Growth Responses of *Avicennia germinans* and *Batis maritima* Seedlings to Weathered Light Sweet Crude Oil Applied to Soil and Aboveground Tissues

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Abstract

Oil spills are a significant stressor to coastal and maritime environments worldwide. The growth responses of *Batis maritima* and *Avicennia germinans* seedlings to weathered *Deepwater Horizon* oiling were assessed through a mesocosm study using a factorial arrangement of 4 soil oiling levels (0 L m⁻², 1 L m⁻², 2 L m⁻², 4 L m⁻²) x 3 tissue oiling levels (0% of stem height, 50% of stem height, 100% of stem height). Overall, growth metrics of *B. maritima* displayed much greater sensitivity to both tissue and soil oiling than *A. germinans*, which exhibited a relatively high tolerance to both routes of oiling exposure. *Batis maritima* in the 4 L m⁻² soil oiling treatment demonstrated significant reductions in cumulative stem height and leaf number, whereas no significant effects of soil oiling on *A. germinans* were detected. This was reflected in end of the study biomass partitioning, where total aboveground and live aboveground biomass were significantly reduced for *B. maritima* with 4 L m⁻² soil oiling, but no impacts to *A. germinans* were found. Tissue oiling of 100% did appear to initially reduce *B. maritima* stem diameter, but no effect of tissue oiling was discerned on biomass partitioning, suggesting that there were no impacts to integrated growth. These findings suggest that *B. maritima* would be more severely affected by heavy soil oiling than *A. germinans*.

Introduction

Salt marshes are valuable habitats that provide a number of ecosystem services, including coastal erosion mitigation, carbon sequestration, water quality enhancement, and faunal support (Pennings and Bertness 2001; Barbier et al. 2011; Duke 2016; Gorman and Turra 2016; CPRA 2017). Vegetation in these salt marshes are subjected to a number of environmental stressors, including elevated flooding, anoxic soils, and increased salinity (Pennings and Bertness 2001; Alleman and Hester 2010; Lonard et al. 2017). However, human activities can present additional stressors to salt marsh vegetation, including those associated with oil spills (Lin and Mendelssohn 2012). Thousands of oil spills occur in waters of the United States (U.S.) annually, and although the vast majority of these incidents are small (<159 L), 44 spills greater than 1.59 million L in volume have occurred in U.S. waters since 1969 (National Oceanic and Atmospheric Administration 2017). Oil spills in salt marshes are particularly problematic not only because of direct physical and chemical impact to biota, but also because of the persistence of oil in flooded soils and the fragility of these habitats precluding subsequent clean up (Pezeshki et al. 2000; Michel and Rutherford 2013). The prevalence of oil spills in coastal areas underscores the need to understand how oiling impacts the valuable species occurring in these important habitats.

Oil spills are relatively frequent in areas where large volumes of petrochemical products are extracted and refined, such as the northern Gulf of Mexico. Coastal wetlands along the northern Gulf of Mexico experienced extensive oiling following the *Deepwater Horizon* spill on April 20, 2010 (Nixon et al. 2016), which was the largest marine oil spill in the U.S. to date (Mendelssohn et al. 2012; Michel et al. 2013). The *Deepwater Horizon* spill impacted coastal wetland vegetation in Louisiana (Hester et al. 2016), as well as in Mississippi and Alabama (Willis et al. 2016). The spill released more than 760 million L of

MC252, a light sweet crude oil, over a 3-month period and resulted in more than 1,040 km of coastal marsh shorelines being oiled, with more than 208 km of this shoreline categorized as moderately to heavily oiled (Lin and Mendelssohn 2012; Nixon et al. 2016). A recent investigation into the persistence of *DWH* petroleum residues in previously oiled Louisiana marshes revealed that oiling contamination continues to be elevated by an order of magnitude over eight years since the spill's occurrence (Turner et al. 2019). Because of the critical roles played by coastal wetlands and the threats posed by oil spills, enhancing the understanding of the effects of oiling on salt marsh vegetation continues to be an important topic of study.

Avicennia germinans (L.) L. (black mangrove) and *Batis maritima* L. (marine saltwort) are common constituents of high marshes of the subtropical Gulf of Mexico, including coastal Louisiana (Alleman and Hester 2010; Lonard et al. 2011). *Avicennia germinans* occurs in the more southern, warmer saline marshes, such that the species has its northernmost boundaries in the Americas along the barrier islands and salt marshes of Louisiana's Gulf of Mexico coast (Alleman and Hester 2010) and in the Guana Tolomato Matanzas National Estuarine Research Reserve of Florida's Atlantic coast (Devaney et al. 2017). *Batis maritima* has a wide distribution that includes high marsh areas and ridges in the coastal areas of the Gulf of Mexico (Lonard et al. 2011). Although *Spartina alterniflora* (smooth cordgrass) is the dominant macrophyte in salt marshes of Louisiana, *A. germinans* is often present as a codominant or subdominant species in these areas (Hester et al. 2005). *Batis maritima* typically occurs at relatively high elevations in salt marshes throughout the Gulf of Mexico coast (Rasser et al. 2013). However, both *A. germinans* and *B. maritima* are commonly present in the high marsh of coastal Louisiana (Hester et al. 2005; Lonard et al. 2017). *Avicennia germinans*, *B. maritima*, and other plant species of mangrove ecosystems in coastal Louisiana and throughout the Gulf of Mexico contribute to important ecosystem services, including protection from erosion, carbon export and sequestration, nursery habitats for fishes and crustaceans, and resources for humans (Katherisan and Bingham 2001; Alongi 2002; Nagelkerken et al. 2008; Everard et al. 2014; Lee et al. 2014; Gormand and Turra 2016).

Laboratory studies of mangrove oiling have yielded useful information about survival and growth responses. Getter et al. (1983) compared effects of unweathered oil and/or dispersants on *A. germinans* and *R. mangle* seedlings and demonstrated that *A. germinans* is more sensitive to oils, dispersants, and oil-dispersant combinations than *R. mangle*. Getter et al. (1983) noted that *A. germinans* osmoregulates by taking up solutes into the roots then excreting solutes through leaf tissue, with oil included in the uptake and becoming deposited on the leaves, whereas *R. mangle* excludes this root uptake (Getter et al. 1983). In the study, effects varied among groups treated variously with a light crude oil, a heavy distillate oil, and a light distillate oil, with lighter oils showing increased effects (leaf shape and width) overall, though the lower concentrations of No. 2 fuel oil and bunker C fuel oil had greater growth rates and foliage production in *A. germinans* than untreated controls. Combining dispersant with oil increased effects in some instances. Getter and Baca (1984) provided further data regarding impacts of unweathered oil and/or dispersant on *R. mangle*, reporting that root-dosing by injection into the soil had lesser effects than tissue dosing, and also that seedlings of *A. germinans* and *R. mangle* obtained from

areas that have been chronically oiled grew more foliage in response to Light Arabian oil. The authors proposed that previous oil exposure may result in an adaptive response (Getter and Baca 1984).

In another controlled study, no *A. germinans* seedlings exposed to fresh motor oil as a one-time application of 120 mL or 15 mL per week survived beyond a few weeks in a 59-week study, while 75% of *R. mangle* survived (Proffitt et al. 1995). A one-time oiling treatment under hot and bright outdoor conditions (as opposed to air-conditioned and diffusely lit laboratory conditions) yielded the greatest mortality (Proffitt et al. 1995). Additionally, acute treatment of *A. germinans* seedlings with 120 mL of Bonny Light crude oil placed on the soil surface of the base of the seedling showed episodic mortality, with 100% survival at 2 weeks, 70% between weeks 3 and 4, 60% between weeks 5 and 10, 50% at week 11, and 30% between weeks 12 and 16 (Chindah et al. 2011). Chronic treatment with 15 mL weekly resulted in reduced survival in weeks 13 and 14, stabilizing in week 15 (Chindah et al. 2011).

Hughes et al. (2018) studied the effects of weathered Louisiana sweet crude oil on mesocosms of *A. germinans* (one-year-old seedlings), *S. alterniflora*, and combined *A. germinans* and *S. alterniflora* assemblages. Oiling treatments of 8 L m⁻² of a weathered crude oil was applied and drained for 5 days, diurnal tide actions were simulated, and response variables were followed for 1 year. Oiling adversely impacted both species, and mixing the species provided no benefit for *S. alterniflora*. However, the mixed community showed reductions in the negative effects of oiling for *A. germinans*. Reduction in *A. germinans* survival and belowground biomass were mitigated to 12% from 21% and to 19% from 71% in the mixed species condition compared to *A. germinans* alone.

The frequency of oiling in coastal wetland habitats necessitates studies that provide insight into how oiling exposure impacts the health and subsequent recovery of specific coastal wetland vegetation species. The effects of oiling exposure on *A. germinans* have been addressed in a number of observational and controlled studies (Getter et al. 1983; Proffitt et al. 1995; Chindah et al. 2011; Hughes et al. 2018). However, knowledge gaps exist in regard to the interactive effect of soil and aboveground tissue oiling for *A. germinans* seedlings. Further, the effect of oiling exposure on *B. maritima* has not been experimentally assessed at the time of this writing. The purpose of this research is to expand the knowledge base of oiling impacts on two high marsh vegetation species relevant to the Gulf of Mexico in order to facilitate coastal management.

Materials And Methods

Seedlings of *A. germinans* and *B. maritima* were collected with intact adjacent soil in April 2018 from the Caminada Moreau Headland, LA. The plants were then transported to the Nicholls State University Farm greenhouse facility and transplanted into 15 cm (diameter) by 17 cm (height) polyethylene pots that were individually placed into 4.73 L polyethylene reservoirs. Reservoir water levels were maintained 10 cm below the soil surface of experimental pots for the duration of the study, with salinities maintained at 18 psu using commercial sea salt (*Instant Ocean* Spectrum Brands, Blacksburg, VA). Salinity levels in reservoirs were checked biweekly using a handheld meter, and adjusted to 18 psu as necessary.

This study employed a 2 species (*A. germinans*, *B. maritima*) x 4 soil oiling levels (0 L m⁻², 1 L m⁻², 2 L m⁻², 4 L m⁻²) x 3 tissue oiling levels (0% of stem height, 50% of stem height, 100% of stem height) completely randomized factorial design with 4 replicates for a total of 96 experimental units. Each experimental unit consisted of a pot containing a single plant of the appropriate species. Experimental plantings were allowed to acclimate for two weeks; thereafter, weathered MC252 oil was applied to achieve oiling treatments. The MC252 oil had previously been weathered by aerating with bubblers in a fume hood until oil volume was reduced to 50% of the initial. Aboveground tissue oiling treatments were applied by dipping a nitrile-gloved hand into a beaker of oil and applying a thin film of oil to the appropriate vertical extent of the plant. Soil oiling treatments were accomplished by individually draining the reservoir floodwaters of experimental units into an empty reservoir container, mixing in the appropriate concentration of oiling, and gently pouring the oil-water mixture directly onto the soil surface of the experimental units. After soil oiling, experimental water levels were restored to the reservoirs containing the pots. The study duration was 5 months, with the experiment initiated in early June 2018 and with harvesting in late October 2018.

Immediately prior to the initiation of the experimental treatments, cumulative stem height, stem diameter, leaf number, and visual chlorosis were determined for all experimental units. Cumulative stem height typically included one stem for *A. germinans* units but often involved multiple stems for units of *B. maritima*, with those multiple stem heights summed into one measurement. Stem height was measured from the soil surface to the highest point on each stem using a meter stick and recorded to the nearest mm. Stem diameter was measured to the nearest mm using calipers on the main stem 5 cm above the soil surface. Chlorosis was visually estimated using a scale developed by Mendelssohn et al. (1990): no impact (naturally speckled chlorosis); trace impact (intensely speckled chlorosis); light impact (50% or less yellowing); medium impact (more than 50% yellowing); heavy impact (all vegetation dead). Cumulative stem height and stem diameter, mortality, leaf number, and visual chlorosis were determined biweekly for both species.

At the conclusion of the study all leaves of *A. germinans* were harvested, rinsed, blotted dry, then scanned into a digital image using a flatbed scanner (Canon LiDE 110, Melville, NY). A small ruler was included in each scanned image for determination of scale. Digital images were analyzed using ImageJ software (Abramoff et al. 2004) to estimate individual leaf area. Following scanning, the leaves were placed into individual, numbered manila coin envelopes, dried to a constant weight at 65°C, and individual leaf weights determined. Specific leaf area (leaf area/leaf mass) was then calculated on a whole plant basis. For all experimental units, aboveground biomass was clipped at the soil surface using shears, rinsed of debris above a screen, partitioned into live and dead components, and dried to a constant mass before weighing.

Data were analyzed using an ANOVA framework with *a priori* contrasts in R 3.6. For metrics measured at one point in time (e.g., biomass metrics collected at harvest), univariate factorial ANOVAs were executed using the `aov` command in R. For metrics collected over time (e.g., cumulative stem height), a mixed

model factorial ANOVA approach with time as the random effect was implemented using the lme command within the nlme package.

Results

Significant main effects of time (Fig. 1; $F = 114.1$, $P < 0.0001$) and species (Fig. 1; $F = 38.0$, $P < 0.0001$) were detected for repeated cumulative stem height measurements. However, significant interactions of time and species (Fig. 1; $F = 47.2$, $P < 0.0001$), time and soil oiling (Fig. 1; $F = 4.3$, $P = 0.0054$), and time, species, and soil oiling (Fig. 1; $F = 2.7$, $P = 0.0477$) were also detected. Interestingly, the significant interaction of time, species, and soil oiling appears to reflect the much slower rate of increase in cumulative stem height for *B. maritima* in the 4 L m^{-2} soil oiling treatment compared to the control, whereas no difference in *A. germinans* cumulative stem height was seen among treatments. This notable impact to *B. maritima* cumulative stem height in the 4 L m^{-2} soil oiling treatment is likely also responsible for the significant time and soil oiling interaction. The greater increase in cumulative stem height for *B. maritima* over time compared to *A. germinans*, which was expected given *B. maritima*'s production of multiple shoots, appears to be driving the significant interaction of time and species, as well as the significant main effects of time and species.

Avicennia germinans exhibited greater stem diameter than *B. maritima* throughout the course of the experiment (Fig. 2; $F = 76.7$, $P < 0.0001$). The effect of time (Fig. 2; $F = 28.1$, $P < 0.0001$) as well as interactions of time and species (Fig. 2; $F = 14.0$, $P = 0.0002$) were both significant, and appear to represent growth over time and interspecific variation in growth over time, respectively. Time and soil oiling approached significance (Fig. 2; $F = 2.6$, $P = 0.0531$), and time and tissue oiling (Fig. 2; $F = 3.9$, $P = 0.0209$) was also significant for the repeated stem diameter measurements. These interactions appear to represent reductions in average stem diameter for *Batis maritima* for the highest dosage of soil oiling (4 L m^{-2}) and level of tissue oiling (100%), while *A. germinans* stem diameter was apparently unaffected by these treatment levels.

Batis maritima had a significantly greater increase in leaf number than *A. germinans* (Fig. 3; $F = 5.5$, $P = 0.022$). However, no effect of soil oiling, tissue oiling, or any interactions were detected for leaf number for either species. *Batis maritima* exhibited a higher visually-estimated chlorotic index than *A. germinans* (Fig. 4; $F = 5.8$, $P = 0.019$), but this likely reflects the normal yellow coloration exhibited by *B. maritima*. No effect of soil oiling, tissue oiling, or any interactions thereof were detected for chlorotic index.

A significant effect of species was detected for both total aboveground biomass (Fig. 5; $F = 7.21$, $P = 0.009$) and live aboveground biomass (Fig. 6; $F = 4.80$, $P = 0.032$) with *B. maritima* having slightly higher total and live aboveground biomass compared to *A. germinans*. No overall effect of soil oiling, tissue oiling, or any interactions were detected for total aboveground biomass or live aboveground biomass for *A. germinans*. However, for *B. maritima* the *a priori* contrast of 0 L m^{-2} with 4 L m^{-2} revealed significantly lower total aboveground biomass (Fig. 5, Contrast $F = 1.57$, $p = 0.0163$) and total live aboveground biomass (Fig. 6,

Contrast $F=1.67$, $p=0.0065$). *Avicennia germinans* specific leaf area (Fig. 7; $F= 3.976$; $P= 0.029$), was significantly higher for the 100% tissue oiling level compared to no tissue oiling, averaged across soil oiling treatments.

Discussion

Oil spills are among the significant threats to coastal marsh regions, as was recently highlighted by the *Deepwater Horizon* incident, the largest marine oil spill thus far in U.S. waters (Mendelssohn et al. 2012). The research presented herein reveals differential tolerance by two important Gulf of Mexico marsh plant species, *A. germinans* and *B. maritima*, to weathered *DWH* oil. Impacts of oiling to short-term, but not integrated, growth responses were discernible for *A. germinans*, whereas both short-term and integrated growth responses in *B. maritima* were clearly impacted by oiling. Importantly, these findings not only confirm the relatively high tolerance of *A. germinans* seedlings to *DWH* oiling recently documented by Hughes et al. (2018) but also refines the oiling dosages (1, 2, and 4 L m⁻²) at which this tolerance can be anticipated. Additionally, this study elucidates hitherto unknown *DWH* oiling exposure growth responses for *Batis maritima*, an important subdominant high marsh species in the Gulf of Mexico. This research also corroborates prior studies (e.g., Meudec et al. 2007; Lin and Mendelssohn 2012) suggesting that, when in isolation, soil oiling is often more detrimental to plant growth than tissue oiling. These findings enhance the existing knowledge base on which estimates of the likely severity of oil spills to Gulf of Mexico coastal plant community are based.

In a similar study, Hughes et al. (2018) applied 8 L m⁻² of Louisiana Sweet crude oil weathered with a bubbler aerator for 5 days to outdoor mesocosms containing either *A. germinans*, *S. alterniflora*, or a mixed planting of the two and compared these to untreated mesocosms. The oil application was repeated and drained daily for 5 days, and a diurnal tidal regime was simulated throughout the one-year experimental duration. Similar to the findings presented herein, Hughes et al. (2018) detected no effect of oiling on *A. germinans* stem height. Further, a consistent negative impact of oiling was not detected for crown volume, a metric representative of *A. germinans* aboveground growth by Hughes et al. (2018), which is consistent with the lack of impacts to *A. germinans* aboveground biomass reported here. Interestingly, reductions to *A. germinans* leaf number were detected with oiling application, but only after a full year of growth under oiled conditions; no impact of oiling to leaf number was detected at the three, seven, or nine month measurement periods (Hughes et al. 2018). Although no effect of oiling on *A. germinans* leaf number was found in the present assessment, the experimental duration was approximately four months, making the findings commensurate between the studies. Importantly, although no effect of oiling on *A. germinans* belowground biomass was found in this study, a reduction in belowground biomass with oiling was reported by Hughes et al. (2018). This again likely reflects the substantial difference in study duration (4 months versus 12 months), and also the higher oil dosage (8 L m⁻²) used by Hughes et al. (2018) compared to this study (4 L m⁻²).

Other studies assessing the survival and growth of *A. germinans* when exposed to oils other than weathered *DWH* oil reveal a range of responses. For instance, all *A. germinans* seedlings died within a few

weeks of being planted in sediment oiled with a 12 L m^{-2} (120 mL in the experiment) of fresh lubricating oil (Proffitt et al. 1995). Similarly, application of 120 mL of Bonny Light crude oil to the base of *A. germinans* seedlings resulted in lower stem height and stem diameter compared to the control over a 16 week study and impacts with respect to leaf length, leaf width, leaf production, leaf senescence, and seedling survival beginning in the third week (Chindah et al. 2011). Although, the experiment reported herein did not indicate such impacts, it employed a weathered oil that would be expected to exhibit reduced toxicity (Grant et al. 1993; Mendelssohn et al. 2012) at lower soil oiling levels. Getter et al. (1983) found that unweathered Bunker C fuel oil, No. 2 fuel oil, and light Arabian crude oil applied in dosages of 12.5; 25; 250; 2,500; and 25,000 μL , to *A. germinans* seedlings demonstrated decreased leaf width with each oil type and at each dose, with a threshold effect on new foliage detected at 2,500 μL dosing (Getter et al. 1983). The experiment described herein used weathered crude oil applications in the amounts of 1,800; 3,500; and 7,100 μL . Although the amounts applied to soil in this study are comparable to those of Getter et al. (1983), the lower toxicity of the weathered oil that was employed likely explains the lack of detection of similar impact.

Oiling impacts to *B. maritima* were detected for multiple growth indicators, indicating that *B. maritima* has a greater sensitivity to weathered DWH oiling than *A. germinans* seedlings. Soil oiling of 4 L m^{-2} exhibited lower cumulative stem height, lower total aboveground biomass, and lower live aboveground biomass than the control for *B. maritima*, and the interaction of 4 L m^{-2} soil oiling and 100% tissue oiling revealed lower *B. maritima* total aboveground biomass, live aboveground biomass, and belowground biomass than the control. Although not statistically significant, similar trends were also notable, including reduced growth of *B. maritima* stem diameter under 100% tissue oiling and increased visual chlorosis with 4 L m^{-2} soil oiling.

A tanker spill of 75,000 to 150,000 gallons of crude oil-water emulsion, which affected 30 miles of shoreline along the Florida Keys, was observed to result in mortality of *Batis* spp. in areas where substrate, stems, or leaves were coated with the contaminant (Irwin et al. 1997). Although the current controlled study did not result in complete mortality of *B. maritima* significant impacts to growth processes were detected with soil and tissue oiling. Tunnell et al. (1995) examined regrowth of vegetation following oil contamination from a ruptured pipeline in the high marsh at Upper Copano Bay, Texas, and the subsequent burning of oil that remained after oil recovery processes. *Distichlis spicata* was the primary recolonizer. Secondary succession by climactic perennial species, such as *B. maritima*, showed a trend, though slow and insignificant, for increasing frequency over time. *Batis maritima* demonstrated an average frequency of 3.1% in the impacted areas and 9.8% in the control areas during the study period of between about 1 and 2.5 years following the spill. The species was shown to contribute to regrowth following oil contamination, recovery techniques, and burning, but it did so slowly and without full recovery of frequency over the time examined.

Conclusions

The current examination of soil and tissue oiling with weathered MC252 did not detect deleterious impacts to the growth of *A. germinans* seedlings but did reveal that some growth responses of *B. maritima* were adversely affected at greater levels of exposure, with lower cumulative stem height at 4 L m⁻² of soil oiling and lower total aboveground biomass, live aboveground biomass, and belowground biomass with the interaction of 4 L m⁻² soil oiling and 100% tissue oiling. The tolerance of *A. germinans* suggests that it may survive and play an integral role in recovery of coastlines contaminated by crude oil spills. The detection of negative effects on *B. maritima* growth with higher levels of oiling are consistent with field observations of oiling impacts to areas where this species occurs. However, the apparent absence of impacts to *Batis maritima* with lower levels of weathered crude oil contamination implies that this species, also, can contribute to spill recovery at lower levels of pollution. These findings provide novel insights into the likely impacts to Gulf of Mexico high marsh habitats under possible future oiling scenarios.

Declarations

Ethics approval and consent to participate

Not applicable

Consent for publication

Not applicable

Availability of data and materials

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request

Competing interests

The authors declare they have no competing interests

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Authors' contributions

JW conceived the research; WH set up and executed the study, and performed data collection; WH and JW equally contributed to statistical analysis and interpretation and the writing of the manuscript

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Figures

Figure 1

The effect of sampling period, species, and soil oiling on cumulative stem height, averaged over tissue oiling (mean +/- s.e.)

Figure 2

The effect of sampling period, species, and soil oiling on stem diameter, averaged over tissue oiling (mean +/- s.e.)

Figure 3

The effect of sampling period, species, and soil oiling on leaf number, averaged over tissue oiling (mean +/- s.e.)

Figure 4

The effect of species and soil oiling level on visual chlorosis over 16 weeks averaged over plant tissue oiling at four soil oiling levels (mean $\pm$ s.e.)

Figure 5

The effect of sampling period, species, and soil oiling on total aboveground biomass, averaged over tissue oiling (mean +/- s.e.)

Figure 6

The effect of sampling period, species, and soil oiling on live aboveground biomass, averaged over tissue oiling (mean +/- s.e.)

Figure 7

Effect of tissue oiling on *A. germinans* specific leaf area averaged across soil oiling (mean $\pm$ s.e.)