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Effect of smoke-derived compounds on the germination of Cerrado ground layer species

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Abstract

Background

Smoke is a key environmental cue for seed germination of species in fire-prone ecosystems. Several smoke-derived compounds influence this process by regulating reactive oxygen species and interacting with plant hormones. We aimed to evaluate the effects of three smoke-derived compounds on the germination of Cerrado ground layer species. We tested cellulose-derived karrikin 1 (KAR_1), lignin-derived hydroquinone (HYD), and mandelonitrile cyanohydrin (MAN) under controlled laboratory conditions.

Results

Different concentrations of KAR_1 and MAN were effective in stimulating germination, while HYD at the highest concentration was detrimental to seed germination. All tested compounds were effective in increasing the germination percentage of at least one species and decreasing the time to 50% germination of the grass *Sorghastrum minarum*. The response to smoke-derived compounds was species-specific and varied depending on their concentration.

Conclusion

It was concluded that the germination response of some Cerrado ground-layer plants is stimulated or inhibited in the presence of smoke-derived compounds, depending on their concentration. The efficacy of smoke-derived compounds should be evaluated in a range of ecological contexts. From a management perspective, the utilization of effective compounds for each species has the potential to enhance the success of seed-based restoration.

Introduction

Fire is linked to the evolution of plant traits in fire-prone ecosystems around the world (Bond and Wilgen 1996; Keeley et al. 2011; Lamont and He 2017; Pausas and Keeley 2009), such as conifer forests (Kilgore and Taylor 1979), eucalypt forests (Christensen and Abbott 1989), grasslands (Tainton and Mentis 1984), Mediterranean vegetation (Moreno and Oechel 2012), and savannas (Trollope 1984; Beerling and Osborne 2006). The Cerrado is a fire-prone ecosystem, where open savannas have a fire frequency of 1 to 5 years (Eiten 1972; Miranda et al. 2009). Fire consumes most of the aboveground vegetation (Rissi et al. 2017; Rodrigues et al. 2021) and alters soil physics and chemistry (Rundel 1981). Physical alterations include soil surface temperature increases during and after the fire (Daibes et al. 2017; Lamont et al. 2019) and an increase in soil hydrophobicity, decreasing water infiltration and increasing surface runoff and erosion (Scholl 1975; Rundel 1981). Soil chemistry is altered due to changes in pH and the distribution of nutrients (Sweeney 1956; Grier 1975). In addition, the smoke released after a fire can promote seed germination (De Lange and Boucher 1990). Smoke is rich in water-soluble chemical

stimulants (Nelson et al. 2012) that are stable at high temperatures and active at very low concentrations (Flematti et al. 2004; Nelson et al. 2012).

Smoke can play a dual role in regulating germination and recruitment in post-fire environments, and this duality is regulated by moisture conditions (Light et al. 2002). Under more limited water availability, smoke-derived compounds may accumulate in the upper soil layers, where inhibitory substances, at high concentrations, can prevent germination (Nelson et al. 2012; Khatoon et al. 2020). Conversely, Soós et al. (2019) suggested that, following rainfall, these highly soluble inhibitory compounds are diluted or leached away, enabling germination-promoting compounds to take effect even at low concentrations.

One of the primary germination-stimulating compounds found in plant-derived smoke is the butenolide karrikinolide (KAR_1), chemically known as 3-methyl-2H-furo[2,3-c]pyran-2-one, which is derived from pure xylose, glucose, or cellulose and is active as a germination stimulant at sub-nanomolar concentrations (Flematti et al. 2004; Van Staden et al. 2004). Structurally similar to strigolactone plant hormones (Nelson et al. 2011), KAR_1 plays a key role in germination and seedling development (Moyo et al. 2022). It also upregulates an aquaporin gene of the TIP (tonoplast intrinsic protein) subfamily, which controls the entry of water and small neutral solutes into the cell (Soós et al. 2010). Furthermore, it is associated with cell cycle activation (Kępczyński et al. 2010) and the expression of light-induced genes (Nelson et al. 2010). Nevertheless, not all smoke-responsive species react to KAR_1 (Downes et al. 2010), indicating the presence of additional non-karrikin stimulants in the smoke (Flematti et al. 2011; Nelson et al. 2012).

Other compounds present in smoke, such as cyanohydrins, e.g., glyceronitrile (Flematti et al. 2011) and potentially related analogues like mandelonitrile (Flematti et al. 2013; Khatoon et al. 2020) and hydroquinone (Kamran et al. 2017), may also influence germination. Cyanohydrins have been identified as components of secondary metabolism in plants, as part of their defense and signaling mechanism (Flematti et al. 2013; Arnaiz et al. 2023). Ecologically, cyanohydrins play a role in plant recovery in post-fire environments, remaining in the topsoil for prolonged periods until conditions become favorable for seed germination (Flematti et al. 2011). Upon contact with water, typically following rainfall, they slowly release cyanide through hydrolysis (Flematti et al. 2013). Cyanide, at low concentrations, breaks dormancy and stimulates seed germination (Major & Roberts 1968; Flematti et al. 2011; Nelson et al. 2012). Hydroquinones are phenolic compounds found in plant secondary metabolites (Giner et al. 2022). They have been identified in plant-derived smoke as a pyrolytic product of lignin and also affect germination (Kamran et al. 2017; Cao et al. 2023). In addition to their potential effect on germination (Kamran et al. 2017), these compounds can inhibit the growth of fungi in seeds (Elwakil 2003) and enhance seedling growth and development (Li et al. 2009). However, the efficacy of hydroquinones also depends on their concentration: low concentrations increase the germination and growth of lettuce, while high concentrations inhibit seedling growth (Kamran et al. 2017).

In the Cerrado, some studies have evaluated the effect of smoke on germination, with some species benefiting from faster or increased germination (Le Stradic et al. 2015; Fichino et al. 2016; Zirondi et al. 2019a, b; Ramos et al. 2019; Gorgone-Barbosa et al. 2020; Fernandes et al. 2021; Sanchez et al. 2022;

Motta et al. 2024). However, to our knowledge, there are no studies evaluating the effect of isolated compounds. It is important to understand the individual effects of each compound to determine which one stimulates germination and at what concentration. This knowledge is particularly relevant for restoration and ecological management. It can guide techniques such as seed priming, in which seeds are pre-soaked in smoke water or treated with its isolated compounds, to increase germination of species in seed-based restoration projects (Kępczyński and Kępczyńska 2023). Therefore, we evaluated the effect of three isolated smoke-derived compounds: hydroquinone (HYD), a lignin-derived compound; karrikin 1 (KAR_1), a cellulose-derived compound; and mandelonitrile (MAN), a cyanohydrin, on the germination of nine Cerrado ground layer species. This study aimed to address the following question: Do smoke-derived compounds influence the germination of the Cerrado ground layer species? We hypothesized that the species would benefit from smoke-derived compounds, as evidenced by the positive response exhibited by species of other fire-prone ecosystems, with the germination response varying according to the concentration (Flematti et al. 2004, 2011; Çatav et al. 2024b; Kamran et al. 2017).

Materials and methods

Study area and seed collection

Seed collection was conducted in areas of the Cerrado in southeastern Brazil, specifically from savanna vegetation and wet grasslands (*campo úmido*). Savannas are characterized by a continuous herbaceous layer dominated by C_4 grasses, with forbs, scattered shrubs, and small trees (Coutinho 1982). These areas have well-drained soils with high concentrations of aluminum combined with a lack of available nutrients (Haridasan 1992). Wet grasslands are characterized by the prevalence of graminoids, with scattered forbs and shrubs. These wetlands are distinguished by their hydromorphic soils and elevated organic matter content (Durigan et al. 2022). Seed collection of savanna species was conducted at the Estação Ecológica de Santa Bárbara (22°47′05″S, 49°14′28″W), while seeds of wet grassland species were collected at the Estação Ecológica de Assis (22°36′36″S, 50°25′23W) and the Estação Ecológica de Santa Bárbara (22°47′22S, 49°14′31W).

Seeds of nine species from the families Cyperaceae, Lamiaceae, and Poaceae were collected, six from the savanna environment and three from wet grasslands (Supplementary Table S1). The study species were selected mainly based on seed availability. For each species, seeds were collected from at least 10 individuals and stored in paper envelopes at room temperature until the start of the experiments. Seeds were selected using a stereomicroscope, and only full and healthy seeds (i.e., visually undamaged) were used in the experiments. Details on vegetation type, collection site, date of collection, and seed storage duration for each species are described in Table S1. The seeds were stored in paper envelopes, in the dark and at room temperature.

Germination experiment

Three smoke-derived compounds were evaluated: hydroquinone (HYD, purchased from Sigma-Aldrich®) was tested at low (9.1 µM), medium (91 µM), and high (908 and 90838 µM) concentrations; karrikin 1 ($KAR_{1,}$ purchased from Biosynth®) was applied at low (0.01 and 0.1 µM), medium (1 µM), and high (10 µM) concentrations; and mandelonitrile (MAN, purchased from Sigma-Aldrich®) was tested at low (0.1 and 1 µM), medium (10 and 50 µM), and high (100 µM) concentrations. The concentrations of isolated compounds were determined based on the studies of Kamran et al. (2017), Long et al. (2011), and Flematti et al. (2011), respectively.

Seeds were placed in Petri dishes (9 cm in diameter), lined with filter paper (with a paper weight of 80 g m^{-2}), and soaked in 3 ml of the following treatments: distilled water (control), HYD, $KAR_{1,}$ and MAN. The seeds were incubated for 24 hours in germination chambers at 27°C and in darkness. After 24 hours, the filter paper containing smoke-derived compounds was replaced with new filter paper, and distilled water was added. Subsequently, the germination chambers were programmed with a 12-hour photoperiod and a constant temperature of 27°C (Fichino et al. 2016).

For all species, four replicates were employed per treatment. The number of seeds per replicate was approximately 25, with the precise number varying by species Table S1. The experiments were monitored daily for 30 days. The number of seeds that germinated was recorded, and those seeds were subsequently removed. Seeds exhibiting a root protrusion of 1 mm were deemed to have germinated. For each replicate, the germination percentage was calculated using the following calculation: $G = (N_g / N_t) \times 100$, where N_g is the number of germinated seeds and N_t is the total number of seeds per replicate.

The time to 50% germination (t50) was calculated using linear interpolation between the two consecutive observation days where cumulative germination crossed the 50% threshold (Farooq et al. 2005). The formula was adapted by defining N as the total number of seeds per replicate rather than the final germination count (Soltani et al. 2015). The calculation was applied as follows: t50 = t□ + [(N/2 - n□) / (n□ - n□)] × (t□ - t□) where: N = total number of seeds per replicate; t□ = last day of observation before reaching 50% germination; t□ = first day of observation after reaching 50% germination; n□ = cumulative number of seeds germinated at time t□; n□ = cumulative number of seeds germinated at time t□. The calculation was performed only for replicates that achieved cumulative germination greater than 50% of the total seeds.

Seed Viability

Seeds that did not germinate were soaked in tetrazolium (1% w/v solution of 2,3,5-triphenyl-2H-tetrazolium chloride, Lakon 1949). The seeds were incubated in the dark at a temperature of 27°C for 72 hours, except for *Bulbostylis fimbriata*, which required a total incubation period of 96 hours. After the designated period, the seeds were dissected using a scalpel and a stereomicroscope to ascertain the coloration of the embryos (Fig. 1). Seeds were classified as viable (pink embryo) or non-viable (unstained embryo, Supplementary Table S2).

Statistical Analysis

To evaluate the germination percentage, we used generalized linear mixed models (GLMM) with a binomial distribution and a logit link function. For the t50, we applied a linear mixed model (LMM) with a Gaussian distribution (identity link). Treatments were coded as categorical factors and included as fixed effects, and species as a random effect. Data were analyzed separately for each compound (Hydroquinone, Karrikin 1, and Mandelonitrile).

To assess the impact of smoke-derived compounds, with each species evaluated separately, we carried out generalized linear models (GLMs). For the germination percentage, we used a binomial distribution with logit link function, the treatment (coded as a categorical factor) as the predictor variable, and the number of germinated and non-germinated seeds as the response variable. An exception was made for *Ctenium polystachyum*, for which a Gaussian distribution was used due to the pronounced difference in germination rates between the control and KAR_1 treatments, which hindered the fit of a binomial model; however, the Gaussian model adequately detected and represented this significant effect. For the t50 we fitted a linear model (LM), the predictor variable was the treatment applied (coded as a categorical factor), while the response variable was the time for 50% of seeds to germinate (in days), and the Gaussian distribution (identity link) was used. Only treatments with three or more valid replicates were considered for statistical analyses.

To assess the suitability of the models fitted to the data, a residual analysis was used with the DHARMa package (Hartig 2022). All analyses were then subjected to the Tukey test, which was conducted using the emmeans package (Lenth 2024). For GLMM and LMM we used the lme4 package (Bates et al. 2015), the estimated parameters can be found in Supplementary Table S3. For GLM and LM we used the stats package, the estimated parameters can be found in Supplementary Table S4. Tests were performed using R software version 4.3.2 (R Core Team 2023).

Results

Treatment of Cerrado seeds with hydroquinone (HYD) did not appreciably affect germination percentage (Gmax), except for the highest concentration (~ 9083 mM), which exhibited an inhibitory effect (Fig. 2a). Karrikin 1 ($KAR_{1)}$ significantly increased the percentage of seeds that germinated at low, medium, and high concentrations (Fig. 2b). Mandelonitrile (MAN) enhanced the germination percentage at medium and high concentrations between 10 µM and 100 µM (Fig. 2c). Considering the t50 for all species together, HYD, KAR_1, and MAN had no significant impact on the speed of germination (Fig. 3a-d). The different species studied not only represent three important Cerrado families from the ground-layer, but also structurally distinct dispersal units (seeds and dry fruits; hereafter referred to as seeds for simplicity), most of which had viable embryos (Fig. 1). According to our germination analysis, most species had 70–90% viable seeds (Table S2).

The germination percentages indicated specific patterns for each species (Fig. 4). The Poaceae species *Aristida megapotamica* showed increased germination percentage in response to high concentrations of HYD (Fig. 4c). *Ctenium polystachyum* (Poaceae) responded positively to KAR_1 and MAN with a notable level of responsiveness in the highest concentration of KAR_1, reaching 100% germination, while the germination in the control treatment was 2% (Fig. 4d). *Saccharum villosum* (Poaceae) responded positively to all isolated compounds (Fig. 4g). Conversely, *Gymneia interrupta* (Lamiaceae) and the Poaceae species *Andropogon leucostachyus*, *Paspalum pectinatum* and, *Sorghastrum minarum* exhibited a reduced germination percentage in response to HYD at high concentrations (Fig. 4a, b, f, h). Furthermore, a detrimental impact of the highest concentration of HYD on *Sorghastrum minarum* (Poaceae) seed viability was observed, as a high proportion of the non-germinated seeds (62%) lost their viability (Table S2). Additionally, high concentrations of HYD were found to induce seed mortality and subsequent fungal infestation in *Andropogon leucostachyus*, *Gymneia interrupta*, and *Paspalum pectinatum* (Table S2). The germination of *Eriochrysis cayensis* (Poaceae) was neutral to smoke-derived compounds (Fig. 4e), and *Bulbostylis fimbriata* (Cyperaceae) did not germinate in any treatment (Table S2).

Regarding the four species that showed cumulative germination greater than 50% of the total seeds (Fig. 5), only *Sorghastrum minarum* showed significant differences compared to the control. The species exhibited faster germination (positive effect) in the presence of HYD at medium concentrations, KAR_1 and MAN at low, medium, and high concentrations (Fig. 5d).

Discussion

Smoke from burning plant biomass contains a variety of compounds that stimulate or inhibit germination, and may exert contrasting effects on seed germination, depending on their chemical nature and concentration (Baldwin et al. 1994; Light et al. 2010; Nelson et al. 2012; Baskin and Baskin 2014). Among these, HYD is a redox-active compound with a biochemical profile characterized by its antioxidant capacity, acting through reversible oxidation-reduction reactions, during which it can oxidize to p-benzoquinone, which has an electrophilic character allowing it to undergo nucleophilic attack, enabling it to modulate reactive oxygen species (ROS) and maintain the redox balance within cells (El-Najjar et al. 2011; Giner et al. 2022). Its influence on ROS levels may also indirectly affect the activity of enzymes involved in endosperm weakening, such as cell wall hydrolases, which facilitate radicle protrusion during germination (Gomes and Garcia 2013; Baxter et al. 2013). Thus, at low concentrations, HYD has been shown to act as an antioxidant, neutralizing ROS and promoting a chemically stable cellular environment that could support germination (Giner et al. 2022; Gomes and Garcia 2013). However, HYD may exhibit dual biochemical behavior, acting as a pro-oxidant at high concentrations, generating excessive ROS, disturbing redox homeostasis, impairing cellular structures, and ultimately disrupting metabolic pathways essential for germination and seed viability (El-Najjar et al. 2011; Gomes and Garcia 2013). In our study, HYD at higher concentrations generally inhibited germination and caused seed mortality. Germination inhibition at HYD high concentrations was also observed in lettuce (Kamran

et al. 2017). Despite these inhibitory effects, we observed positive germination responses in certain species. In *Saccharum villosum*, HYD increased germination percentage at all concentrations, and *Aristida megapotamica* at the highest concentration. This suggests that species-specific physiological responses, such as redox buffering capacity or sensitivity to oxidative stress, likely modulate the balance between beneficial and detrimental effects (Oracz et al. 2009; Leymarie et al. 2012; Gomes and Garcia 2013). Different species-dependent responses were also observed for another lignin-derived smoke compound, syringaldehyde, which is a positive germination cue for germination in *Nicotiana attenuata* (Cao et al. 2021), despite being ineffective in stimulating the germination of species native to the Mediterranean Basin (Çatav et al. 2024a).

In addition, following a fire occurrence, both karrikins and cyanohydrins can be deposited in the soil and remain until the next rainfall event (Flematti et al. 2011, 2013). The presence of these smoke compounds in the soil signals that pre-existing vegetation has been removed, and that competitive pressure is temporarily reduced, creating optimal conditions for germination and seedling establishment (see, Nelson et al. 2012; Morffy et al. 2016; Soós et al. 2019). Despite sharing this ecological context, they belong to distinct chemical classes and act through different biochemical pathways. KAR_1 is a butenolide that is perceived via the KAI2-MAX2 signaling cascade (Guo et al. 2013). It induces the expression of key genes associated with seed germination (Nelson et al. 2009, 2010, 2012; Meng et al. 2016, 2017; Morffy et al. 2016, Kępczyński 2018) and also plays a role in controlling ROS levels (Cembrowska-Lech et al. 2015). In contrast, MAN is a cyanohydrin that acts on germination through the slow release of cyanide in contact with water (Flematti et al. 2011, 2013), a mechanism that modulates both ROS levels (Oracz et al. 2009) and ethylene-related signaling pathways (Oracz et al. 2008; Gniazdowska et al. 2010).

The evolutionary history of grass species is closely linked to fire (Edwards et al.2010; Lamont 2023). Our findings indicate that both KAR_1 and MAN increased the germination percentage of *Ctenium polystachyum* and *Saccharum villosum*. Our results corroborate studies conducted with species from the Mediterranean Basin that reported positive effects of these compounds on smoke-responsive species (Çatav et al. 2024a, b). Furthermore, all smoke-derived compounds effectively reduced the t50 of *Sorghastrum minarum*. This decrease may confer a competitive advantage by facilitating earlier seedling establishment in post-fire environments, where light, space, and nutrients are temporarily more abundant (Keeley and Fotheringham 2000, Daibes et al. 2019). Nevertheless, treatments involving smoke-derived compounds were ineffective in alleviating seed dormancy in *Bulbostylis fimbriata* and *Eriochrysis cayensis*, suggesting that the germination responses may be regulated by other ecological or physiological factors.

Some species may respond to smoke, but not to its isolated compounds, or vice versa (Daws et al. 2007; Downes et al. 2010; Keeley and Pausas 2018; Kępczyński 2018). The diverse range of compounds present in smoke (Baldwin et al. 1994; Burger et al. 2018) may explain why some species respond uniquely to smoke rather than to individual compounds, likely due to the synergistic interactions between them, as exemplified by KAR_1 and MAN (see Çatav et al. 2018; 2024a). The responses to isolated

compounds may facilitate the identification of specific compounds responsible for promoting or inhibiting germination in particular species. These contrasts highlight the importance of examining different smoke-derived compounds, as well as their effects across species and at different concentrations, since both germination-promoting effects and toxicity are species- and dose-dependent.

Conclusion

The present findings demonstrate that germination responses in certain Cerrado ground-layer plants are stimulated by smoke-derived compounds such as KAR_1 and MAN. The species-specific response is dependent upon the concentration of isolated compounds: for example, HYD harms seed germination and viability at high concentrations; however, it has stimulatory effects on germination in certain species. This shows that we must better understand the individual effects of the smoke-derived compounds, as they vary by species and concentration. We emphasize the importance of studying the effects of smoke-derived compounds in different ecological contexts. From a management perspective, identifying which compounds are most effective for each species can enhance the success of seed-based restoration practices.

Declarations

Supplementary information

The data generated in this study are available online in the electronic supplementary material.

Conflict of interest declaration

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Author Contribution

G.S.T.M.: conceptualization, methodology, visualization, writing-review and editing. H.L.Z.: visualization, writing-review and editing. A.F.: funding acquisition, project administration, resources, writing-review and

editing. T.S.: funding acquisition, visualization, writing-review and editing. J.O.C: conceptualization, funding acquisition, methodology, writing-review and editing. G.L.-M.: funding acquisition, resources, supervision, visualization, writing-review and editing. R.M.K.: conceptualization, methodology, funding acquisition, resources, supervision, visualization, writing-review and editing. The authors gave final approval to the submitted version.

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Data Availability

The data generated in this study are available online in the electronic supplementary material.

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Figures

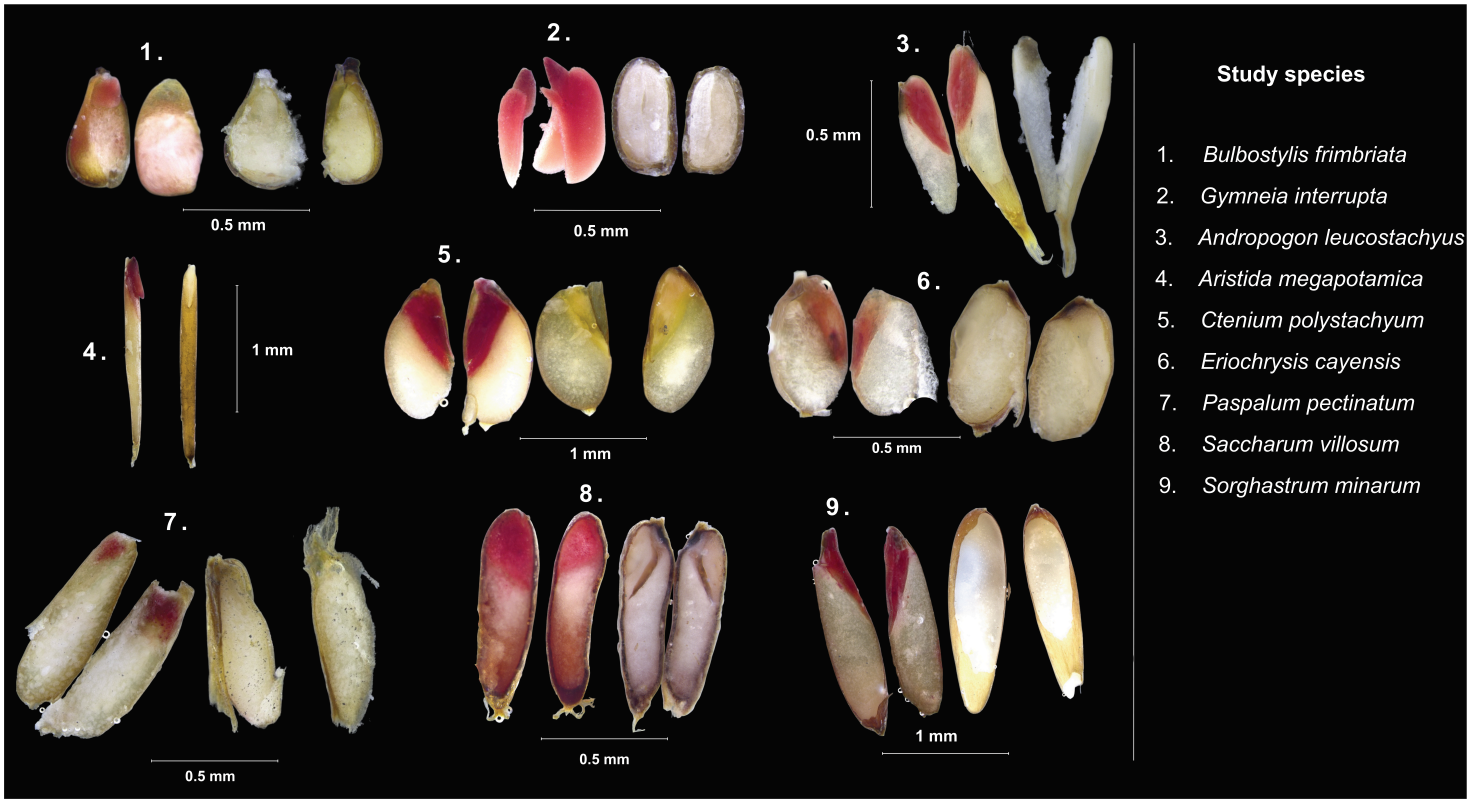


Figure 1

Embryo staining results using the tetrazolium test. The image illustrates a viable embryo (left), displaying characteristic staining indicative of metabolic activity, and a non-viable embryo (right), showing no staining due to lack of viability.

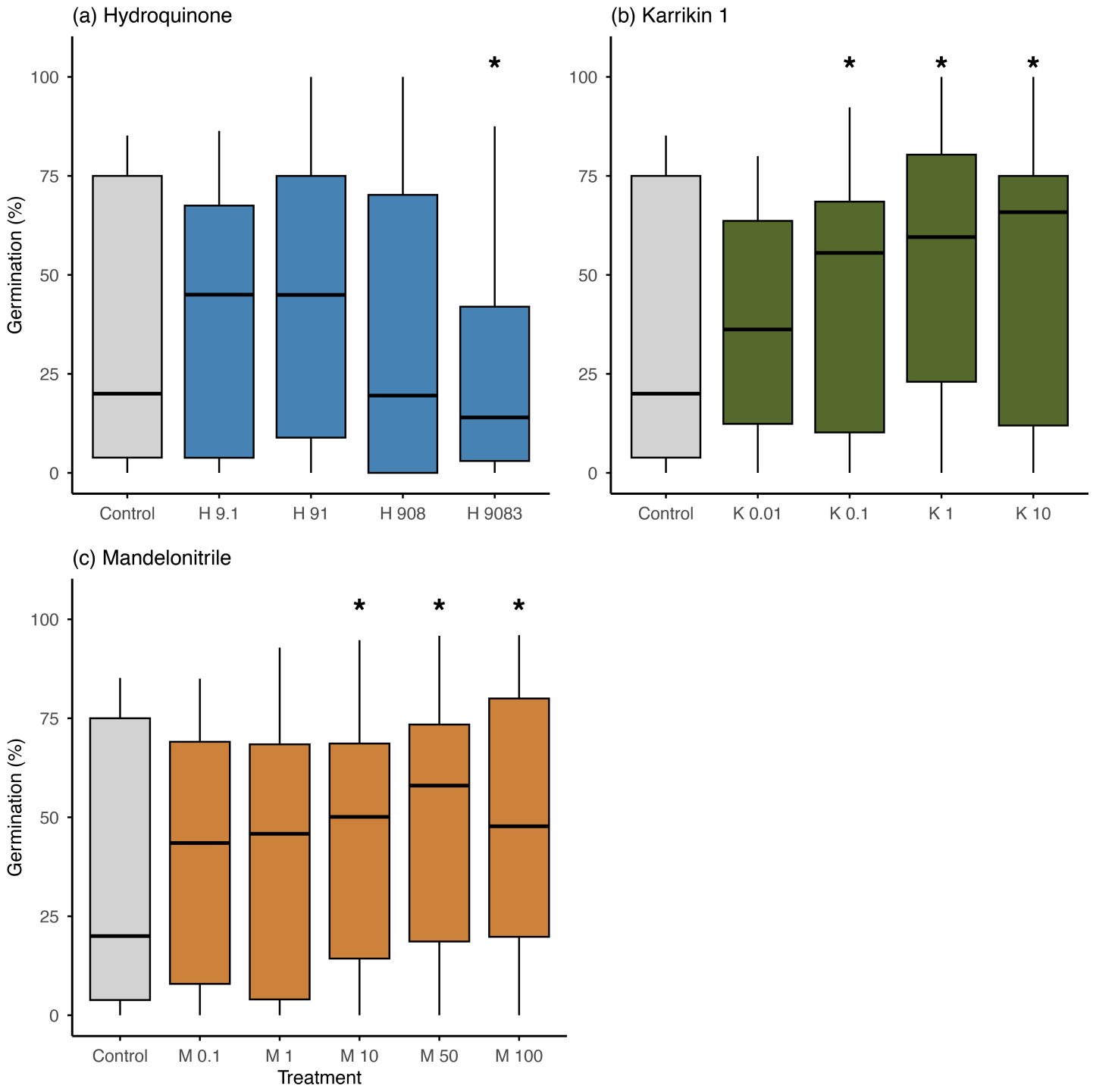


Figure 2

Effect of smoke-derived compounds on germination percentage across species, (a) hydroquinone (blue, H), (b) Karrikin 1 (green, K), and (c) mandelonitrile (orange, M) in different concentrations of µM. The boxes show the quartiles, and the lines show the median. Asterisks represent significant differences between treatments compared to the control (distilled water, light gray) according to GLMM and Tukey tests ($p \leq 0.05$).

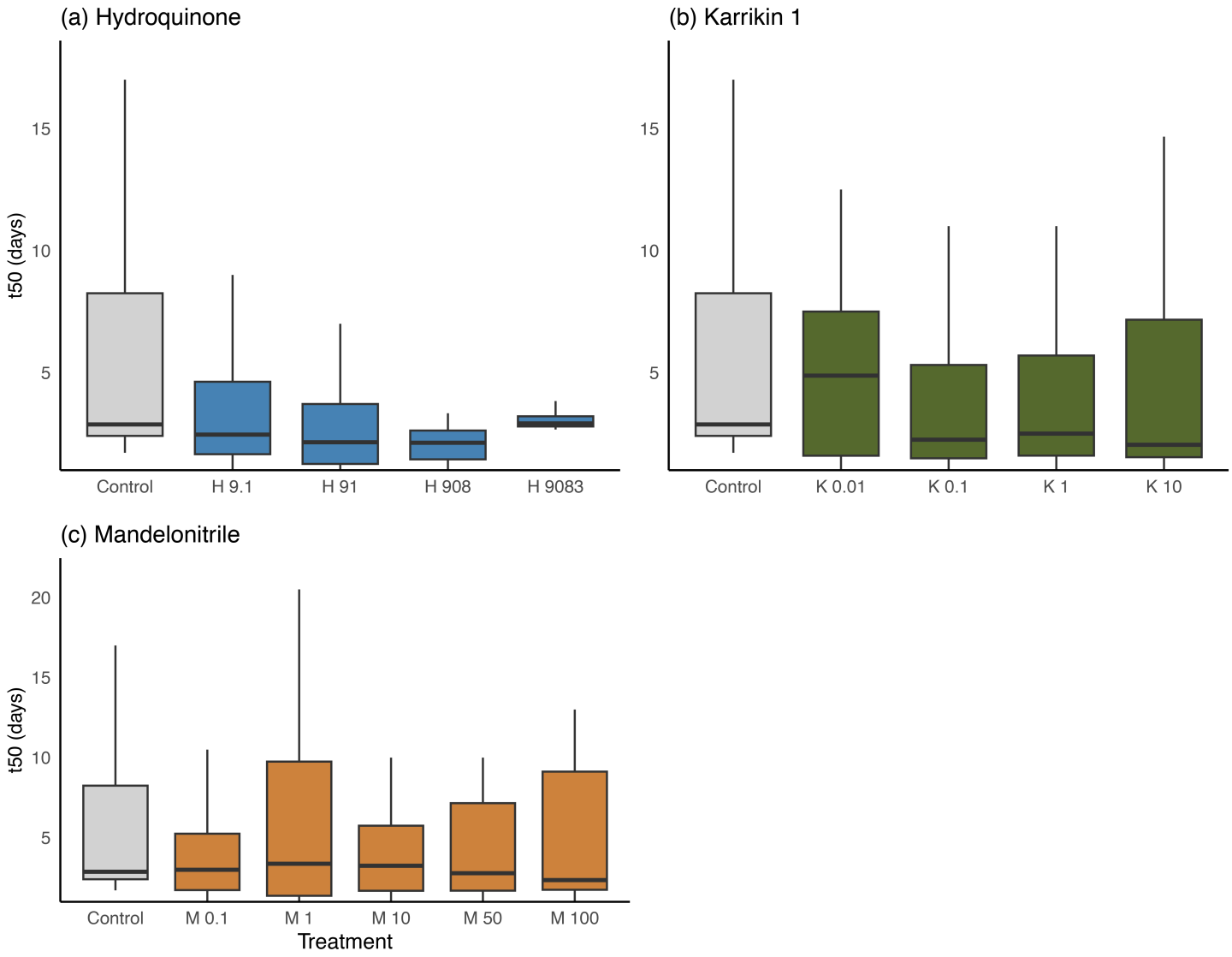


Figure 3

Effect of smoke-derived compounds on t50 across species, (a) hydroquinone (blue, H), (b) Karrikin 1 (green, K), and (c) mandelonitrile (orange, M) in different concentrations of μM. The boxes show the quartiles, and the lines show the median. There was no significant difference between treatments compared to the control (distilled water, light gray, C) according to GLMM and Tukey tests ($p \leq 0.05$).

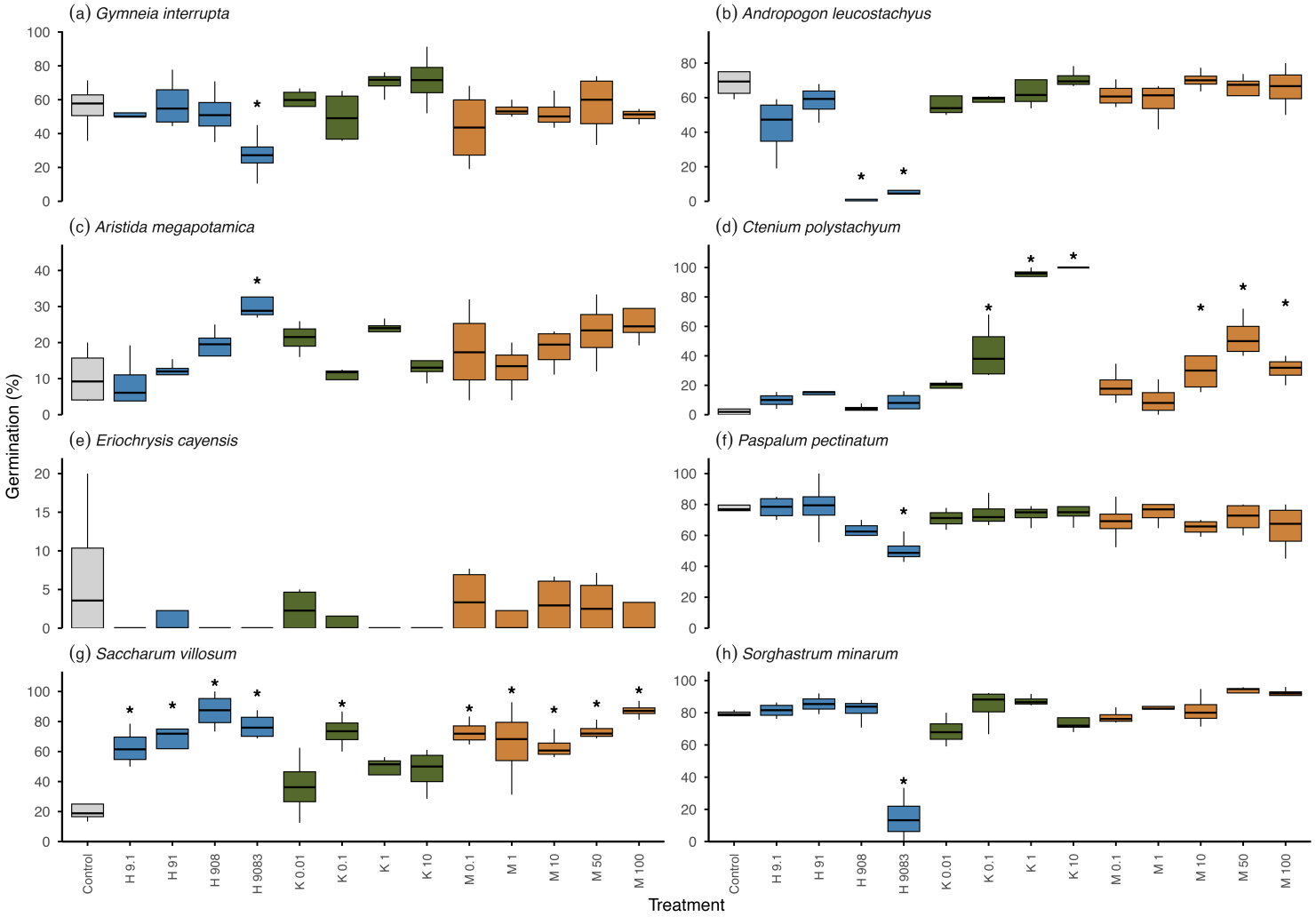


Figure 4

Effect of smoke-derived compounds on germination percentage, considering hydroquinone (blue, H), Karrikin 1 (green, K), and mandelonitrile (orange, M) in different concentrations of μM. Data for *Bulbostylis fimbriata* were not shown, as there was no germination in the treatments. The boxes show the quartiles, and the lines show the median. Asterisks represent significant differences between treatments compared to the control (distilled water, light gray) according to GLM and Tukey tests ($p \leq 0.05$).

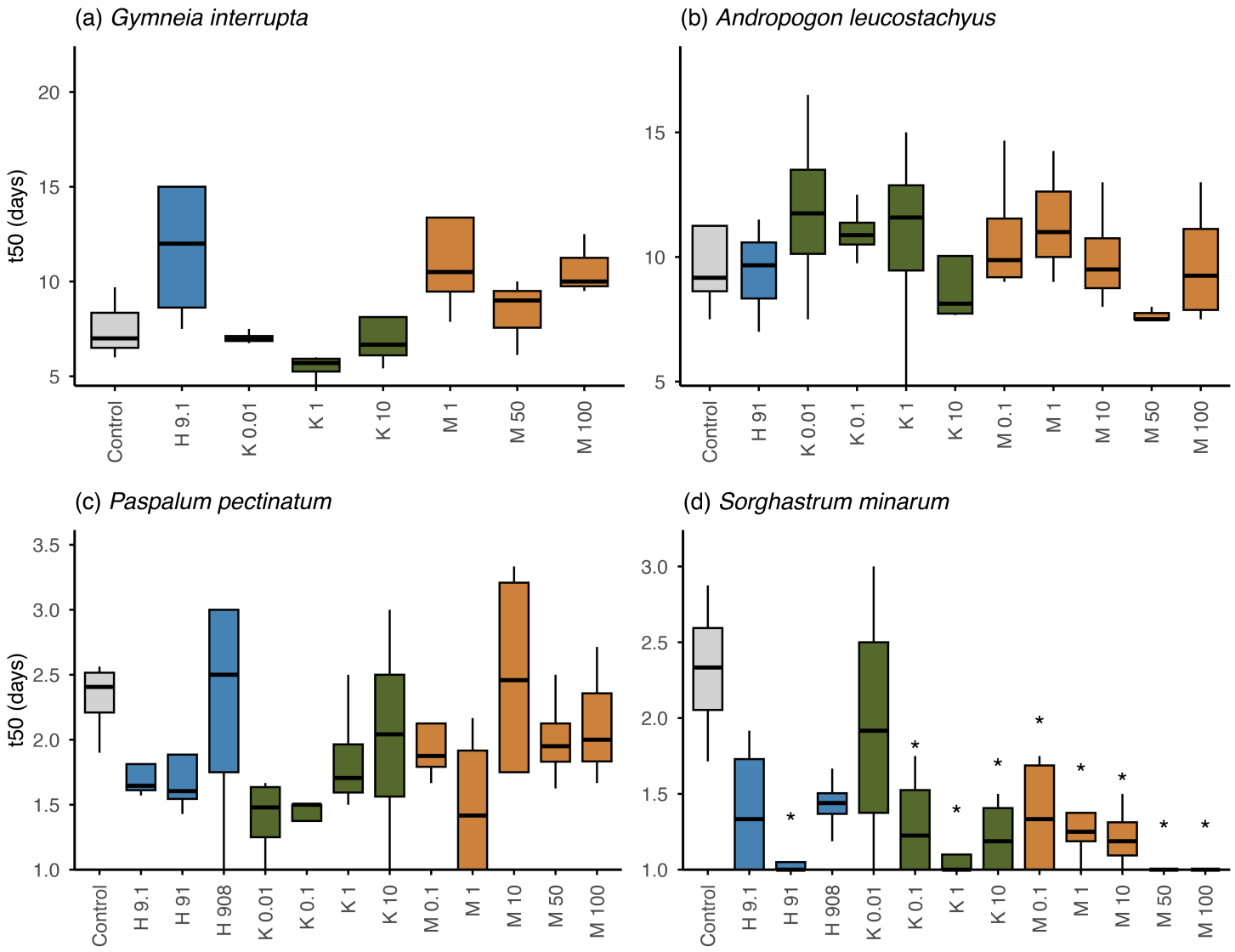


Figure 5

Effect of smoke-derived compounds on t50, shown only for species with valid data, considering hydroquinone (blue, H), Karrikin 1 (green, K), and mandelonitrile (orange, M) in different concentrations of µM. The boxes show the quartiles, and the lines show the median. Asterisks represent significant differences between treatments compared to the control (distilled water, light gray) according to LM and Tukey tests ($p \leq 0.05$).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Motta2026Supplements.pdf