Aquatic Botany 137 (2017) 30–38

Contents lists available at ScienceDirect

Aquatic Botany

journal homepage: www.elsevier.com/locate/aquabot

Title: Influences of reduced iron and magnesium on growth and photosynthetic performance of *Phragmites australis* subsp. *americanus* (North American common reed)

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ARTICLE INFO

Article history:
Received 28 April 2016
Received in revised form 31 October 2016
Accepted 8 November 2016
Available online 9 November 2016

Keywords:
Wetland plants
Propagation
Nutrient limitation
Chlorosis
Fe(II)
Mg(II)

ABSTRACT

Studies of the North American native wetland plant *Phragmites australis* (Cav.) Trin. ex Steud. subsp. *americanus* Saltonst., P.M. Peterson & Soreng have been hindered by chlorosis – tissue yellowing due to reduced chlorophyll production – while grown in controlled conditions, resulting in reduced growth. This study tested the effects of reduced iron [Fe(II)] and magnesium [Mg(II)], both important nutrients for chlorophyll production, on photosynthetic performance and growth of North American *Phragmites*, a plant of interest for wetland restoration and management. Plants were exposed to five treatments in a 13-week greenhouse experiment. Four of the treatments consisted of a factorial arrangement of Fe(II) and Mg(II) treatments (0.0002 M FeSO4, 0.0002 M MgSO4, both, or none, in supplied water), supplemented with a slow-release fertilizer. A fifth treatment received no fertilizer, Fe(II), or Mg(II). Treatments that received added Fe(II) had significant increases in biomass, stem height, leaf and root production, chlorophyll B, and fluorescence parameters compared to non-Fe(II) treatments. Adding Mg(II) did not significantly improve plant health (growth, morphology, or resource capturing) compared to the fertilized control, and suppressed the stimulatory effect of Fe(II), possibly by interfering with root Fe(II) uptake. Under greenhouse conditions and normal fertilization practices, Fe(II) deficiency can sharply reduce growth and photosynthetic performance of *Phragmites australis* subsp. *americanus*. Supplementing plants with freshly prepared solutions of Fe(II) will alleviate limitation and facilitate experimental study and propagation under controlled conditions. The strong effect of iron limitation on native *Phragmites* suggests there could be a link between Fe(II) limitation and plant competitive interactions in some wetlands.

1. Introduction

Effective plant propagation for food, nursery production, or research has long been an important area of scientific exploration (Allen and Pearsall, 1963; Marsh et al., 1963; Steucek and Koontz, 1970; Wei et al., 1994; Thoiron et al., 1997; Rout, 2015). Considerable research has focused on growth effects of secondary macronutrients and micronutrients such as magnesium (Mg) and iron (Fe) (Marsh et al., 1963; Steucek and Koontz, 1970; Thoiron et al., 1997; Overman and Scholtz, 2004; Rout, 2015). When nutrient requirements are not met or are poorly understood, growth under controlled conditions may prove difficult or impossible.

Studies on nutrient limitation in wetland plants have primarily focused on macronutrients, especially nitrogen, under natural conditions in the field or under simulated conditions in the greenhouse (Baldwin, 2013). Fewer studies have analyzed the effects that secondary nutrients (e.g., Mg) and micronutrients (e.g., Fe) have on growth of wetland species. Limited Mg may hinder wetland plant growth, as indicated by Ding et al. (2006), who found that Mg deficiencies were linked to reduced shoot growth and chlorophyll concentration. Rozbrojová and Hájek (2008) studied nutrient levels – including Fe – in a mountain fen, and found that poor vegetation growth was correlated with high levels of Fe (above 150 μg/g in vegetation tissue). Of the limited studies published on the relationship between wetland plant growth and Fe or Mg, only a few have focused on their effects on *Phragmites australis* (Snowden and Wheeler, 1993; Weisner, 1996; Vretare-Strand and Weisner, 2002, 2003; Gigante et al., 2014).

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http://dx.doi.org/10.1016/j.aquabot.2016.11.005
0304-3770/

Phragmites australis, the common reed, is a perennial wetland grass and one of the most widespread plant species in the world (Haslam, 1972; Clevering and Lissner, 1999). It reproduces sexually through seed dispersal and asexually by rhizomal expansion, and can be found in many fresh and brackish water wetlands, including fens, lake and river shorelines, and marshes (Saltonstall and Stevenson, 2007; Ward, 2010; Kettenring et al., 2011). Three taxonomic lineages of *Phragmites* have been described in North America: *Phragmites australis* (Cav.) Trin. ex Steud. subsp. *australis* (Eurasian, invasive); *Phragmites australis* subsp. *americanus* (native to USA and Canada); and *Phragmites australis* (Cav.) Trin. ex Steud. subsp. *berlandieri* (E. Fourn.) Saltonst. & Hauber (native to the USA subtropics and southward) (Saltonstall, 2002; Saltonstall et al., 2004). This study specifically examined *Phragmites australis* subsp. *americanus*, hereafter referred to as native *Phragmites*.

Differences in morphological and physiological traits affect the competitive abilities of the native and invasive lineages. Native *Phragmites* is shorter and exhibits lower stem density than invasive Eurasian *Phragmites*, which has a longer growing season as well as greater primary productivity, nitrogen uptake, and leaf litter build-up (League et al., 2006; Saltonstall and Stevenson, 2007; Mozdzer and Zieman, 2010; Holdredge and Bertness, 2011; Price et al., 2014). These traits, along with invasive *Phragmites*' tendency to proliferate and be stimulated by anthropogenic factors such as disturbance and nutrient pollution have allowed it to invade wetlands formerly dominated by native plants, including native *Phragmites* (Saltonstall, 2002; Mozdzer and Zieman, 2010; Guo et al., 2013; Hazelton et al., 2014). The invasive lineage decreases biodiversity in wetlands through competitive displacement (Weisser and Parsons, 1981; Marks et al., 1994; Ailstock et al., 2001), whereas the native lineage allows for more diversity due to its scattered shoot and root growth pattern (Meyerson et al., 2000; Hershner and Havens, 2008; Price et al., 2014). Soil microbial communities also differ between the native and invasive lines (Yarwood et al., 2016), suggesting differences in biogeochemical function. Aside from the differential impacts on biodiversity, both lineages have phytoremediatory and soil stabilization properties, which are highly valued in constructed and restored wetlands (Hershner and Havens, 2008; Faure et al., 2012).

Genetic confirmation of the existence of native *Phragmites* (Saltonstall, 2002; Saltonstall et al., 2004) has led to greater interest in research on its biology and ecology. However, propagating it under controlled conditions for experiments or planting trials has proven more difficult than propagating Eurasian *Phragmites*. The invasive lineage produces prolific lateral shoots from stems placed horizontally in tanks of water and has high productivity if supplemented with commercial fertilizers in greenhouse pots and tanks ('Personal observation'; Achenbach et al., 2013). Native *Phragmites*, in contrast, does not produce abundant lateral shoots under flooded conditions ('Personal observation' and Eller, 'Personal communication'). When grown in the greenhouse, chlorosis, a lack of chlorophyll production in plants that leads to the yellowing of the leaves and stems, develops even when pots are supplemented with commercial fertilizers or Hoagland's solution (Marsh et al., 1963; Wei et al., 1994; 'Personal observation'). Hoagland's solution contains a suite of macro- and micronutrients, including Mg(II) and chelated Fe(III).

Other researchers studying native *Phragmites* have reported "apparent nutrient deficiency" (Mozdzer and Megonigal, 2013) or alleviated chlorosis using reduced Fe(II) (Eller et al., 2013) or reduced Mg(II) (as Epson salts, $MgSO_4$; Saltonstall, 'Personal communication'). Chlorosis due to Fe limitation has been studied in rice (*Oryza sativa*), another wetland plant in the same family (Poaceae). This limitation is thought to be due to an inability of rice to reduce oxidized Fe(III) to Fe(II) in the rhizosphere, because of radial oxygen leakage (Jones et al., 1982; Mori et al., 1991; Abadia et al., 2011). Magnesium deficiency has also been shown to reduce growth and chlorophyll content in rice, which can be aggravated by increased potassium (K) supply (Jones et al., 1982; Ding et al., 2006).

The objective of this study was to understand how two nutrients necessary for chlorophyll production, iron and magnesium, affected the growth of native *Phragmites* from rhizomes to a healthy, non-chlorotic, adult stage. Magnesium sulfate ($MgSO_4$) and iron sulfate ($FeSO_4$) were used as supplements based upon anecdotal and published evidence suggesting some success in reducing chlorosis in native *Phragmites* and other wetland plants (Vretare-Strand and Weisner, 2002; Saltonstall and Stevenson, 2007; Eller et al., 2013). We hypothesized that plants given additional Fe(II) and/or Mg(II) would produce more aboveground and belowground biomass and have little or no evidence of chlorosis or other impairment of photosynthetic performance.

2. Methods

2.1. Experimental setup

A greenhouse experiment was established in a randomized block design of five treatment combinations of Fe(II), Mg(II), and fertilizer. Four of the treatments consisted of a factorial arrangement of Fe and Mg treatments (0.0002 M $FeSO_4$ (Eller et al., 2013), 0.0002 M $MgSO_4$ (Saltonstall and Stevenson, 2007), both, or none, in tap water). Pots receiving these treatments were supplemented once before the start of the experiment with a slow-release pelletized fertilizer at the recommended application rate (Osmocote®, Scotts, Marysville, OH, N:P:K = 15:9:12, about 27.5 g per pot). The fifth treatment was an unfertilized control consisting only of unamended tap water. The five treatments are abbreviated as: Fe (only Fe(II) added); Mg (only Mg(II) added); MgFe (both Mg(II) and Fe(II) added); FC (fertilized control; no Fe(II) or Mg(II) added); and UC (unfertilized control; no Osmocote, Fe(II), or Mg(II) added). Five replicate blocks were established in the University of Maryland Research Greenhouse to test for any potentially confounding light or humidity gradients. Each block contained five pots (the experimental unit) planted with one native *Phragmites* rhizome in each pot. Each of the five blocks contained one Fe, Mg, MgFe, FC, and UC treatment, applied randomly to experimental units within each block, totaling five replicates.

The rhizomes were collected from tidal freshwater and oligohaline wetlands on the Choptank River, in the U.S. state of Maryland, in October 2014 (N38° 42′ 8″, W76° 41′ 48″, map datum: WGS84). After rinsing the rhizomes in the field, they were kept refrigerated (4 °C) and moist for 4 months. Prior to planting, 5.6-l circular pots with a surface area of 410.43 cm^2 were prepared for facilitated water drainage by drilling three holes halfway up the side of each pot. On 6 February 2015, rhizomes with five or more nodes were then planted 3 cm below the surface of a 1:1 peat:sand mixture and the pots were placed inside of a 19-l bucket. Water levels were maintained 5 cm below the soil surface. Each week, the plants were watered through the following procedure: the pot was removed from the bucket, the remaining bucket water was poured through the pot (to flush soil of precipitated solutes), and allowed to completely drain; the pot was placed back into the bucket and half of a 3-l solution of treatment water (mixed immediately prior to application) was poured onto the surface of the growth media; and the other half was poured into the exterior bucket. As the experiment progressed, additional water was needed due to increasing evapotranspiration rates, but the total Fe and Mg loads remained the same. Evapotranspiration rates in individual experimental units were not tracked. On 12 February 2015, Osmocote was added to the fertilized treatments. The plants were allowed to sprout and