. This makes the fidelity of the entire process quite variable. So, while SW or aerosol smoke is much more cost-effective, KARs are preferable in many cases because they are chemically pure. However, KARs are currently very costly, so the adoption of this approach relies on a making a compromise between cost and convenience[31]. A general practical advantage is that KARs are active at such low concentrations. It has been estimated that 2–5 g/ha KAR_1 are sufficient to stimulate the germination for many responsive species in field settings[115]. While no specific stipulations exist at present for the use of KARs or SW in organic production, SW is a naturally derived product and could therefore be compatible with organic practices. The applicability of KARs is more unclear, since KARs can be produced synthetically or extracted from SW. However, laboratory purifications techniques typically use a range of chemical solvents and reagents, which would violate organic production principles[19]. Biochar is widely used in organic agriculture and has also been found to contain KARs[120]. Biochar would pose the same problems as SW in that the agricultural applicability would be limited by the inhibitory compounds present, but its use does offer a guiding light to how SW could be used in the organic production framework.

Based on the findings in this study, KAR_1 treatments at 1 µM and 10 µM concentrations are recommended to improve FGP of *Lavandula angustifolia*. Lower KAR_1 concentrations (0.001–0.1 µM) yielded only marginal improvements in germination so are not recommended. GA_3 treatment remains the most effective for both FGP and speed but may have limitations for organic or sustainable production contexts. Smoke water and food-grade liquid smoke treatments did not significantly increase lavender seed germination and so are not recommended.

For *Veronica officinalis*, KAR_1 treatments across a broad concentration range (0.001–10 µM) are recommended to maximise germination. Smoke water dilutions between 1:10 and 1:1000 and food-grade liquid smoke at 1:100 and 1:1000 dilutions also increased germination effectively. Stronger concentrations of smoke products (undiluted or 1:10 dilution for liquid smoke) inhibited germination and should be avoided.

For the other species (*Alchemilla xanthochlora*, *Rhodiola rosea*, *Verbena officinalis*) that did not respond positively to KAR_1 and smoke water, further research with different treatment concentrations or other smoke water formulations is needed before concluding that these treatments are not beneficial for these species.

Conclusion

To our knowledge, these are the first studies conducted on dormancy in cultivated species of aromatic, medicinal and perfume plants using smoke-derived compounds, either in the form of a pure compound (KAR_1) or in the form of smoke water. The positive responses from two of the species studied are encouraging.

Despite the promising effects of KAR_1 and SW, further research is required to optimise the formulations. Testing a wider range of KAR_1 concentrations and exploring different SW formulations, potentially derived from different plant materials, could yield more effective treatments for germination improvement and dormancy alleviation in these species. Going forward, other active chemicals in smoke, such as other karrikins and cyanohydrins, could be tested alongside and in conjunction with KAR_1 for a more comprehensive understanding of the dynamics of the germination response to smoke. Emphasis should be placed on optimising SW formulations, as they provide an organic-friendly solution for improving germination. This approach offers an avenue for developing a sustainable production system. Future studies should also vary the environmental conditions to better simulate field settings. Overall, this research demonstrates the potential for smoke-derived compounds in overcoming dormancy or improving germination in the selected MAP species, which could confer significant advantages in optimising their cultivation. The promising results on non-fire-followers should also prompt testing other MAP species of economic or agronomic interest. Furthermore, if smoke treatments do elicit a stress response in plants, the downstream effects on secondary metabolite production, which is relevant for MAPs, should be assessed and documented.

Data availability

The datasets supporting the current study is available from the corresponding author upon request.

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Conceptualization and methodology: SSR, XS; formal analysis and investigation: SSR, XS, ADN; data curation: SSR; writing-original draft preparation: SSR; writing-review and editing: SSR, LN, LS, ADN, AB, XS; supervision: XS, LN. All authors have read and approved the manuscript.

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Declarations

Competing interests
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