


RESEARCH ARTICLE

The sweet and musky scent of home: biogenic ethylene fine-tunes seed germination in wetlands

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Gas; germination niche; mud; reproduction; sediment; seed; trigger.

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ABSTRACT

- Wetlands are known for comparatively high production of biogenic ethylene from decomposed sediment. Because the gas has various well-documented effects on seed physiology, we asked whether it can be a vital seed germination cue for wetland plants. Specifically, we explored whether ethylene plays an ecological role in (i) breaking/weakening seed dormancy, (ii) broadening the germination niche width, (iii) promoting germination speed or (iv) altering the germination requirements of six plant species with different occurrence along a hydroperiod gradient.
- In a factorial experiment, both ethylene-treated and untreated seeds were incubated in combinations of temperature (constant *versus* fluctuating), illumination (light *versus* darkness) and oxygen (aerobic *versus* hypoxia) with and without cold stratification.
- Our results revealed seed exposure to ethylene did not weaken or break dormancy without cold stratification treatment. However, ethylene helped to broaden the germination niche width, increased overall germination percentage and speed of cold-stratified (non-dormant) seeds. This indicates that ethylene helps those seeds that lost dormancy (non-dormant) to sense favourable water-saturated *versus* flooded substrate depending on their requirement for aerobic *versus* hypoxic conditions to trigger germination.
- We conclude that ethylene does not interfere directly with the dormancy-breaking process in autumn-dispersed seeds that are naturally cold-stratified in winter and germinate in spring/summer. However, ethylene plays a crucial ecological role as a 'flood detector' for different wetland plant communities (reed, mudflat, swamp, shallow water) to synchronize germination of non-dormant seeds in the most suitable habitat at the right time.

INTRODUCTION

Seed germination is an irreversible process, once it starts, a seedling must establish or die (Fenner & Thompson, 2005). Accordingly, plants have evolved several adaptations in seeds allowing them to avert or trigger the germination process through sensing certain (non-optimal/optimal) environmental cues that correspond with the least or most optimal environments for successful germination and seedling establishment. Sensing accurate germination cues also plays a crucial role in selecting a suitable habitat where adult plants can survive and perform better until reaching the reproductive stage (Donohue *et al.* 2010; Phartyal *et al.* 2020a). Consequently, understanding the sensitivity of the germination process to environmental cues has become a dynamic field of ecological research. Establishing seed–environment relationships in space and time helps us to better understand plant population dynamics, species distribution patterns and community assembly rules for the past, present and future (Grubb, 1977; Walck *et al.* 2011; Poschlod *et al.* 2013; Larson & Funk, 2016; Saatkamp *et al.* 2019).

In wetlands, the habitat of interest in this study, light, temperature, substrate moisture, oxygen conditions and combinations thereof, are among the most important environmental cues that control the seed germination process (Galinato & van der Valk, 1986; Baskin *et al.* 1993; Baskin & Baskin, 2014). For example, our recent studies on germination ecology of Central European wetland plants highlight that seeds of species with contrasting ecological niches along a hydroperiod gradient respond differently to a common environment through their seed germination triggers (Phartyal *et al.* 2018, 2020a,b; Rosbakh *et al.* 2020). Specifically, seed germination of mudflat and reed plants (*i.e.* communities at the 'dry' end of the gradient) is largely stimulated by the presence of oxygen and light, irrespective of temperature fluctuation, whereas the seed germination of plants occurring in permanently flooded parts of the gradient was found to have no specific germination triggers (Phartyal *et al.* 2018; 2020a,b; Rosbakh *et al.* 2020). Together with other research (*e.g.* Pons & Schröder, 1986; Shipley & Parent, 1991; Baskin *et al.* 2004; Peralta Ogorek *et al.* 2019), these findings highlight that a highly specific germination niche can help seeds of wetland species to better sense an environment

conducive to successful seedling establishment (Thompson & Grime, 1983; Baskin *et al.* 1993), and thereby play an important role in community assembly in these ecosystems (Grubb 1977; Rosbakh *et al.* 2020).

Wetland ecosystems are well-known for the production of biogenic gases, as they are generally favourable environments for microbial growth under hypoxic or anoxic conditions in submerged or flooded sediments (Smith & Restall, 1971; Smith & Dowdell, 1974; Primrose & Dilworth, 1976; Qiu *et al.* 2005; Keddy, 2010). In particular, freshwater wetlands, with shallow water and muddy substrates rich in decomposed organic matter, harbour a diverse and active microbial community that produces methane, carbon dioxide, hydrogen and ethylene in large quantities (Smith & Dowdell, 1974; Keddy, 2010; Cross *et al.* 2014). Since some of these gases could influence plant physiological processes, previous research suggested that they also play a crucial role in controlling the timing and course of seed germination in wetland plants (Morinaga, 1926; Egley & Dale, 1970; Eplee, 1975; Taylorson, 1979; Baskin *et al.* 2003). Among these gases, the physiological response of seeds to ethylene is best understood (Linkies & Leubner-Metzger, 2012; Baskin & Baskin, 2014; Corbineau *et al.* 2014; Ahammed *et al.* 2020). Initially, ethylene was found to break dormancy and/or enhance seed germination in wetland species, such as *Nymphoides peltata* (Darbyshire & Francis, 2008), *Epilobium hirsutum* (Etherington, 1983), *Schoenoplectus hallii* (Baskin *et al.* 2003) and five Australian ephemeral rockpool species (Cross *et al.* 2014). Further, ethylene is known to broaden germination niche width, especially of seeds in non-optimal environments (*e.g.* hypoxia, osmotic stress, supra-optimal temperature, salinity, etc.) (Katoh & Esashi, 1975; Esashi *et al.* 1989; Corbineau *et al.* 1990; Khan *et al.* 2009; Wang *et al.* 2011; Silva *et al.* 2014). Moreover, ethylene can accelerate germination in cold-stratified seeds of *Echinacea angustifolia* (Macchia *et al.* 2001). In contrast, seeds of several other wetland plants (*Echinochloa crus-galli*, *Digitaria sanguinalis*, *Ipomoea purpurea*, *Persicaria pensylvanica*) remained unresponsive to ethylene treatment (Taylorson, 1979), whereas in *Chenopodium rubrum*, *Plantago major*, *P. maritima* and *Potentilla norvegica* seed germination is inhibited (Olatoye & Hall, 1973; Suzuki & Taylorson, 1981). Yet, although the physiological effects of ethylene on seed germination are well studied – it promotes seed germination through antagonistic interaction with abscisic acid (ABA) and synergistic action with gibberellins (GAs; Ahammed *et al.* 2020) – its ecological role as a seed germination trigger is poorly understood.

In this study, we explored the role of biogenic ethylene as a seed germination trigger in wetland plants. Given that recruitment of many wetland plants is typically constrained by desiccation (Hay *et al.* 2000; Phartyal *et al.* 2018), we expected the seed germination of wetland plants to be sensitive to ethylene, as its high concentrations in/on sediments correspond with the time most favourable for seedling establishment (Smith & Restall, 1971; Qiu *et al.* 2005; Keddy, 2010; Fig. 1). Thus, in addition to light, temperature fluctuations and oxygen availability, ethylene would serve as a reliable germination trigger in this habitat. Based on known complex effects of ethylene on seed dormancy and germination, we specifically focused on two questions:

1 Do seeds with different degrees of dormancy, *i.e.* non-stratified (fresh ‘summer/autumn’ seeds) and cold-stratified

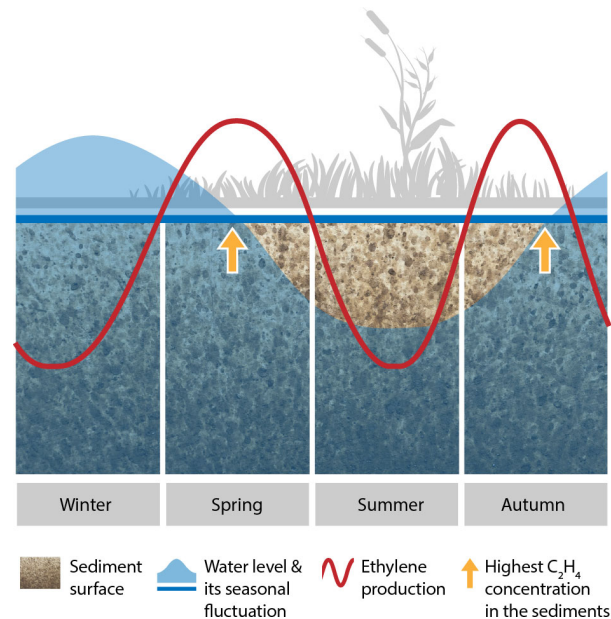


Fig. 1. Seasonal water level fluctuations and biogenic ethylene production in a muddy, sediment-rich, temperate wetland ecosystem.

(overwintered ‘spring’ seeds) respond differently to ethylene exposure? Seasonal water level fluctuations can be very dramatic and unpredictable between seed germination and seedling establishment. We assumed that exposure to biogenic ethylene, which is highest in the middle of a flood–drought cycle (Jäckel *et al.* 2004; Cross *et al.* 2014; Fig. 1), might broaden the germination niche width, *i.e.* the habitat-specific germination response to light, temperature fluctuations and oxygen availability, and additionally stimulate germination and help wetland plants rapidly complete their life cycle.

2 Is the role of biogenic ethylene subordinate or leading in wetland seed germination response to light, oxygen and fluctuating temperatures as key germination triggers? Although non-dormant seeds of wetland species usually respond to very specific environmental cues for germination (Phartyal *et al.* 2018, 2020a,b; Rosbakh *et al.* 2020), we expected that exposure of wetland seed to biogenic ethylene would reduce these specific requirements and open a temporarily available germination window.

MATERIAL AND METHODS

Study species

To determine the effect of ethylene on the seed germination process, we selected six typical herbaceous plants occurring in temperate, freshwater wetlands of Central Europe (Appendix S1). These species are confined to one or two wetland communities along the hydroperiod gradient and have distinct seed germination behaviour that reflects adaptations to community-specific levels of light, temperature and oxygen availability (Rosbakh *et al.* 2020).

Fresh seeds were collected from typical natural populations in Croatia, Germany and Slovenia (Appendix S1). Seeds of

Rorippa palustris were obtained from the University of Regensburg experimental water basins or field greenhouses that cultivate mudflat sediments for soil seed bank studies under similar conditions as at the field sites (Poschlod & Rosbakh, 2018).

Fully ripened fruits/seeds (hereafter 'seeds') of the selected species were collected at maturity in 2012 and 2015 (Appendix S1) from several randomly chosen individuals at least a step distance from each other, then thoroughly mixed. After collection, seeds were air-dried for several days, cleaned and dry stored in a cold room at 4 °C until the start of the germination experiments (Baskin & Baskin, 2014). Seeds collected in 2012 were germinated in 2016, whereas seeds collected in 2015 were germinated in 2018.

Seed germination experiments

The effects of ethylene on seed germination in the focal species were assessed following the method of Cross *et al.* (2014) with slight modifications. Briefly, 800 seeds of each species in fine organza mesh bags were soaked in deionized water for 24 h to allow imbibition, blotted dry and placed in 100 cm³ glass vials. The vials were sealed with gas-tight rubber septa and ethylene gas injected (final concentration 100 ppm). This ethylene concentration (100 ppm) reflects the measured gas concentration in soils rich in organic matter under hypoxia conditions, similar to values in/on wetland sediments in the field (Smith & Restall, 1971; Smith & Dowdell, 1974; Primrose & Dilworth, 1976). Seeds were incubated in the ethylene atmosphere for 24 h prior to germination experiments in a fridge at + 4 °C. Another 800 seeds of each species were treated as controls, by injecting air into the vials instead of ethylene. Vials for the dark treatments (see below) were wrapped in aluminium foil and opened after the ethylene exposure under green safe light.

Ethylene concentration in the vials was verified using gas chromatography, following Wiesenack *et al.* (2002) with FID detection and on-column injection (GC 7820A; Agilent, Frankfurt, Germany). The oven and inlet temperatures were kept constant at 70 °C, while the carrier gas, helium, was used in combination with a DB624 Megabore (30 m × 0.53 mm × 3 µm) column (J&W Scientific, Agilent, Frankfurt, Germany) at 60 kPa. The quantification of ethylene was accomplished with an external calibration curve (linear $R^2 = 0.9993$) in the range 50–10,000 ppm. Ethylene standard was purchased from Sigma-Aldrich (Messer CANGas 99.95%; Sigma-Aldrich, Taufkirchen, Germany) and diluted with air. A 50-µl headspace gas volume from each sample was injected with a gastight syringe (100F-AG-GT-0.63/0.47 100 µl; SGE, Ringwood, Australia). Ethylene eluted at a retention time of 0.63 min, with a signal to noise (s/n) ratio of 62/1 at 100 ppm. The lower limit of quantification was estimated at a s/n ratio of 10/1 at 15 ppm. Intra-series variability at 100 ppm was 4.5% (n = 5).

To assess effects of ethylene exposure on seed germination, we germinated untreated and treated seeds in a three-factorial experiment, with two combinations of temperature (constant *versus* fluctuating), illumination (light *versus* dark) and oxygen availability (aerobic *versus* hypoxic). These three factors are considered the most important seed germination triggers in wetlands (Baskin & Baskin, 2014) and represent environments in wetland communities over the hydroperiod gradient that seeds may encounter (Phartyal *et al.* 2020a,b; Rosbakh *et al.* 2020).

In each germination test, 20 seeds were placed on two layers of wet filter paper in a Petri dish; each treatment was replicated five times. All experiments were conducted in climate chambers (RUMED 1301; RubarthApparate, Germany) at constant (22 °C) or diurnal fluctuating temperature (22/14 °C), with 14 h light supplied by white fluorescent tubes. To test the ability of seeds to germinate in the dark, the Petri dishes were immediately wrapped with four layers of aluminium foil. Germination for all dark treatments were recorded once, on the last day of the experiment. For light treatment, the Petri dishes remained unwrapped throughout the experiment. To simulate hypoxic conditions, the Petri dishes were supplied with vents to provide consistent gas exchange and then placed in a desiccator, where the air was replaced with pure nitrogen. We repeated this procedure each time germination was scored. Control treatments are referred to as aerobic.

The Petri dishes were first examined twice a week and then once a week and any seeds with a protruding radicle or evidence of cotyledons were classed as germinated; these were removed once the checks had been completed. After 6 weeks, the experiments were terminated, and viability of non-germinated seeds checked by inspection of embryos. Seeds with white and firm embryos were considered viable (Baskin & Baskin, 2014); only data for viable seeds were used in the statistical analysis (see below). Abbreviations used for different seed treatments were: L – light, D – dark; C – constant temperature, F – fluctuating temperature; A – aerobic, H – hypoxic.

These seed germination experiments were conducted with both non-stratified (but dry-stored) and moist cold-stratified seeds ('Non-stratified' and 'Cold-stratified', respectively; Appendix S1). Cold stratification involved keeping seeds in Petri dishes lined with wet filter paper at 4 °C for 6 weeks to overcome physiological dormancy, if any (Baskin & Baskin, 2014).

Data analysis

Germination of seeds from control and ethylene treatments was assessed using three traits: (i) germination percentage (ii) germination niche width and (iii) germination speed. Percentage seed germination, *i.e.* final germination percentage in each of the eight combinations of temperature, light and oxygen, characterizes species' ability to complete germination at a given position along the hydroperiod gradient (Rosbakh *et al.* 2020). Seed germination niche width was estimated as Pielou's evenness index (J; Thompson *et al.* 1999; Rosbakh *et al.* 2020), calculated from final germination percentage in each of the eight treatments. Low J values indicate high sensitivity to a treatment (narrow germination niche) and values close to one indicate evenly distributed germination among treatments (wide germination niche).

The number of days to 50% seed germination (T_{50}) was used as a proxy for seed germination speed (lower T_{50} values indicate faster germination). T_{50} values were only calculated for most optimal germination conditions (*i.e.* experimental treatment with highest germination percentage).

All statistical analyses were conducted in R version 4.0.0 (R Core Development Team, 2021). As several assumptions of parametric statistical methods were violated (*e.g.* homogeneity of variances or lack of variance in experimental data), we used a non-parametric rank-based approach (Konietschke *et al.* 2015), to test for significance of ethylene exposure in each seed germination characteristic. The group comparison was made

using the R *nparcomp* package (Konietschke *et al.* 2015). To estimate T_{50} , two-parameter log-logistic models were fitted to the data using the parametric event time framework of Ritz *et al.* (2015) and Jensen *et al.* (2017) implemented in the R package *drc* (Ritz *et al.* 2015).

RESULTS

Ethylene concentrations

The ethylene measurements confirmed that it was present in all vials containing seeds. Mean ethylene concentration in vials was 93.9 ± 3.5 ppm ($n = 80$).

Seed germination percentage and germination niche width

Final germination percentage and germination niche width in both control and ethylene treatments for single species are presented in Fig. 2 and Appendix S1. T_{50} for optimal germination is reported in Fig. 3 and Appendix S1. The study species had different germination responses to ethylene treatment. Ethylene-exposed non-stratified seeds of a reed species *Lythrum salicaria* had significantly higher germination under LFA and LCA treatments. Yet, these positive ethylene effects did not significantly affect the germination niche width (J-values, control: 0.30, ethylene: 0.39). Similarly, J-values of cold-stratified seeds

from control and ethylene treatments did not differ significantly (0.81 in both cases), despite a significant positive effect of ethylene treatment on seed germination in the DFH treatment. The seed germination in the second reed species; *Rorippa palustris* differed from the patterns detected for *L. salicaria*. Specifically, ethylene did not affect final germination percentage in non-stratified seeds, but significantly increased final germination percentage of cold-stratified seeds in four out of eight incubation treatments (LCA, LFH, DCA, DFH), resulting in a significantly broader germination niche width (Jc = 0.38 versus Je = 0.60; Fig. 2).

The effects of ethylene treatment were more consistent in two mudflat species, *Carex bohemica* and *Pericaria lapathifolia*; in both cases, non-stratified seeds exposed to ethylene has significantly higher germination percentages under optimal germination condition (LFA). These positive ethylene effects also significantly broadened the germination niche width (J) of both species (Fig. 2). Ethylene treatment of cold-stratified seeds of both species was significantly effective only under a few sub-optimal incubation conditions (Fig. 2). This positive response was also reflected in the germination niche width (J).

The response of *Sagittaria sagittifolia* and *Typha latifolia*, two wetland species mainly found in flooded swamps and shallow water (Rosbakh *et al.* 2020), to ethylene treatment was moderate, and the germination niche width was not affected by exposure to ethylene.

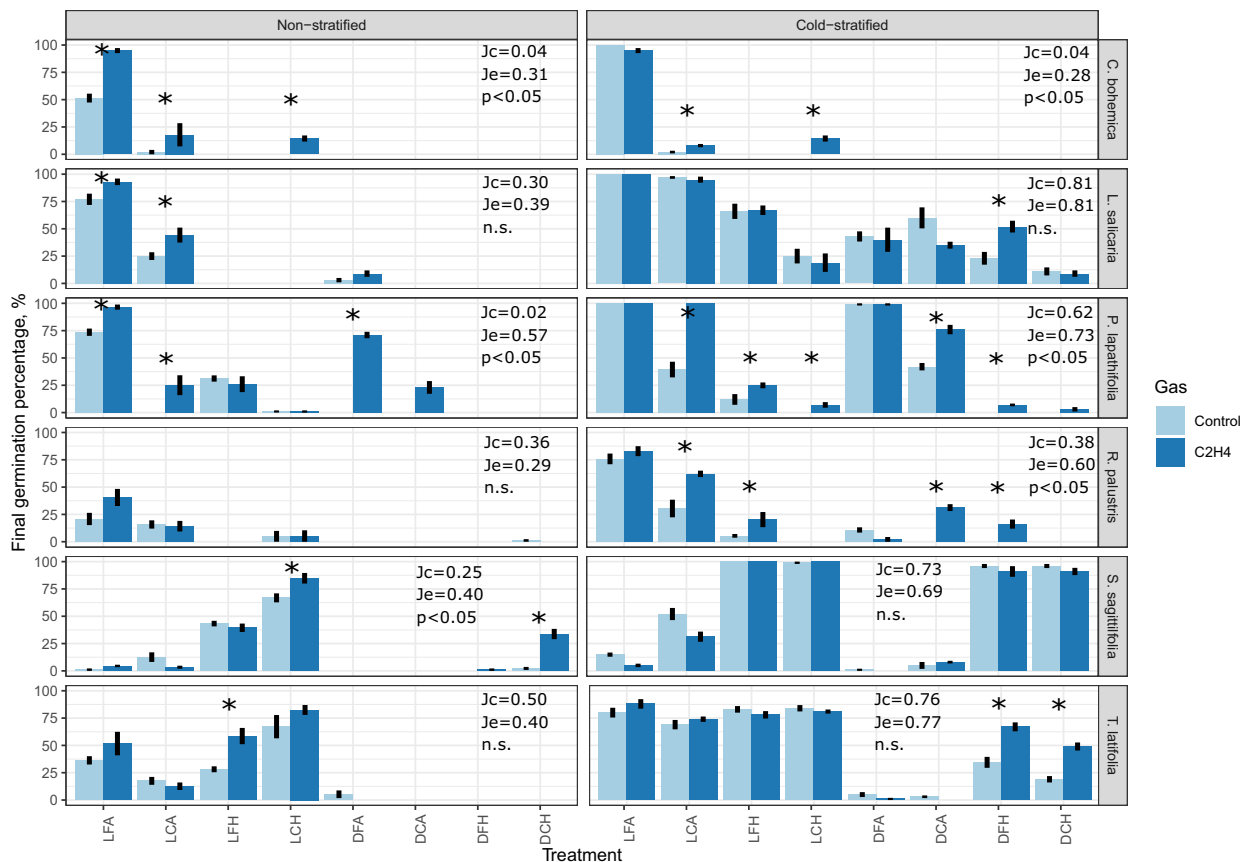


Fig. 2. Effect of ethylene exposure on seed germination percentage and germination niche width in six wetland species. L = light; D = dark; C and F = constant and fluctuating temperature; A and H = aerobic and hypoxic conditions, respectively; Jc and Je = germination niche width for control and ethylene-treated seeds, respectively. Asterisks indicate statistically significant differences ($P < 0.05$; non-parametric rank-based test).

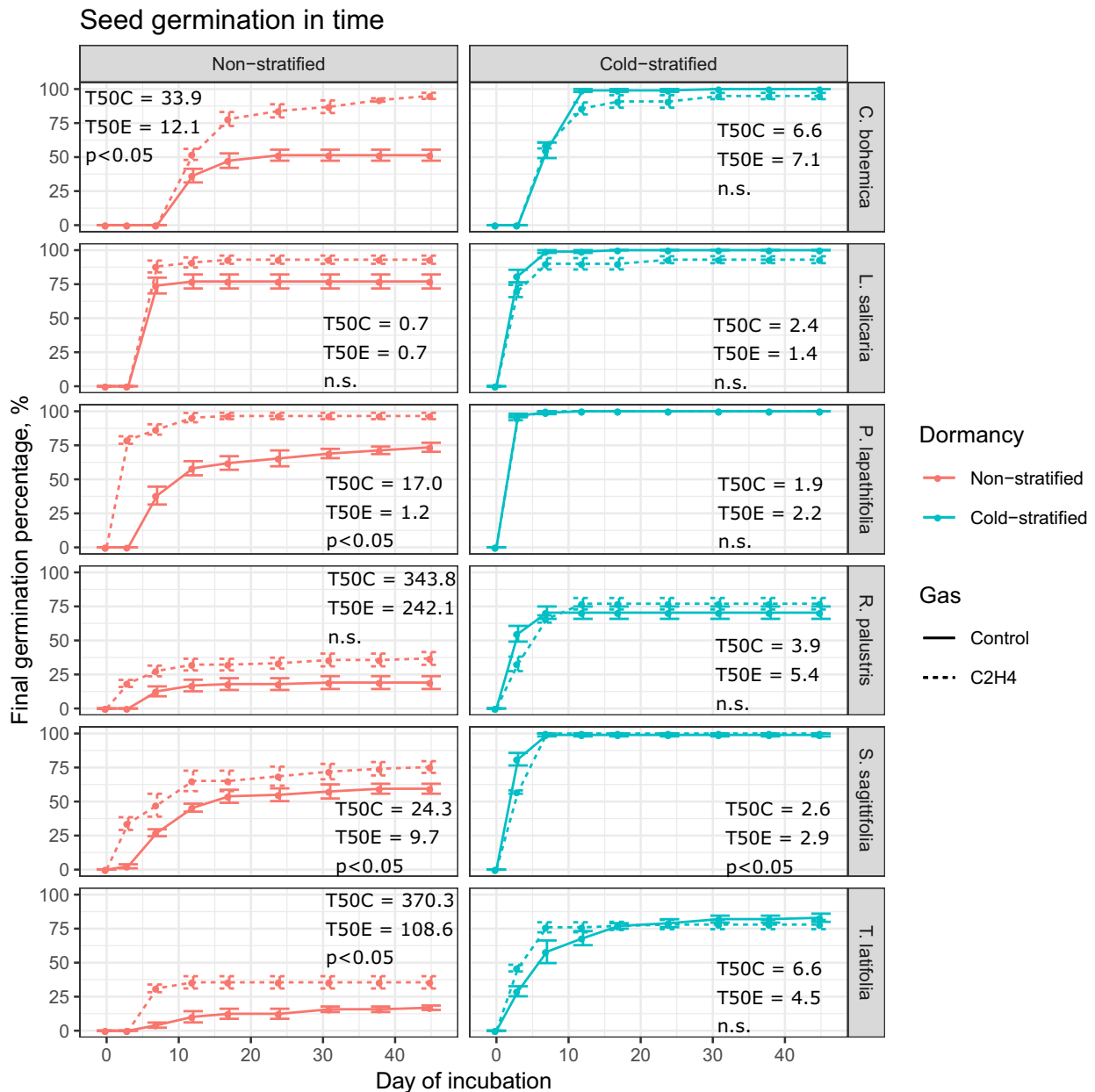


Fig. 3. Effect of ethylene (C₂H₄) exposure on seed germination speed (T₅₀) in six wetland species at optimal germination conditions (see Appendix S1). T₅₀C and T₅₀E = germination speed of control and ethylene-treated seeds, respectively. Asterisks indicate statistically significant differences ($P < 0.05$; non-parametric rank-based approach).

Seed germination speed (T₅₀)

The effect of ethylene on seed germination speed under optimal germination conditions differed among species from different parts of the hydroperiod gradient in wetlands. Specifically, T₅₀ in control and ethylene treatment did not differ in the reed species (*L. salicaria* and *R. palustris*; Fig. 3), whereas non-stratified seeds of mudflat species (*C. bohemica* and *P. lapatifolia*) germinated significantly faster after exposure to ethylene. Cold-stratified seeds of the latter two species had no differences in germination speed between control and ethylene treatment. Ethylene had a significant effect on germination speed of *S. sagittifolia* both in non-stratified and cold-stratified seeds, but

this positive effect was only for non-stratified seeds in *T. latifolia* (Fig. 3).

Overall, cold-stratified seed performed much better than non-stratified seed in terms of germination percentage, germination niche width and speed, irrespective of ethylene treatments.

DISCUSSION

Biogenic ethylene production of microbial communities is stimulated by wetting and has been suggested as an important factor regulating seed germination in wetland communities adapted to flooding/non-flooding cycles (Baskin *et al.* 2003;

Baskin & Baskin, 2014; Cross *et al.* 2014). We expected that in such ecosystems, enhanced biogenic ethylene production acts as a 'flood-detecting' mechanism and an indirect signal of water availability for rapid completion of plant life cycles in a short temporal window and/or in the absence of competing species. Previous, albeit limited, research on ethylene effects on wetland plant germination has been done either on single species (Eplee, 1975; Etherington, 1983; Baskin *et al.* 2003; Darbyshire & Francis, 2008) or on highly specialized species (Australian ephemeral rock pool habitats; Cross *et al.* 2014). Thus, the present study provides a next step in understanding the role of ethylene as a seed germination cue for temperate wetland species with different ecological niches.

Ethylene effects on seed germination with different dormancy patterns

Seed exposure to ethylene led to a mixed response, depending on species ecology and seed dormancy state (non-stratified–summer/autumn seeds *versus* cold-stratified–spring seeds). This can be explained by the fact that the four study species are primarily inhabitants of reed (*L. salicaria*) and mudflat (*C. bohemica*, *P. lapathifolia*, *R. palustris*) communities and require specific germination cues. Physiological dormant (PD) seeds (Klips & Peñalosa, 2003; Baskin & Baskin, 2014; Phartyal *et al.* 2020a) generally disperse in summer/autumn, undergo cold stratification in winter and germinate in spring/summer, when the environment is favourable for seedling establishment. Our earlier studies highlighted that mudflat species have a very narrow germination niche, requiring fluctuating temperatures, full illumination and sufficient oxygen supply, conditions present immediately after water drawdown (Phartyal *et al.* 2020a; Rosbakh *et al.* 2020). In contrast, non-dormant seeds of typical reed species (in this case *L. salicaria*) have a wide germination niche and seeds germinate in aerobic conditions irrespective of light and temperature fluctuations, and only a hypoxic environment inhibits their seed germination (Rosbakh *et al.* 2020). The response of ethylene exposure on swamp and shallow-water species (*S. sagittifolia* and *T. latifolia*) was relatively moderate as they both produce a mixed cohort of non-dormant (Ekstam & Forseby, 1999; Royal Botanic Gardens Kew, 2021) and dormant (Pollux *et al.* 2005; Meng *et al.* 2016) seeds and have a moderate germination niche width (Rosbakh *et al.* 2020).

It is well known that ethylene acts via a complex hormone signalling network to regulate seed germination (Matilla & Matilla-Vázquez, 2008; Arc *et al.* 2013; Corbineau *et al.* 2014); however, its role in seed dormancy is less clear (Matilla & Matilla-Vázquez, 2008). Our results confirm that ethylene had an effect in weakening seed dormancy or broadening germination niche width of non-stratified seeds as compared to cold-stratified seeds. In all species, except *C. bohemica*, cold-stratified seeds performed much better in terms of overall germination percentage and germination niche width (Fig. 3). It should be noted that we used seeds stored dry for several months, with supposedly weakened seed dormancy (Baskin & Baskin, 2014), suggesting that a proportion of the non-stratified seeds that germinated under control and ethylene treatments might have already lost their dormancy. It further proves the weak role of ethylene exposure in dormancy-breaking in seeds of wetland species. Specifically, ethylene does

not stimulate germination after seed dispersal in autumn, because such seeds overwinter to avoid the harsh climate and then synchronize seed germination in spring/summer. Cross *et al.* (2014) argued that alleviation of seed dormancy is not only dependent on ethylene, but also on a combination of other intrinsic (after-ripening, seasonal-dormancy cycling) and extrinsic (light, temperature, oxygen, hydroregime) germination stimulating factors. In their study, fresh seeds of seven rock pool species were exposed to ethylene in the laboratory, and ethylene failed to stimulate seed germination in fresh mature seeds. These authors also observed high seedling emergence from sediments collected from rock pools and treated with ethylene, suggesting a small role of ethylene in alleviating dormancy in freshly dispersed seeds. They suggested that the sediment seed bank may comprise a population of mixed seed age having varying degrees of dormancy. Similarly, Baskin *et al.* (2003) reported that seed dormancy of *Schoenoplectus hallii* breaks during winter under non-flooded conditions and that non-dormant seed then require flooding, ethylene and light for germination.

Triggering seed germination at the right moment is of paramount importance for plants as they cannot afford to break dormancy at an inappropriate time. If biogenic ethylene alleviates dormancy and triggers germination before the start of the growing season, it will be fatal for seedling survival in a harsh and unfavourable environment. Further, seeds do not always depend on the presence of ethylene for dormancy-breaking. Thus, we speculate that ethylene plays a very small or no role in dormancy breaking, but a major role in fine-tuning germination of shallow-dormant (non-stratified) or non-dormant (cold-stratified) seeds in a favourable growing season. This seems true especially for seeds of the mudflat–reed community (*e.g.* *C. bohemica*, *P. lapathifolia*, *R. palustris*), as ethylene exposure of their non-dormant seeds was not very effective in broadening germination niche width or increasing germination speed. Production of ethylene is maximum when microorganisms are under well aerated conditions, but not under anoxia (de Bont, 1976). Mudflat–reed communities often grow very rapidly after water drawdown on well-aerated and water-saturated sediments. In the present study, the mudflat–reed species responded considerably better to ethylene exposure compared to typical reed, swamp and shallow-water species. This further indicates the ecological role of ethylene in fine-tuning seed germination of those species that perform best in water-saturated sediments.

The relative role of ethylene as a seed germination trigger in wetlands

Ethylene is also known to stimulate germination of non-dormant seeds in non-optimal environments (Abeles, 1986; Esashi *et al.* 1989; Gallardo *et al.* 1991; Baskin *et al.* 1993; Corbineau & Come, 1995; Silva *et al.* 2014). All of our study species have very specific germination niches (Phartyal *et al.* 2020a; Rosbakh *et al.* 2020). Germination of some species was inhibited by non-optimal (constant temperature, darkness, hypoxia) conditions, and in others fluctuating temperature and aerobic conditions were non-optimal. However, as we hypothesized, exposure of seeds to ethylene can alter their germination response to light, oxygen and temperature fluctuations. For

example, seeds of *C. bohemica* and *P. palustris* exposed to ethylene germinated even under hypoxic and dark conditions, which are otherwise non-optimal for these species.

Based on these results and published literature, we conclude that ethylene plays a crucial role in broadening germination niche width, promotes germination percentage and germination speed, but seems less effective in weakening or breaking dormancy of summer/autumn-dispersed seeds of wetland plant communities. Additionally, non-dormant seeds are highly sensitive to biogenic ethylene compared to shallow-dormant (non-stratified) seeds. Overall, ethylene seems to play an important ecological role in plant sensing of a favourable water-saturated substrate for those species that require aerobic conditions or serves as a 'flood detecting' mechanism for species that require hypoxic conditions to trigger germination. Further research is needed to better understand the triggering role of ethylene in wetland seed germination. Our treatment involved comparatively short (24 h) exposure of seeds to ethylene at a comparatively low concentration (100 ppm), which may not be optimal. Under natural conditions, seeds of wetland species can be exposed to ethylene at higher concentrations and/or for longer periods, which might have stronger effects on dormancy and germination patterns.

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AUTHOR CONTRIBUTIONS

PP conceived the study; SR and SSP performed the seed germination experiments; MG made the ethylene measurements; SR analysed the dataset; SSP led the writing process. All authors helped in critically revising the manuscript and gave final approval for publication.

SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Appendix S1. Effect of ethylene exposure on seed germination patterns in six wetland species.

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