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Germination of 14 freshwater wetland plants as affected by oxygen and light

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ABSTRACT

Oxygen and light are factors that can affect seed germination of wetland plants. We selected seeds from 20 plant species found in temperate eastern North American marsh and wet meadow wetlands, ranging from obligate wetland (OBL), facultative wetland (FAC) and upland (UPL) species. In growth chambers we tested germination success under controlled manipulations of O_2 concentration (low [<1%] and atmospheric [~20%]) and light levels (full light, half light and dark). Our objective was to (1) determine whether oxygen, light and their interaction facilitate germination of wetland seeds; (2) whether timing of germination is affected by oxygen and light; and (3) whether germination is related to wetland functional groups. Six species did not germinate. Of the 14 species that did germinate, a non-native ruderal (*Echinochloa crus-galli*) had the greatest germination success. However, we found that low O_2 reduced germination success of all but three species (*Rudbeckia triloba*, *Sagittaria latifolia* and *Typha latifolia*). Reduction in light levels only reduced germination success of *S. latifolia*. We conclude that the physiological constraints that control germination operate independently for oxygen and light. It is important to know and anticipate oxygen and light levels when designing a wetland restoration project so that the proper species can be sown that can germinate under the specific conditions. Restoration planners should be aware that anoxic and hypoxic conditions seem to promote the germination of weedy and potentially invasive native and non-native species.

1. Introduction

Wetland plants have adapted to the stressful anaerobic environment imposed by flooding and the resulting consequence of hypoxic and anoxic soil conditions. Much research has been done to study how wetland plants cope with submergence, with the principle mechanism being the evolution of air spaces or lacunae formed within plant tissue (Crawford, 1992, 1996; Kozlowski, 1984; Crawford and Brandle, 1996; Voesenek et al., 2006; Mitsch and Gosselink, 2007). However, it has been shown that life-history strategies of mature freshwater marsh plants do not necessarily reflect the strategies of seeds and seedlings (Shipley et al., 1989). The strategy of seeds is important because hydrologic changes often leave large areas of unvegetated soils open to colonization (van der Valk and Davis, 1978; Keddy and Reznicek, 1986). Without regeneration from surviving individuals (e.g., Grubb, 1977), seed banks and dispersal are responsible for the establishment of new plant communities (Leck, 1989; Baskin and Baskin, 1998; Fenner and Thompson, 2005). Since seed banks of freshwater wetlands often differ substantially from the plant community (van der Valk, 1981; Leck, 1989), disturbances have the potential to result in dramatic changes in vegetation. Species-specific germination responses will depend on site-specific environmental conditions such as hydrology, hypoxic soil conditions, and shade from remnant vegetation or litter.

Generally, seeds of plants that grow on well-drained soil have higher germination rates under ambient atmospheric O_2 concentrations than with reduced O_2 levels (Heichel and Day, 1972; Côme et al., 1991). However, wetland plant seeds can often germinate under hypoxic conditions, with some requiring hypoxia or anoxia (Leck, 1996). Lorenzen et al. (2000) found an increased germination percentage of *Typha domingensis* at low concentration of O_2 (4.34%) compared to atmospheric O_2 levels. Wijte and Gallagher (1996) found that *Phragmites australis* can germinate under hypoxic conditions of 2.5% O_2, but not under anoxic conditions; whereas *Spartina alterniflora* was able to germinate under anoxic conditions thereby giving that species a competitive advantage over *P. australis* in poorly drained marshes.

The effect of light on germination of wetland plant seeds may either promote or inhibit germination (Leck, 1989; Kettenring et al., 2006). Emergence from the seed bank of species requiring light for germination is triggered by a disturbance, such as lowering

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of water level or gaps in the plant canopy (Leck, 1989). There is often a connection between light levels and O_2 concentration on hydric soils with water level fluctuations such that a decline in water depth can increase light levels and O_2 on wetland soils. However, a physical disturbance, such as grazing, can increase light levels with little or no effect on the concentration of O_2 in the soil. We could not find any studies that investigated the interaction between O_2 concentration and light levels on wetland plant seed germination. Understanding species-specific germination requirements, and the potential interaction between light levels and O_2 concentration, is necessary for predicting how wetland species respond to disturbance.

With an understanding of how O_2 and light affect seed germination, revegetation efforts can be improved in freshwater wetland restorations. Wetland functional groups have been used as a predictive tool for restoration and management. Boutin and Keddy (1993) categorized wetland marsh plants into three groups based on 27 traits relating to adult and seedling growth and physiology: 'ruderals' flower in the first year, 'interstitials' are perennial plants with a clumped growth form, and 'matrix' species are perennial plants with a robust clonal growth form. Ruderals allocate a greater proportion of their resources to reproduction, rather than growth or storage, compared to interstitials and matrix species (Grime, 1979; Boutin and Keddy, 1993). Matrix species allocate most of their resources to growth, while interstitials allocate most resources to storage, and the least amount of resources to reproduction (Grime, 1979; Boutin and Keddy, 1993). Based on these findings, we might expect that germination success is related to functional group (Shipley and Parent, 1991).

Our study examined the effects of O_2 and light on twenty freshwater marsh species. The objective was to (1) determine whether oxygen, light and their interaction facilitate germination of wetland seeds; (2) whether timing of germination is affected by oxygen and light; and (3) whether germination is related to wetland functional groups. We hypothesized that low oxygen concentration and low light levels would inhibit germination success. We could find no published research that manipulates the interaction between oxygen and light, but it would be reasonable to expect that the combination of low oxygen and low light would have the greatest negative effect on germination. Finally, we hypothesized that ruderals would have the highest rate of germination, followed by matrix and then interstitial.

2. Materials and methods

Twenty species were selected to represent a broad range of plant life forms typically found in temperate eastern North American marsh and wet meadow wetlands (Crow and Helquist, 2000) (Table 1). Most of the species are commonly used in wetland restoration projects (Hammer, 1996; Cronk and Fennessy, 2001), while others are non-native (*Echinochloa crus-galli*, *Festuca ovina* and *Agrostis stolonifera*) and *Phalaris arundinacea*, while native, is highly invasive. *Typha latifolia* and *Leersia oryzoides* are also native, but can be invasive. The selected species represent three functional groups (matrix, interstitial, and ruderal). Seeds were either purchased through Ernst Seed Company (Meadville, PA) or collected in the field. The seed source from Ernst Seed Company is a combination of field-collected and cultivated plants that are bulk processed so that there is genetic variability. Field-collected seed were taken from multiple individuals and from at least three sites in northeast Ohio, which were then combined by species. Seed was placed in mesh bags, buried in containers of damp sand and stored in a refrigerator at 5 °C for at least two months but no longer than one year (Shipley et al., 1989). Immediately prior to setting up the experiment, seed viability was measured by placing 100 seeds of each species on moist filter paper in separate petri dishes exposed to a 14-h photoperiod provided by two 1000 W, High Pressure Sodium bulbs for thirty days (Table 1).

The experiment was a two by three factorial design with oxygen and light as treatments, and with three replicates. A single experimental unit was a 'pot' (a 20.3 cm diameter PVC pipe with a length of 16 cm). The bottom was sealed and three 18.5 cm Whatman™ general purpose filter papers were layered inside. Each pot had two 2 cm holes in the side, drilled 3 cm from the top and equidistant from each other. One hole was plugged with a rubber stopper containing an inflow tube. The other hole was plugged with a rubber stopper containing two small diameter holes for the constant release of air, for the sampling of air inside the pots, and for the occasional addition of water to ensure the filter papers remained saturated. The top of the pot was covered with one of three materials depending on the light treatment.

The oxygen treatment included an ambient atmospheric O_2 concentration and a N_2, anoxic treatment. A regulated constant flow of N_2 or ambient air was provided to each of the 36 pots. The light treatment included full, half light, and dark. For the full light treatment we used Tufflite IV™, 6 mil, 0.152 mm thick plastic (Tyco Plastics and Agricultural Films, Monroe, LA, USA), with 93% PAR transmission. For the half light treatment we used a layer of the same Tufflite as used in full, plus a layer of red Saran™ Premium Wrap. A red filter was used as a neutral shade because while it reduces general PAR transmission, the red-light spectrum is transmitted, which has been shown to be more responsive to germination (Shinomura et al., 1996). For the dark treatment we used the same Tufflite plus a layer of 0.024 mm aluminium foil.

A set of six pots, two of each light treatment, was placed within a single plastic container (80 cm long by 40 cm wide by 15 cm deep); the plastic container was used to facilitate handling of the pots. Each pot could contain seeds from only ten species when using 50 seeds per species, so two paired treatment pots were needed to accommodate the 20 species. Three of the six containers received the ambient oxygen treatment while the other three received the anoxic N_2 treatment. The arrangement of containers, pot position within each container, and seeds of species' groups within each pot were randomized at the beginning of the experiment.

Seeds from each species were arranged in separate species groups on saturated filter paper. A daily, 14-h photoperiod was provided by two 1000 W, High Pressure Sodium bulbs providing an average photosynthetically active radiation (measured with a LiCor LI-250 light metre) of 140.5 $\mu mol\ s^{-1}\ m^{-2}$ ($\pm$8.2 SD) on the experimental plants. Windows in the room were covered to prevent incident solar radiation. Temperature was maintained at 22 ± 2 °C. Humidity ranged between 40 and 50% and was self-maintained due to the evaporation of the water from the saturated filter paper. Filter paper was watered to maintain saturation when needed.

The experiment ran for 30 days and was monitored at least every second day to ensure adequate N_2 and air supplies. Light was measured twice, day 1 of the experiment and day 30, within each pot. Atmospheric oxygen concentration within each pot was measured every second day. Date of germination was recorded throughout the experiment. During monitoring of germination, the only light source was UV-A black light; all growth lights and room lights were turned off. At the end of the experiment, percent germination was calculated for each species within each pot.

A one-way ANOVA by species was done to test overall germination success, followed by a post hoc Tukey's HSD test. A two-way, fixed-effects ANOVA was conducted for the oxygen and light treatment effect on percent germination of all species that had any germination. Percent germination was arcsine square root transformed to meet assumptions of normal distribution. Tukey's HSD test was run to determine statistical significance between means. A Bonferroni correction was applied in order to adjust for the number of simultaneous tests (Rice, 1989). A three-way ANOVA was done

Table 1
Twenty selected wetland species, with authorities (Gleason and Cronquist, 1991), included in experiment, and (1) their national wetland classification category (Wetland, 2012), and their wetland functional group (Boutin and Keddy, 1993). *Abbreviations*: OBL – obligate wetland; FACW – facultative wetland; FAC – facultative; FACU – facultative upland; UPL – upland; M – matrix; I – interstitial; R – ruderal. Initial seed viability is noted as germination viability (%). For seed source, 1 = collected by authors; 2 = purchased from seed company.

Species	Wetland indicator status	Wetland functional group	Germination viability (%)	Seed source
Agrostis stolonifera L.	FACW	I	52	1
Allium cernuum Roth[a]	FACU	I	23	2
Andropogon gerardii Vitman	FACU	M	47	1
Carex lurida Wahlenb.	OBL	I	40	1
Echinochloa crus-galli (L.) Beauv.	FAC	R	95	2
Eleocharis palustris (L.) Roem. & Schult.[a]	OBL	I	36	1
Eupatorium perfoliatum L.	FACW	I	35	1
Festuca ovina L.	UPL	I	52	1
Glyceria Canadensis (Michx.) Trin.[a]	OBL	I	22	2
Juncus tenuis Willd.[a]	FAC	I	20	1
Juncus torreyi Coville	FACW	I	34	2
Leersia oryzoides (L.) Sw.[a]	OBL	I	16	2
Liatris spicata (L.) Willd.	FAC	I	57	1
Phalaris arundinacea L.	FACW	M	79	1
Rudbeckia hirta L.	FACU	R	73	2
Rudbeckia triloba L.	FACU	I	18	2
Rumex orbiculatus Grey	OBL	I	70	1
Sagittaria latifolia Willd.	OBL	I	36	2
Sparganium americanum Nutt.[a]	OBL	I	25	1
Typha latifolia L.	OBL	M	24	1

[a] Seeds of species that achieved no germination in the experimental treatments.

for oxygen, light and wetland functional group on germination. Tukey's HSD test was used to determine difference between means. All statistical analyses were conducted using Systat 13 (SYSTAT, 2009).

3. Results

Oxygen and light measurements did not differ between time periods (every second day of the experiment for oxygen, and day 1 and day 30 for light), so we show mean values of measurements by treatment for the first day of measurement. Oxygen readings by treatment were as follows: ambient 17.54% (±0.09 SE) and N_2 0.81% (±0.06 SE). Light readings by treatment were as follows: full light 124.68 μmol s^{-1} m^{-2} (±7.37 SE); half light 95.45 μmol s^{-1} m^{-2} (±5.07 SE); and, dark 0.43 μmol s^{-1} m^{-2} (±0.06 SE).

Six species had no germination success and were therefore excluded from further analysis (Table 1). For the remaining 14 species, *E. crus-galli* had the greatest mean germination success for all treatments combined, followed by *P. arundinacea* and *Rumex orbiculatus* (Fig. 1). *Eupatorium perforliatum*, *Juncus torreyi*, *T. latifolia*, and *Rudbeckia triloba* had the lowest germination success (Fig. 1).

The first species to germinate were *Andropogon gerardii*, *E. crus-galli*, *Eupatorium perfoliatum*, *Liatris spicata*, and *R. orbiculatus* on day 3 (Table 2). All 14 of the species that germinated had started germinating by day 11. *E. crus-galli* started germination on day three, regardless of treatment, except for the dark treatment with reduced oxygen, in which case the start of germination was delayed by 2 days. The other species that had any germination in reduced oxygen (*R. orbiculatus*, *Sagittaria latifolia*, and *T. latifolia*) were delayed in their start time of germination by between 2 and 20 days in the reduced oxygen treatment (Table 2).

The germination of all but three species, *R. triloba*, *S. latifolia*, and *T. latifolia* was negatively affected by the oxygen treatment (Table 3), such that lack of oxygen reduced germination (Table 4). The only species affected by the light treatment was *S. latifolia* (Table 3), which had greater germination under full light and half light compared to the dark treatment (Table 4). There was no interaction effect between light and oxygen (Table 3).

Wetland functional group differed in their germination response (Table 5), with the ruderal species having the greatest germination success and interstitial species the least (Fig. 2). There was a significant interaction between the oxygen treatment and wetland functional group with respect to germination (Table 5).

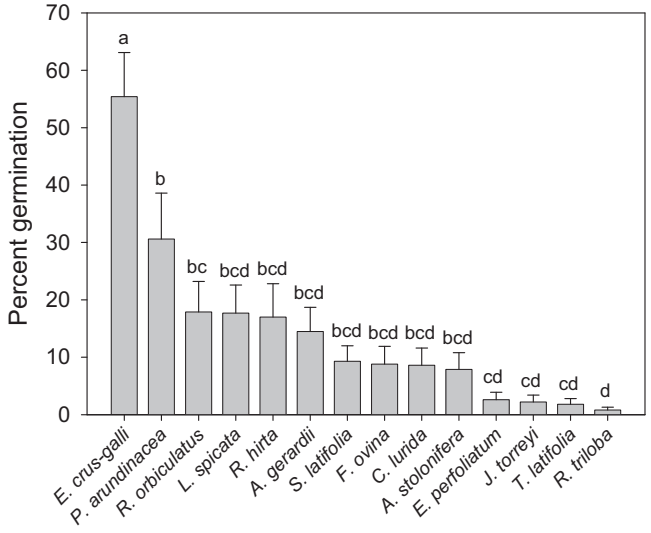

Fig. 1. Mean percent germination of 14 freshwater wetland species. Error bars represent ±SE, and bars sharing the same letter are not significantly different according to Tukey's HSD post hoc analysis.

4. Discussion

We found that the lack of oxygen, or atmospheric O_2 concentration below 1%, will generally reduce germination success of freshwater wetland species compared to atmospheric concentrations of O_2. Ten of the fourteen species that germinated in ambient atmospheric O_2 levels had no germination success when O_2 was reduced. It would seem that most seeds not only have a greater germination success under atmospheric O_2 concentration, but that seeds require a minimum concentration of O_2 above 1% to germinate. *E. crus-galli* and *R. orbiculatus* had seeds germinate under low O_2, but germination success was less than at ambient O_2. *T. latifolia* and *S. latifolia* also germinated under low O_2, but were

Table 2
Number of days to the start of germination in the oxygen (present/absent) and light (full/half/dark) treatments for fourteen herbaceous plants. A blank space indicates zero germination.

Species	Full light		Half light		Dark	
	$+O_2$	$-O_2$	$+O_2$	$-O_2$	$+O_2$	$-O_2$
Agrostis stolonifera	7		7		7	
Andropogon gerardii	3		5		5	
Carex lurida	9		7		11	
Echinochloa crus-galli	3	3	3	3	3	5
Eupatorium perfoliatum	3		22			
Festuca ovina var glauca	5		9		5	
Juncus torreyi	9		11			
Liatris spicata	3		5		5	
Phalaris arundinacea	5		5		5	
Rudbeckia hirta	5		5		3	
Rudbeckia triloba	11				11	
Rumex orbiculatus	5	15	3	15	3	
Sagittaria latifolia	11	13	11	13		
Typha latifolia	7	27	5		11	

Table 3
2-Way ANOVA results testing the effects of oxygen (present/absent) and light (full/half/dark) on each of the 14 species, and all species combined. Significant interactions ($P<0.05$, after Bonferonni correction) are shown in bold.

Species	Oxygen		Light		Oxygen × Light	
	F	*P*	*F*	*P*	*F*	*P*
Agrostis stolonifera	**33.061**	**<0.001**	0.140	0.871	0.140	0.871
Andropogon gerardii	**112.450**	**<0.001**	1.700	0.224	1.700	0.224
Carex lurida	**23.070**	**<0.001**	1.291	0.311	1.291	0.311
Echinochloa crus-galli	**11.982**	**0.005**	0.289	0.754	0.920	0.426
Eupatorium perfoliatum	**14.549**	**0.002**	4.387	0.037	4.387	0.037
Festuca ovina var glauca	**64.75**	**<0.001**	3.695	0.056	3.695	0.056
Juncus torreyi	**10.977**	**0.006**	3.249	0.075	3.249	0.075
Liatris spicata	**124.541**	**<0.001**	0.036	0.965	0.036	0.965
Phalaris arundinacea	**325.752**	**<0.001**	1.841	0.201	1.841	0.201
Rudbeckia hirta	**86.966**	**<0.001**	5.978	0.016	5.978	0.016
Rudbeckia triloba	3.494	0.086	0.898	0.433	0.898	0.433
Rumex orbiculatus	**84.960**	**<0.001**	2.433	0.130	1.622	0.238
Sagittaria latifolia	1.905	0.193	**9.115**	**0.004**	0.643	0.543
Typha latifolia	5.995	0.031	1.326	0.302	0.548	0.592
Combined plants	**121.215**	**<0.001**	1.422	0.243	0.128	0.880

not affected by lack of oxygen. *S. latifolia* and *T. latifolia* are obligate wetland plants found in marshes; therefore they often grow in hypoxic or anoxic soil conditions, suggesting an ecological significance of the response of the germination of *S. latifolia* and *T. latifolia* under anoxic soil conditions. Perhaps it is more surprising that the other wetland species tested either failed to germinate, or had significantly less germination, under reduced O_2 concentration, especially the other obligate wetland species (*Carex lurida*, *R. orbiculatus*). These results suggest that using seeds can be problematic for restoration projects without a full understanding of the abiotic conditions presented within the restoration site, and the germination requirements of the seeds included in the restoration.

Comparing our results to those in the literature, the ability to germinate under reduced oxygen concentrations has been shown for *Scirpus juncoides* (Pons and Schröder, 1986), *Scirpus lacustris* and *Scirpus maritimus* (Clevering, 1995), *T. latifolia* (Bonnewell et al.,

Table 4
Mean percent germination response to light (full/half/dark) and oxygen (present/absent) treatments (±1 standard error in parenthesis) of fourteen herbaceous wetland species, and all plants combined.

Species	Full light		Half light		Dark	
	$+O_2$	$-O_2$	$+O_2$	$-O_2$	$+O_2$	$-O_2$
Agrostis stolonifera	16.0 (13.0)	0	18.7 (8.7)	0	12.7 (1.8)	0
Andropogon gerardii	20.8 (7.0)	0	41.1 (2.9)	0	24.9 (10.0)	0
Carex lurida	14.7 (11.8)	0	27.3 (1.8)	0	9.9 (5.0)	0
Echinochloa crus-galli	77.1 (9.0)	20.7 (9.4)	70.0 (8.1)	47.9 (22.3)	85.1 (1.5)	31.9 (24.3)
Eupatorium perfoliatum	10.7 (5.2)	0	4.7 (2.4)	0	0	0
Festuca ovina var glauca	8.0 (4.2)	0	12.5 (6.2)	0	32.0 (7.3)	0
Juncus torreyi	8.6 (4.8)	0	4.7 (3.7)	0	0	0
Liatris spicata	38.4 (12.6)	0	33.3 (5.9)	0	34.3 (9.0)	0
Phalaris arundinacea	46.6 (12.6)	0	68.1 (5.2)	0	68.9 (8.8)	0
Rudbeckia hirta	62.7 (4.7)	0	25.6 (11.1)	0	13.8 (6.3)	0
Rudbeckia triloba	2.7 (2.7)	0	0	0	2.0 (1.2)	0
Rumex orbiculatus	31.3 (8.7)	1.3 (0.7)	51.8 (14.3)	0.7 (0.7)	22.2 (5.3)	0
Sagittaria latifolia	13.3 (9.6)	24.3 (2.3)	4.1 (0.1)	14.0 (7.2)	0	0
Typha latifolia	6.7 (5.7)	0.7 (0.7)	2.7 (0.7)	0	0.7 (0.7)	0
Combined plants	25.5 (3.9)	2.3 (1.9)	26.1 (3.9)	4.5 (2.4)	21.9 (4.1)	3.4 (1.4)

Table 5
Three-way ANOVA to test the effects of oxygen, light and wetland functional group on germination. Significant factors and interactions are indicated in bold ($P < 0.05$).

Source	df	Mean squares	*F*-ratio	*P*-value
Oxygen	**1**	**7.334**	**148.300**	**<0.001**
Light	2	0.052	1.054	0.350
Wetland Group (WG)	**2**	**1.640**	**33.152**	**<0.001**
Oxygen × Light	2	0.021	0.421	0.657
Oxygen × WG	**2**	**0.299**	**6.050**	**0.003**
Light × WG	4	0.013	0.259	0.904
Oxygen × Light × WG	4	0.060	1.205	0.310
Error	234	0.049		

1983), *Rumex crispus* and *Rumex maritimus* (Voesenek et al., 1992) and *Peltandra virginica*, *Pontederia cordata*, and *S. latifolia* (Leck, 1989). Bonnewell et al. (1983) found maximum germination of *T. latifolia* when the O_2 concentration was reduced to between 2.3 and 4.3%, and no germination at 1%. Although we found *T. latifolia* germination at <1% O_2, germination success was less than 1%. Pons and Schröder (1986) found maximum germination at ~88% of *S. juncoides* peaked at 5% O_2 levels, but 0% O_2 also had high germination success (~77%), especially compared to atmospheric O_2 concentration (20%) with a germination success of ~25%. The physiological factors leading to inhibition under anaerobic conditions depend on plant seed tolerance of ethanol, a terminal product of anaerobic metabolism (Rumpho and Kennedy, 1981, 1983). If a seed is unable to tolerate high concentrations of ethanol it will not germinate under anaerobic conditions. It would seem that each species may have an optimal O_2 concentration for germination success, but it is only a few that can germinate under extremely low O_2 concentration (<1%). Early emerging seedlings have a competitive advantage and probability of survival is higher compared to seedlings that emerge later (Miller, 1987; Stockey and Hunt, 1994). Therefore, those species that can germinate at <1% O_2 would likely have a competitive advantage as seedlings over species unable to germinate at low O_2 concentrations, provided that O_2 becomes available to the seedling shortly after germination.

Reduced light levels only affected one species, *S. latifolia*, and caused a reduction in germination. We tested for light levels because wetland plant seeds often experience reduced light through flooding, through burial via sedimentation, and through tall vegetative canopies (Jurik et al., 1994; Leck and Brock, 2000; Peterson and Baldwin, 2004; Kettenring et al., 2006). The fact that the germination success of only one species was affected by low light, while many were affected by low oxygen, suggests that the physiological constraint of anaerobic conditions has a potentially greater detriment than light on seed germination and seedling emergence. Or, that seedling survival in light-limited environments is influenced by a different set of plant strategies than germination (Carlyle and Fraser, 2006). There was no interaction effect of oxygen and light on germination. Oxygen and light may not physically operate independently in natural wetland systems; that is, flooding and sedimentation caused by flooding can reduce O_2 concentration as well as light levels. However, based on our results, we think that the physiology of seed germination seems to operate independently of oxygen and light levels.

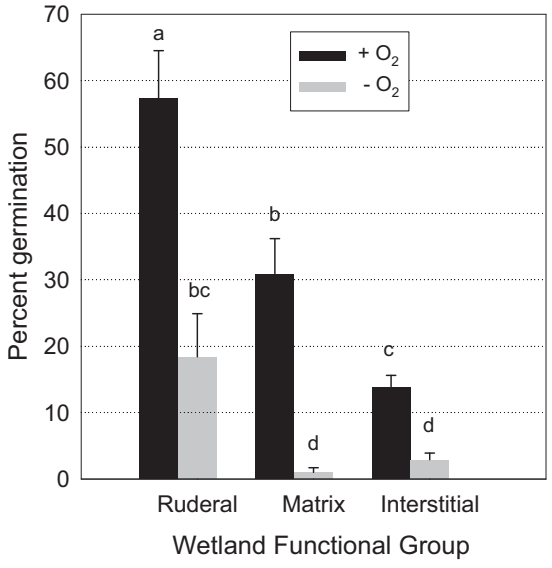

Fig. 2. Mean percent germination of 14 freshwater wetland species by wetland functional group and by oxygen treatment (ambient at ~17.5% O_2 and reduced at ~0.8% O_2). The number of species within each functional group were 2 ruderal, 3 matrix, and 9 interstitial.

Timing of germination was affected by O_2 concentration for three of the four species that germinated under low O_2. There was a delay of 2 days for *S. latifolia*, 10 days for *R. orbiculatus* and 20 days for *T. latifolia*; at low O_2 compared to ambient O_2. It is interesting that germination of *S. latifolia* was only delayed by two days since the germination success of *S. latifolia* was not affected by low O_2. The timing of germination for *E. crus-galli* was generally not affected by low O_2, except under the dark treatment, in which case there was a two day delay. *E. crus-galli* is an annual with a high reproductive potential; it germinated early and had the highest germination success. Even though *E. crus-galli* had reduced germination under low O_2 treatment, the percent germination was still relatively high compared to the other species germination success under atmospheric O_2.

Germination success of wetland functional groups differed, such that ruderals had the highest percent germination, followed by matrix and interstitial groups. Others have also found that ruderals have high germination rates (Galinato and van der Valk, 1986; Boutin and Keddy, 1993), likely due to ruderals higher allocation of resources to reproduction compared to longer-lived plant strategy types (Fenner and Thompson, 2005), which include interstitial and matrix functional groups. We also found that low O_2 reduced germination success across all wetland functional groups, but that the germination success of ruderals at low O_2 was equivalent to the success of matrix and interstitial at atmospheric O_2. It would seem that ruderal seeds can germinate under the stressful conditions imposed by low O_2 concentration. Whether the emerged seedling can persist is another question, but see Voesenek et al. (2006). The coleoptiles of *Echinochloa* are colourless when germinated in an oxygen-free environment, but turn green after exposure to oxygen (VanderZee and Kennedy, 1982; Bozarth and Kennedy, 1985). Regardless of whether a wetland plant seedling can tolerate hypoxic or anoxic conditions when submerged, the first step is germination. If a species can germinate under low O_2 concentrations, and survive as a seedling under the same conditions, establishment will likely succeed.

Why did 6 species fail to germinate? Our pre-experiment seed viability analysis showed that all 20 species were viable. However,

the viability of the 6 species that failed to germinate in the experiment generally had lower rates of germination than the 14 species that germinated. We ran our experiment for 30 days, which was 14 days longer than a wetland plant germination study done by Shipley et al. (1989) on many of the same species, so duration of the study was likely adequate. It is possible that germination was inhibited by leachates from neighbouring seeds (Cope, 1982). Seeds can contain phytotoxic compounds that can inhibit seed germination. Cope (1982) tested the effects of leachates from seeds on the germination of 8 plant species (4 grasses and 4 legumes) and found that all effects were interspecific, not intraspecific. Legume germination was almost completely inhibited by grass seed leachate, but only one grass species showed signs of germination inhibition, which was from one of the legumes (Cope, 1982). A useful follow-up experiment would be to test the effects of leachates on inhibition of all 20 species included in our study, but particularly on the 6 species that failed to germinate.

In conclusion, our results suggest that the physiological constraints related to oxygen depletion controlling germination are operating independently of the constraints related to light levels. Low O_2 (<1%) is generally an impediment to germination success of the wetland species tested. Only a few species were able to germinate under low O_2, and those species that could had suppressed rates of germination and delayed timing of germination. Although low light levels reduced germination in three of our tested species, the differences in germination among light levels were not great. In terms of restoration, it is important to know which species can tolerate low O_2. Restoration planners should be aware that anoxic and hypoxic conditions seem to promote the germination of weedy and potentially invasive native and non-native species. Therefore, it is important to control the hydrology on the site to minimize low oxygen conditions, thus promoting more desirable wetland species.

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