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Is the reduced growth of the halophyte *Suaeda maritima* under hypoxia due to toxicity of iron or manganese?

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ABSTRACT

For most plants, submergence in water is a rare occurrence, but for plants that grow on salt marshes flooding with seawater may be a twice-daily event. This is the case for plants of the halophyte *Suaeda maritima*, growing at low elevations on salt marshes. These plants are, however, smaller than those growing at higher elevations, where flooding is less frequent and the soil better drained. We investigated whether the reduced growth brought about by flooding with saline water was a consequence of toxicity of manganese or iron. Seedlings of *S. maritima* were grown both in a solid medium (a mixture of salt-marsh mud and sand) that was either submerged twice a day or continuously flooded with half-strength seawater and in a hydroponic solution where the oxygen concentration was adjusted by bubbling with nitrogen or air. Hypoxia, reduced the growth of plants in both solid and liquid media and resulted in increases in manganese and iron in the shoots and roots. Experiments in culture solution showed that elevated levels of manganese were unlikely to be toxic, but that iron did reach toxic concentrations in flooded plants.

1. Introduction

Suaeda maritima is a plant that grows in both the upper and lower regions of salt marshes, although plants are larger on upper than lower elevations (Wetson, 2008; Wetson and Flowers, 2010). The hypoxic conditions that exist in the lower marsh compared to the normoxic conditions of the upper marsh (Colmer et al., 2013) are likely to result in reduced ATP production, as oxygen is in poor supply to the roots. *S. maritima* has no aerenchyma to facilitate diffusion of oxygen from the shoots (Hajibagheri et al., 1985; Wetson, 2008), although it does accumulate high concentrations of lactate in both normoxic and hypoxic conditions (Colmer et al., 2013; Wetson et al., 2012). Reduced ATP supply could reduce the uptake of ions that determine the growth rate (Yeo and Flowers, 1986) and so reduce growth. There is also the possibility that hypoxia influences the bioavailability and accumulation of metal ions, leading to deficiency or toxicity, which might explain the difference in growth between upper and lower elevations of a salt marsh – the subject of this paper.

Coastal salt marshes are heavily influenced by daily tidal inundations that waterlog the soil for different lengths of time depending on elevation. Waterlogging affects the availability of micronutrients for plants, as periodic and prolonged flooding of soil results in biological and chemical processes that are very different from those that happen in well-drained and aerated soils. When a soil is flooded, oxygen diffuses from the air into the soil around 10,000 times more slowly than in well-drained soil, so the concentration of oxygen can decrease to very low levels (Ponnamperuma, 1972), reducing the redox potential of the soil and altering its elemental profile. Once oxygen is depleted, respiring soil microbes use nitrates as electron acceptors, followed by oxides of manganese, then iron and then sulphate. The conversion of Mn (IV) and Fe (III) oxides to Mn (II) and Fe (II) oxides, increases the solubility of both elements with a sharp decline in redox potential (Ponnamperuma, 1972). The end result of changes in oxidation state in the soil is a significant increase in soluble Fe^{2+} and Mn^{2+}, even at high pH (see Millaleo et al., 2010) with potential consequences for plant growth.

The concentration of Mn in agricultural soils is highly variable (by some 40 fold; Nagajyoti et al., 2010) with values, on a soil water basis, ranging from 20 nM to 72 μM (Mansfeldt, 2004; Goss et al., 1992). The concentrations of Fe in aerobic soils at normal pH values (pH 5–7) are very low (in the nM range; see Marschner, 1986) and can limit the growth of plants. However, high external

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concentrations of both elements (Mn^{2+} and Fe^{2+}, the forms in which plants take up Mn and Fe) are toxic (Millaleo et al., 2010; Marschner, 1986). Poor aeration in salt-marsh soils leads to high, potentially toxic, concentrations of both Fe (Otero et al., 2009) and Mn (Otero et al., 2009; Singer and Havill, 1985). For example, at low elevation of a salt marsh in southern Brazil, Mn reached concentrations of about 300 μM, 10–20 cm below the surface of a zone dominated by *Spartina alterniflora*; Fe concentrations were about 200 μM in the same zone (Otero et al., 2009). In salt marshes from N. Carolina, Fe concentrations ranged from about 20–700 μM (Adams, 1963). Such high concentrations could lead to reduced growth directly due to Mn or Fe toxicity or as a consequence of the costs of adapting to such high concentrations.

Unfortunately, the literature does not provide a consensus on the effects of changed Mn and Fe concentrations on the growth of salt-marsh species. Cooper (1984) reported that the shoot dry weights of *Plantago maritima, Armeria maritima and Juncus gerardii* were reduced by Mn concentrations greater than 250 μM (the results for *Salicornia europaea*,*Puccinellia maritima*, *Triglochin maritima*, *Aster tripolium*, and *Festuca rubra* were less clear). *S. europaea* and *A. tripolium* have been reported sensitive to Mn concentrations greater than 160 μM in solution culture, but in the absence of salt (Singer and Havill, 1985). Singer and Havill (1993) later claimed that although Mn concentrations were relatively high in the upper 1 cm of salt-marsh soils and that salt-marsh species have considerable tolerance to Mn, the concentration did not correlate with elevation or species distribution. Whether plants of *S. europaea*, *P. maritima*, *J. gerardii* or *A. maritima* were grown under flooded or drained conditions had little effect on the Fe or Mn concentrations in their shoots (Rozema and Blom, 1977). Adding NaCl (170 mM) to the culture solution in which *P. maritima* and *A. tripolium* were grown reduced the uptake of Mn (Singer and Havill, 1993), a result in line with the finding that halophytes sampled from salt marshes had lower Fe and Mn concentrations than plants from non-saline habitats (Gorham and Gorham, 1955). Data in the literature do not answer the question of whether high Mn or Fe concentrations might be responsible for differences in growth of *S. maritima* between upper and lower elevations of salt marshes.

We investigated the effects of external Fe and Mn concentrations on the growth of *S. maritima* and its content of these elements under normoxic and hypoxic conditions, using both a soil-based medium and a hydroponic solution (for details see below) in order to elucidate metal bioavailability and its consequences for *S. maritima* growing on the varying conditions of a salt marsh. We examined the hypothesis that the accumulation of Mn and Fe in *S. maritima* plants growing in hypoxic conditions, characteristic of the lower marsh, was sufficient to result in toxicity and so reduce growth relative to plants growing more aerobic conditions.

2. Materials and methods

2.1. Plant material, germination and initial growth of seedlings

Seeds of *S. maritima* from Cuckmere Haven, East Sussex (UK National Grid Reference 551400098500, TQ515978 were germinated in plastic trays containing silver sand irrigated with half-strength nutrient solution (Stout and Arnon, 1939; supplementary Table 1) and grown for four weeks, in a growth chamber (Weiss 2400E/+5 JU-Pa-S; Weiss Technik, Gmbh, Reiskirchen–Lindenstruth, Germany) with a 16 h photoperiod at 200 μmols $m^{-2} s^{-1}$ and 22 °C and 60% relative humidity; during the dark period, the temperature was 17 °C and the relative humidity 70%.

2.2. Plant growth

Since in the majority of previous research on *S. maritima*, plants have been grown hydroponically at pH values below 7, this practice was continued in some of the experiments described in this study. Plants were grown in a half-strength culture solution (Stout and Arnon, 1939) made up in a dilution of an artificial seawater (Harvey, 1966), in order to provide the necessary nutrients that are low in seawater (N and P) while maintaining the ratios of the major ions (Cl, Na, Mg and Ca) present in natural seawaters. In order to investigate the effects of hypoxia, some of the solutions contained 0.1% agar. Preliminary tests showed that a 0.1% agar solution more effectively simulated the situation in waterlogged soils and in the rhizosphere, as compared to N_2 flushed or non-flushed agar-free nutrient solutions (see also Wetson, 2008). We recognise that there may be a contrast with plants growing in natural salt-marsh soils, but attempting to grow plants at a high pH with hydroponics, means that many micronutrients precipitate from solution, so that the solution has to be changed daily or other ways found of supplying micronutrients, such as by foliar spray (Singh et al., 2002). Experiments were also conducted in a medium based on a natural salt-marsh soil for comparative purposes (see below). All experiments were repeated at least once with representative data being presented here.

2.3. Experiment 1: the effect of aerobic and hypoxic conditions on growth and trace metal contents under controlled conditions in a growth cabinet

Seeds were germinated as described above. Plants were transplanted at 4 weeks into nutrient solution in artificial seawater diluted to 350 mM Na^+containing agar (see below) and grown in black plastic-lidded beakers (500 ml, 15 cm high and 7 cm diameter). There were 15 plants per treatment (5 beakers per treatments; 3 plants per beaker), each plant being suspended through a hole in the lid and held in place with non-absorbent cotton wool. Agar-nutrient solution was prepared by dissolving 10 g of agar (Sigma, Plant Cell Culture A 1296) in 2 L of distilled water and autoclaving at 120 °C for 15 min. After cooling, this solution was added to 7.39 L of full-strength artificial seawater, then distilled water was added to make a final volume of 10 L, so producing 350 mM Na^+ with 0.1% w/v agar. The solution was stirred thoroughly to avoid lumps of agar forming. For normoxic treatments, compressed air was bubbled through the solution to obtain good aeration in the solution prior to filling the beakers (pre-bubbled). For hypoxic treatments, nitrogen gas was bubbled through the solutions to reduce oxygen to less than 0.5 mg L^{-}. Gas was not bubbled through the solutions during the eight weeks of treatment as this can damage the roots (Wetson, 2008), but the solutions were changed twice a week.

Plants were harvested after 8 weeks in the Weiss cabinet with one of two treatments.

(a) Normoxic nutrient solution (pre-bubbled with air) with 350 mM Na^+ (350 N).
(b) Hypoxic nutrient solution (pre-bubbled with N_2) with 350 mM Na^+ (350 H).

Oxygen concentrations were recorded before and after changes of the culture solution with an oxygen meter (HI 9142 oxygen meter, HANNA Instruments); pH values and electrical conductivity (EC) were measured before growth medium solutions were renewed.