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Physiological relationship between oil tolerance and flooding tolerance in marsh plants

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ABSTRACT

Coastal salt marshes are highly susceptible to accidental oil spills. Oil can cover root tissues, inhibiting gas exchange and increasing root oxygen stress. This is similar to flooding; both conditions reduce the availability of oxygen to a plant. Therefore, it was hypothesized that tolerance to both flooding and oil are related in plants, as both impede gas exchange between roots and their environment. We investigated effects of oil on respiration and photosynthesis in nine marsh species ranging from flooding sensitive (hypothesized oil sensitive) to flooding tolerant (hypothesized oil tolerant). Plants were subjected to $6\,\mathrm{L\,m^{-2}}$ light motor oil in greenhouse experiments for six weeks. Oil exposure caused root oxygen deficiency in all but the most flooding tolerant species. Alcohol dehydrogenase activities increased in roots of flooding sensitive species and moderately flooding tolerant plants under oil exposure, indicating oxygen stress. In contrast, flooding tolerant plants showed no sign of oxygen deficiency under oil treatment. Additionally, decreases in photosynthetic rates were measured in some species following oil exposure. Photosynthesis was reduced by non-stomatal limitations, suggesting toxic effects of oil. It was concluded that tolerance to oil relates to flooding tolerance when measured in terms of biochemical enzyme activity. Root alcohol dehydrogenase activity was a sensitive indicator of oil susceptibility, whereas leaf-level photosynthesis was less sensitive to oil. Relating flooding and oil tolerance based on physiological measures could help future efforts to protect marsh plants prior to an oil spill as well as manage coastal marshes following an oil spill.

1. Introduction

Coastal marshes contain a rich diversity of both flora and fauna (Culbertson et al., 2008; Lin and Mendelssohn, 2012), but many factors threaten marshes (Ko and Day, 2004; Mendelssohn et al., 2012). One prominent anthropogenic stress that is highly relevant to coastal marshes is oil spills (Hester and Mendelssohn, 2000). Off-shore oil drilling (DeLaune et al., 1979), transportation (Fiedler et al., 2009), and refining (Lin and Mendelssohn, 1998) pose serious risks to the health of coastal salt marshes (Lin et al., 2002), particularly due to a rise in accidental oil spills (Lin and Mendelssohn, 1996). Inland systems are also at risk of oil spills. Used motor oil is one of the most commonly spilled petroleum products in the United States, both along shorelines and inland urban areas (National Park Service, 1997). Oil can impact plants physiologically from toxic components in the oil or by preventing gas exchange with surroundings (Baker, 1970). Numerous studies have shown photosynthetic rates to decrease from oil exposure (Pezeshki and DeLaune, 1993; Lin and Mendelssohn, 1996). However, little is known about the effects of oil on respiration (Baker, 1970; Pezeshki et al., 1998), with varying results between studies.

When considering effects of oil on plant respiration, it is important to consider availability of oxygen. Following an oil spill, oil covers root tissue, making it difficult for a plant to access oxygen from its surrounding soil (Pezeshki et al., 2000). In a similar manner, oxygen exchange in flooded tissues is also inhibited (Maricle et al., 2006), meaning oil might lead to conditions similar to flooding. We hypothesize plant tolerance to oil exposure is related to flooding tolerance, as oxygen availability to a plant is reduced in both of these conditions (LaRiviere et al., 2003; Maricle et al., 2006). Accordingly, adaptations that confer flooding tolerance might provide tolerance to oil. Many flooding tolerant plants can survive hypoxic soils by variation in anatomical and physiological mechanisms, such as aerenchyma formation in roots (Maricle and Lee, 2002), metabolic adjustments (Maricle et al., 2006), or variations in oxygen uptake kinetics (Maricle and Lee, 2007). The status of oxygen in below-ground tissues is especially meaningful, as these tissues would be oxygen limited in oil-affected plants

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(Pezeshki et al., 2000). Consequently, measures of respiration can be instructive in oil-impacted plants, either with direct measures of gas exchange (Maricle and Lee, 2007), or with indirect measures of metabolic capacity with enzyme assays (Maricle et al., 2006).

Under hypoxic (low oxygen) conditions (e.g., flooding, oil), oxygen might be limiting for aerobic respiration (Drew, 1997). In the most common form of anaerobic respiration in plants, alcohol dehydrogenase (ADH) is the terminal enzyme that oxidizes NADH during fermentation (Drew, 1997). High ADH activity indicates a plant is routing metabolism through anaerobic respiration (Maricle et al., 2006); therefore, ADH activity is commonly used as an indicator for oxygen stress due to its sensitivity to hypoxic conditions. In a similar manner, if plants are deficient of oxygen during oil exposure (Stebbings, 1970; LaRiviere et al., 2003), ADH activities in roots could be used to assess oil tolerance.

Measures of photosynthesis can also be instructive to evaluate plant tolerance to spilled oil, as sensitive plants are expected to reduce photosynthesis. Low-oxygen conditions in flooded soil commonly limit gas exchange between leaves and the environment (Waring and Maricle, 2012; Caudle and Maricle, 2012). Similarly, low-oxygen conditions in oil-impacted soil could be expected to cause a decrease in photosynthetic rates (Pezeshki and DeLaune, 1993). Reductions in photosynthesis caused by flooding can come from stomatal closure (Yordanova et al., 2005) or non-stomatal limitations (Else et al., 2009; Polacik and Maricle, 2013). Similar results have been documented in plants exposed to oil. Numerous studies have shown oil to inhibit gas exchange between leaves and the environment due to blocking of stomata with oil (Pezeshki and DeLaune, 1993; Armstrong et al., 2009). Additional studies have shown oil to cause non-stomatal limitations of photosynthesis from light absorption by oil or from cell-level toxicity (Baker, 1970).

The preceding indicates oil causes a lack of oxygen available for the plant and a decrease in photosynthesis that might or might not relate to this lack of oxygen. Considering the mechanisms of oil toxicity, physiological studies of photosynthesis and respiration become meaningful when assessing plant responses to oil exposure. If oil influences plants by a lack of oxygen, then anaerobic respiration should be increased in oil-impacted tissues. If oil influences plants by a cell-level toxicity, then other metabolic indicators should become evident, such as decreases in photosynthesis and respiration.

In the present study, plant species ranging from flooding tolerant (*Phragmites australis* (Cav.) Trin. ex Steud. and *Spartina alterniflora* Loisel.), moderately flooding tolerant (*Phalaris arundinacea* L., *Spartina patens* (Aiton) Muhl., and *Spartina pectinata* Bosc ex Link), and flooding sensitive (*Ammophila breviligulata* Fernald, *Panicum virgatum* L., *Schizachyrium littorale* (Nash) E.P. Bicknell, and *Solidago sempervirens* L.) were exposed to spilled oil in greenhouse treatments (Table 1). We sought to determine if the proposed relationship between flooding tolerance and oil tolerance could be assessed based on changes in ADH activities and photosynthesis in response to oil exposure. Since both flooding and oil conditions limit oxygen availability to a plant, the flooding tolerant (hypothesized oil tolerant) category of species was expected to have unchanged photosynthetic rates and unchanged ADH activities under oil exposure; the moderately flooding tolerant (hypothesized moderately oil tolerant) category of species was expected to have a minor decrease in photosynthetic rates as well as a minor increase in ADH activities under oil exposure, and the flooding sensitive (hypothesized oil sensitive) category of species was expected to have significantly decreased photosynthetic rates and increased ADH activities under oil exposure.

2. Materials and methods

2.1. Plant material and growing conditions

Plants were obtained through the USDA (2012) Natural Resources Conservation Service Plant Materials Program (Table 1). Plugs of *A. breviligulata* “Cape,” *S. sempervirens* “Monarch,” New Jersey ecotype of *S. patens* “Avalon,” *S. pectinata*, *P. virgatum* “high tide,” as well as seeds of *S. alterniflora* “Bayshore” and *S. littorale* “Dune Crest” were obtained from the Cape May Plant Materials Center of Cape May, New Jersey, USA. Plugs of the Florida ecotype of *S. patens* “Sharp” were obtained from the Brooksville Plant Materials Center of Brooksville, Florida, USA. Rhizomes of *P. australis* “Southwind” were obtained from the Manhattan Plant Materials Center of Manhattan, Kansas, USA. Additionally, *P. arundinacea* was collected from sites of growth near Hays, Kansas, USA (38°57′ N, 99°23′ W).

Seeds of *S. alterniflora* and *S. littorale* were planted in potting soil (Miracle Gro Potting Mix; 0.21% N, 0.07% P, 0.14% K; Scotts Company, Marysville, OH, USA) and allowed to germinate under greenhouse conditions for two months. Pots were 11 cm × 11 cm × 11 cm with 8–10 seeds per pot. All plugs and rhizomes were transplanted into pots (11 cm × 11 cm × 11 cm) of potting soil and allowed to establish under greenhouse conditions for two months. The greenhouse was in Hays, Kansas, USA (38°52′ N, 99°20′ W) with natural lighting. Maximum photosynthetic photon flux density was near 540 μmol m^{-2} s^{-1} at midday during the study. Greenhouse temperatures were variable, but were typically near 30 °C during afternoons and 21 °C at night. Plants were watered daily with tap water.

At the end of the two month establishment period, plants were divided into control and treatment groups. Plants were arranged on plastic trays (61 cm × 40 cm × 2 cm) for proper drainage and containment of oil. Some individuals died during the experiment. Others had too little leaf material for a photosynthesis measure, but enough roots to harvest for enzyme assays, leading to uneven sample sizes across treatments, species, and measures (3–9 individuals per species in each treatment).

For oil treatments, 6 L m^{-2} (60 mL per pot) of used light motor oil was applied to the soil surface surrounding each individual plant in the treatment group. Choice of oil was based on data provided by the National Park Service (1997). Oil remained in soil but did not contact leaf tissue. Amount of oil applied to individual plants

Table 1
A list of marsh species tested in this study. Species are grouped according to their relative flooding tolerance, which is hypothesized to correspond with tolerance to oil.

Experimental species		
Flooding tolerant Hypothesized oil tolerant	Moderately flooding tolerant Hypothesized moderately oil tolerant	Flooding sensitive Hypothesized oil sensitive
Phragmites australis[a,b]	*Phalaris arundinacea*[b,e]	*Ammophila breviligulata*[e]
Spartina alterniflora[c,d]	*Spartina patens*[d,f]	*Panicum virgatum*[e]
	Spartina pectinata[b,d]	*Schizachyrium littorale*[e]
		Solidago sempervirens[e]

Sources for determining flooding tolerance: [a] Armstrong et al. (2009); [b] Waring and Maricle (2012); [c] Culbertson et al. (2008); [d] Maricle et al. (2009); [e] USDA (2012); [f] Bertness (1991).