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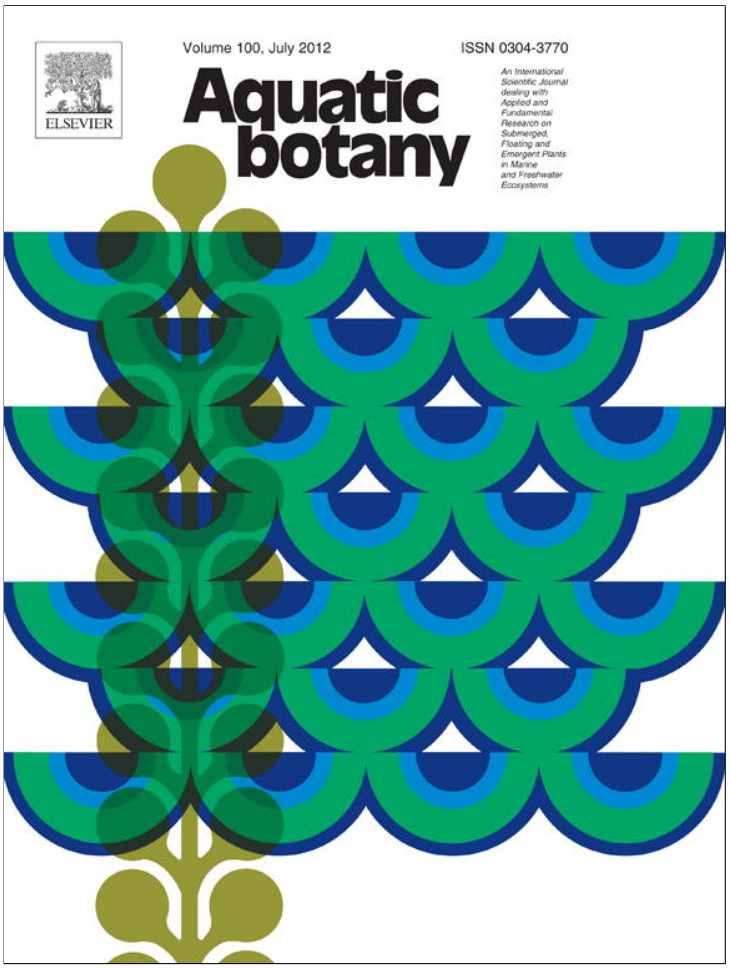


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Short communication

# Establishing a moss cover inhibits the germination of *Typha latifolia*, an invasive species, in restored peatlands

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ABSTRACT

Invasion of *Typha latifolia* L. into man-made pools in restored North American peatlands may represent a serious barrier to the establishment of a plant community typical of natural pool edges. As no classical method of population management appears applicable in the context of peatlands, our aim was to determine the ability of three environmental factors to inhibit *T. latifolia* germination, namely peat type, shade level and moss cover. A split-plot experiment conducted in a growth chamber investigated the effects of three substrates (fibric peat, mesic peat and filter paper) and six shade levels (including total obscurity) on germination rates of *T. latifolia*. In a second, greenhouse experiment, the effect of three increments of moss cover (null, fragmented and full) growing on two peat types (fibric and mesic) was examined for six corresponding seedbeds. Our results show that peat type was the major factor affecting germination, as almost none occurred on fibric peat while germination rates reached 84% on mesic peat. However, germination on mesic peat decreased with increasing moss cover: the germination rate dropped from $36 \pm 3\%$ on bare peat to $1 \pm 0\%$ in full moss carpets. Germination of *T. latifolia* was initiated by very low light levels (as low as 6%) but was inhibited by total obscurity. The low pH of fibric peat as well as the modification of environmental factors (e.g. light or substrate access) by moss carpets appear to be factors explaining the results. Establishing a dense moss cover and digging pools to a depth that prevents the exposure of peat with a pH favorable to seed germination (above 4) might be efficient methods to reduce *Typha latifolia* invasions in restored peatland pools.

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## 1. Introduction

Widely distributed in pristine wetlands of the circumpolar boreal hemisphere, *Typha latifolia* L., or broadleaf cattail, shows a high invasive ability in North America (McNaughton, 1966; Shih and Finkelstein, 2008). Its ecology and life-history traits, as well as environmental changes, contribute to the expansion of this eutrophent and ubiquist macrophyte (Galatowitsch et al., 1999; Olson et al., 2009). It can rapidly develop monospecific populations by both seed dispersion and rhizome growth and has been associated with biodiversity impoverishment, particularly in temperate wetlands like swamps, marshes or peatlands (Yeo, 1964; Houlahan and Findlay, 2004). The exploitation of peatlands for horticultural peat has created new surfaces for *T. latifolia* to invade (Poulin et al., 2005). Indeed, in peatlands abandoned after peat extraction activities have ceased, *T. latifolia* easily colonises former drainage ditches and water-logged areas. Using the moss layer transfer technique, the restoration of peatlands helps raise the water table (Rochefort et al., 2003), but can also further promote the expansion of existing *T. latifolia* colonies, notably when peat is more minerotrophic or where pools have been dug (Fontaine et al., 2007).

Pool creation has been a recent focus of peatland restoration. This type of micro-habitat strongly increases alpha diversity by sheltering specialist species (Fontaine et al., 2007). Pool creation is now recognized as constituting an ecological improvement, and is becoming common in peatlands restoration projects. Studies on active plant introduction along the margins of man-made pools have been carried out in Europe and North America using both seeds and rhizomes (Poulin et al., 2011; Falk et al., 2006). However, even six years after restoration, the establishment of

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specialized pool species remained sporadic notably because of the rapid expansion of *T. latifolia* populations (Fontaine et al., 2007). Techniques commonly used to actively control established *T. latifolia* populations in wet meadows, marshes or lakeshores, such as fire, water salinization, grazing and mowing, are not applicable to restored peatlands (Baldwin and Cannon, 2007). Furthermore, large scale modifications of microclimatic factors, using shade cloths or mulching, appear unable to control *T. latifolia* expansion since neither reduction of the full light flux by 80% nor a controlled temperature between 10 and 30 °C decreased *T. latifolia* germination (Lombardi et al., 1997; Kellogg et al., 2003). Among all ecological factors previously tested, including photoperiod, oxygen and nitrates concentrations, only total obscurity and pH lower than 3.5 have been shown to completely stop *T. latifolia* germination and root growth (Morinaga, 1926; Sifton, 1959; Bonnewell et al., 1983; Brix et al., 2002).

This study aimed to determine whether peat types, shade level or moss cover are ecological factors able to restrict the germination of *Typha latifolia* in a peatland context. We first hypothesized that lower peat pH and dense moss cover limit *Typha latifolia* germination. We also hypothesized a drop in germination rate below 80% of the full light flux. These three ecological factors were assessed during two experiments in controlled environments.

## 2. Methods

### 2.1. Plant and peat material

Mature spikes of *Typha latifolia* were collected in November 2010 around created pools of two restored peatlands located in southern Québec (47°58′N 69°26′W and 48°19′N 68°50′W). Seeds were separated from bristle hairs by spraying follicles with a strong jet of distilled water (adapted from Yeo, 1964) and were then settled in deionised water to select the most viable ones, which sink, whereas non-viable ones float (McNaughton, 1968). Seeds stored in cold (2.8 °C) and moist conditions for two months were used to perform both experiments because they showed germination rates 2.5 times higher than seeds stored at room temperature and in dry conditions (results not shown, $F_{\text{stratification}} = 27.12$, $p < 0.001$). Two types of peat were used: (1) fibric commercial peat, which had a pH of 3.7, a von Post decomposition index of H3 (Malterer et al., 1992; Buteau, 1985) and a corrected electrical conductivity of 86 μS cm$^{-1}$ (Sjörs, 1950); and (2) mesic peat, collected from an abandoned peatland, which had a pH of 4.3, a von Post decomposition index of H4 (Malterer et al., 1992; Buteau, 1985) and a corrected electrical conductivity of 117 μS cm$^{-1}$. These two peat types were chosen because fibric peat is the main substrate characterizing abandoned peatlands after peat mining while mesic peat outcrops are more common in the deeper peat layers left exposed after exploitation. Prior to experiments and physicochemical measurement, both types of peat were pasteurized at 60 °C, to deplete their seed bank.

### 2.2. Effect of substrate and shade on germination rate

The effect of six shade levels and three substrates on germination rate was assessed by a split-plot experiment conducted in a growth chamber. Main plots corresponded to 0, 50, 75, 88, 94, and 100% of the initial photosynthetic photon flux density of the growth chamber (50 μmol m$^{-2}$ s$^{-1}$). These shade levels were determined by superimposed layers of fiberglass nets, each one dividing the incoming light flux by half. Subplots corresponded to three substrates (fibric peat, mesic peat and filter paper) placed in Petri dishes. Filter paper, a substrate commonly used for germination experiments, was set as control. Fifty seeds of *T. latifolia* were placed in each Petri dish. Each treatment was replicated six times for a total of 108 experimental units. All substrates were moistened daily with deionised water to keep saturation. Temperature was fixed at 22 °C and a 18/6 h (day/night) photoperiod was provided by cold white fluorescent tubes. Germinated seeds, from which a hypocotyle had emerged, were counted using binoculars after seven days of growth.

### 2.3. Effect of moss cover on germination rate

A factorial experiment was performed inside a greenhouse to determine the effect of moss cover and peat types on germination rates of *T. latifolia*. We used three densities of moss cover (null, fragmented and full cover) and two underlying peat types (fibric or mesic). One particular species of moss was associated with each peat type to approximate natural growth conditions. *Campylium stellatum* (Hedw.) Lange & C.E.O. Jensen, collected in natural fens, was set on mesic peat while *Sphagnum rubellum* Wils., collected in natural bogs, was set on fibric peat. Full and fragmented covers were respectively obtained by recreating a moss carpet with natural stem density and by spreading moss diaspores on peat substrate until a cover of 75% was achieved. Bare peat corresponded to null moss cover. The experiment was set up in a complete randomized block design. Each of the six treatments was replicated in 15 experimental units which consisted of gardening pots 12 cm in height and 12.7 cm in diameter for a total of 90 experimental units. Water level was maintained 6 cm below the surface of the seedbed (combination of peat and bryophyte) to reflect hydrological conditions along natural pool margins. During the first six weeks, all emerging seedlings were weeded out to deplete the seed bank. Afterwards, 150 seeds of *T. latifolia* were sown on seedbeds and watered profusely with rainwater to bury them in the moss carpets. Environmental conditions were maintained at 20 °C and 70% of relative humidity (RH) during 16 day hours, alternating with 8 h of night at 15 °C and 50% RH. Experimental units were watered with rain water twice a week. Germinated seeds, from which a hypocotyle had emerged while in the seedbed, were counted 8 times during the 33 days of growth.

### 2.4. Statistical analyses

To assess the effects of substrates and shade levels, germination rates were compared among treatments by least square means, after having performed a two-way ANOVA using the Kenward-Rodger method to estimate degrees of freedom. To assess the effect of moss cover, a repeated measures ANOVA using a compound heterogeneous symmetry structure of covariance was first achieved to define when germination rates reached their maximum values and to prevent germination differences related to a delaying effect caused by seedbeds in the subsequent analysis. As maximum germination was attained at the end of the experiment (day 33) for all treatments ($F_{\text{peat type}\times\text{moss cover}\times\text{day}} = 45.54$, $p < 0.0001$), these final rates were used to compare seedbed effects using a two-way ANOVA, followed by least square means. Statistical significance was determined at $p \leq 0.05$. Prior to all analyses, the normality and homogeneity of variance were verified. Analyses were conducted with SAS (version 9.2, SAS Institute Inc., Cary, NC).

## 3. Results

After seven days in a growth chamber, germination rates ranged from 0 ± 0% (mean ± standard error) to 92 ± 3% (Fig. 1) and were significantly influenced by shade level and substrates (Table 1). Minimal seed germination rates (between 0 ± 0% and 5 ± 2%) were observed in total obscurity regardless of peat type. On the other hand, maximal rates (between 65 ± 7% and 92 ± 3%) were observed on mesic peat and filter paper for all shade levels from 0 to 94%

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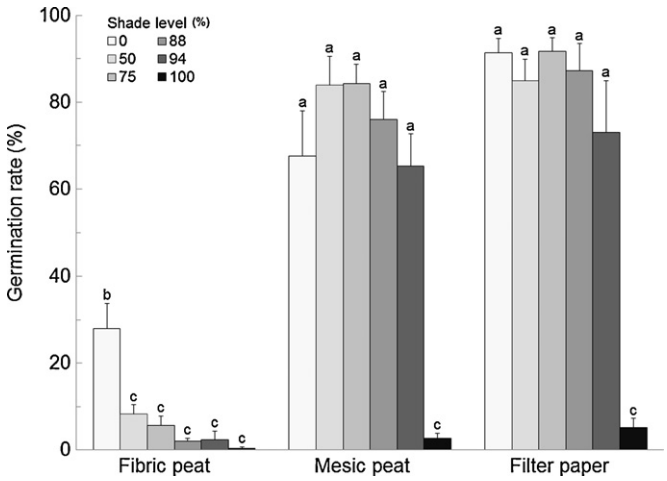


**Fig. 1.** Average germination rates (±SE; $n = 6$) of *Typha latifolia* seeds on three different substrates (fibric peat, mesic peat and filter paper) at six different shade intensities ranging from total obscurity to full light (see top left of the figure for the exact intensities). Different letters indicate significant differences among treatments (LS-means, $p < 0.05$).

**Table 1**
Effects of shade level (0 – 50 – 75 – 88 – 94 – 100%) and substrate (fibric peat – mesic peat – filter paper) on germination rates of *Typha latifolia* after seven cultivation days in growth chamber analyzed with a two-way ANOVA (df: degrees of freedom; %SS: percentage of sum squares).

| | df | %SS | *p*-Value |
|---|---|---|---|
| Shade level | 5 | 51.19 | <0.0001 |
| Peat type | 2 | 380.37 | <0.0001 |
| Shade level × peat type | 10 | 15.73 | <0.0001 |

**Table 2**
Effects of peat type (fibric peat – mesic peat) and moss cover (null cover – fragmented cover – full cover) on germination rates of *Typha latifolia* after 33 cultivation days in greenhouse analyzed with a two-way ANOVA (df: degrees of freedom; %SS: percentage of sum squares).

| | df | %SS | *p*-Value |
|---|---|---|---|
| Peat type | 1 | 180.11 | <0.0001 |
| Moss cover | 2 | 82.91 | <0.0001 |
| Peat type × moss cover | 2 | 82.46 | <0.0001 |

(Fig. 1). On fibric peat however, germination rates were comparable to those recorded in total obscurity (ranging from $2 \pm 1\%$ and $8 \pm 2\%$) for all shade levels except for full light, for which rates were significantly higher (Fig. 1; $28 \pm 6\%$ on average).

At the end of the greenhouse experiment (day 33), germination rates were differently impacted by moss cover depending on peat type (Table 2). Cotyledon emergence was highest on mesic peat in the absence of moss cover (Fig. 2; $36 \pm 3\%$; mean ± standard error). As moss cover increased, germination rates decreased strongly. Intermediate rates ($10 \pm 1\%$) were observed with fragmented moss cover, while very low rates ($1 \pm 0\%$) were observed with dense carpets of *Campyllium stellatum* (Fig. 2). No germination was observed on fibric peat, independently of moss cover.

## 4. Discussion

Results of both experiments showed that the nature of the substrate was a key factor in controlling germination of *Typha latifolia* seeds. Fibric peat inhibited germination almost completely, whereas maximal germination rates occurred on mesic peat and filter paper. Similar tendencies were observed previously for germination of *Typha domingensis* Pers., which was reduced from a maximum of 90% on filter paper to as low as 55% on Everglades peat (Stewart et al., 1997). The lower germination rates on fibric peat were likely due to its physicochemical properties. Since germination rates on filter paper were as high as those on mesic peat watered with deionised water, the lower nutrient availability of fibric peat cannot explain the near absence of germination. Peat pH remains the factor most likely to have controlled germination rates. Indeed, previous studies have determined that pH levels lower than 3.5 completely stop growth of *T. latifolia* rhizomes, by affecting root cell integrity and preventing nitrogen absorption (Brix et al., 2002). Conversely, no reduction in germination rates or in seedling growth of *T. latifolia* has been observed at pH levels higher than 4 (Rivard and Woodard, 1989). This physiological threshold most likely explains the very low germination rates of *T. latifolia* on fibric peat with a pH of 3.7. Nevertheless, allelopathic effects cannot be excluded as an explanation of the almost null germination rate on fibric peat, because at this stage of its life

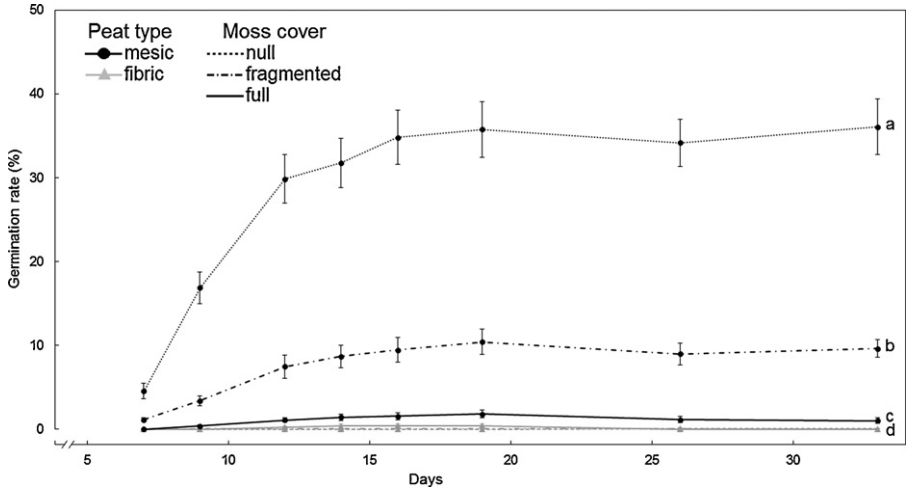


**Fig. 2.** Temporal evolution of *Typha latifolia* germination rates on two types of peat (fibric vs mesic) on which three different bryophyte covers were applied (bare peat, bryophytes established from fragments and dense carpet of bryophytes). Bryophyte species were *Sphagnum rubellum* on fibric peat and *Campylium stellatum* on mesic peat. Significant differences among treatments at day = 33 (LS-means, $p < 0.05$) are indicated by different letters. The letter d corresponds to the three curves of fibric peat which are superimposed and all belong to the same group. *X*-axis is shortened.

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cycle, *T. latifolia* is sensitive to specific toxic sterols, chemical compounds that can also be found in peat moss (Ives and O'Neill, 1958; McNaughton, 1968; Della Greco et al., 1990).

On mesic peat, for which no pH-limitation occurred, germination was reduced by the presence of mosses, especially as their cover increased, culminating in an absence of germination on complete moss cover. Previous studies have obtained similar results for vascular wet meadow species, which had higher germination rates after aboveground vegetation was mowed and the moss layer underneath removed (Kotorova and Leps, 1999). Moss cover's effect on vascular plant germination has been partly attributed to its role as a physical barrier, since moss reduces availability of germination sites as well as preventing direct contact between seeds and substrate (During and Van Tooren, 1990). Moreover, the absence of germination cannot be attributed to light competition with mosses, at least on fibric peat, on which almost no germination occurred even without moss cover. On mesic peat, watering the seeds buried them, and the moss may have intercepted all available light, creating conditions of total obscurity and thereby preventing seed germination. These results are in accordance with Kellogg et al. (2003), who showed that germination was not affected by 80% shade, and are also consistent with the germination break observed in total obscurity in *T. latifolia* (Sifton, 1959).

Results from our controlled experiments allow us to define recommendations for peatland pool restoration, even if further experiments are needed to confirm peat pH-limitation of seed germination. Before pool digging, it is advisable to characterize the variations of physicochemical properties along the peat profile, in order to avoid the exposure of mesic peat (with a pH higher than 3.7) favourable to seed germination. After pool creation, if any mesic peat is exposed, the rapid establishment of a complete moss carpet is recommended, to create ecological conditions detrimental to *Typha latifolia* germination. Furthermore, planting vascular specialist species, which are absent from the seed bank of mined peatlands, should be considered to reestablish a complete community of typical plants. The high invasive ability of *Typha latifolia* also implies a regular monitoring of the plant composition in restored pools to identify individuals of this species early and limit their expansion.

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