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Factors Controlling the Impacts of South Louisiana Crude Oil on the Vegetation and Revegetation of Coastal Wetlands.

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FACTORS CONTROLLING THE IMPACTS OF SOUTH LOUISIANA CRUDE OIL ON THE VEGETATION AND REVEGETATION OF COASTAL WETLANDS

A Dissertation

Submitted to the Graduate Faculty of the
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ABSTRACT

The impacts of south Louisiana crude oil on three types of marsh, salt, brackish, and freshwater, dominated by *Spartina alterniflora*, *S. patens*, and *Sagittaria lancifolia*, respectively, were studied in the greenhouse. The influence of a number of factors such as marsh type, plant species, oil dosage, oil coverage, soil composition, leaf surface structure, season and meteorological conditions, on the impact of crude oil to marsh vegetation and revegetation were investigated. Vegetative sensitivity to south Louisiana crude oil increased in the order of *S. lancifolia*, *S. alterniflora*, and *S. patens*. Photosynthetic rates, stem densities, and the regrowth of aboveground biomass for the two *Spartina* species significantly decreased, while those for *S. lancifolia* were either not detrimentally affected or enhanced by oil dosages up to 24 l m^{-2}. *Sagittaria lancifolia* showed relative resistance to oil coverage because its smooth cuticle on the leaf surface prevented oil from absorbing into internal tissue. However, the two *Spartina* species were sensitive to oil coverage of their aerial portions, with decreased live and total biomass production and stem density. Oil absorbed into the furrows on the adaxial surface of the two *Spartina* species completely inhibited their photosynthetic capabilities. However, oil contact with the lower aerial portions of the stems did not cause long-term damage to any of the species studied. In contrast, oil incorporation into the substrate caused both short and long term damages to all three plant species, with a reduction in photosynthetic rate, biomass, stem density and regrowth. Furthermore, all three plants species were affected by oil application to soil with high organic matter and coarse texture. The influence of season was dramatic. *Sagittaria lancifolia* and *Spartina alterniflora* were very resistant to applications of crude oil to the substrate during the fall, but they were least resistant in the summer. Vegetative transplants could be effectively used to revegetate oil contaminated soil with oil concentrations as high as 250 mg g^{-1}. Fertilizer increased the biomass of the transplants in the oiled soil, and it also increased

the oil degradation rate, suggesting that fertilization could be a valuable tool for restoring oil contaminated marshes.

INTRODUCTION

Louisiana coastal wetlands comprise 41% of the wetlands in the contiguous United States (Turner & Gosselink, 1975). The importance of these ecosystems and their benefits to society have been well documented. Wetland functions, which are quite diverse, and vary with wetland type, quality and location, include nurseries for fisheries, habitat for wildlife, flood mitigation, protection from shoreline erosion, wastewater treatment, and water quality enhancement (Mitsch & Gosselink, 1986; Dahl, 1990).

The Gulf of Mexico is among the most intensive oil-producing areas in the world, and is also heavily traveled by tankers transporting oil products (Fletcher, 1977; Geyer, 1980). In 1985, offshore oil wells in the Gulf of Mexico accounted for 92% of the total U.S. offshore oil production (EPA, 1985). A network of pipeline and navigation canals in coastal Louisiana and its outer continental shelf provide a conduit for transportation of more than 70% of the oil from all U.S. coastal waters (Turner & Cahoon, 1987). Because of all the oil-related activities in the Gulf of Mexico, there is a proportionately higher risk of oil spills. An accident involving an oil tanker, production well, pipeline, or storage tank could cause huge volumes of oil to be released into coastal areas or directly into the adjacent wetlands, affecting vegetation. A report by the National Academy of Sciences (1985) estimated that average oil inputs into the world's oceans were between 1.7 and 8.8 million metric tons per year, most occurring in coastal areas by human-factors. Thus, oil pollution is a major concern in protecting coastal wetlands.

An oil spill can cause widespread impacts to coastal wetlands. A number of studies have been conducted concerning the impact of oil spills on wetland vegetation (Burk, 1977; Delaune *et al.*, 1979; Ferrell *et al.*, 1984; Alexander & Webb, 1985 & 1987; Li *et al.*, 1990; Mendelssohn *et al.*, 1990). Oil spills cause various acute and

chronic damages including reduced stem heights, stem density and aboveground biomass, increased mortality, and impaired growth and regrowth (Gebhart, 1973; Krebs & Tanner, 1981; Ferrell *et al.*, 1984; Alexander & Webb, 1987; Mendelssohn *et al.*, 1990). There is also evidence that oil may do little damage or even stimulate growth (Burk, 1977; Hershner & Moore, 1977; Delaune *et al.*, 1979; Li *et al.*, 1990).

FACTORS THAT MIGHT AFFECT THE IMPACT OF OIL ON WETLAND VEGETATION

Various studies have shown that the impact of oil on wetland plants varied with a number of biotic and abiotic factors, such as oil type, degree of oil weathering, amount and persistence of oil, plant species, degree of oil coverage on the plant, penetration of oil into the soil, soil substrate composition, season and meteorological condition during an oil spill (Baker, 1970; Boech, 1974; Burk, 1977, Hershner & Moore, 1977; Getter *et al.*, 1984; Ferrell *et al.*, 1984; Alexander & Webb, 1985 &1987, Mendelssohn *et al.*, 1990). Variation in these factors has resulted in conflicting results, even for the same species. Presently, there is insufficient information available for predicting oil impacts on marshes. The factors that might control the impact of oil on wetlands are generally cited as follows:

Oil Type and Degree of Weathering

Biological and ecological effects of an oil spill are greatly influenced by the type of oil spilled (Boech, 1974; Baker, 1970). The spilled oil can usually be classified as crude oil or refined products. Crude oil is a complex mixture of hydrocarbons together with small amounts of sulfur, oxygen, and nitrogen compounds, and trace amounts of inorganic and organometallic compounds (Baker, 1970; Boesch, 1974; Freedman, 1989). The main hydrocarbon classes are aliphatic hydrocarbon (open chain compounds, such as paraffin), alicyclic hydrocarbons (some or all of their carbon atoms arranged in a ring structure, such as naphthenes), and aromatics (hydrocarbon that contain at least one six-carbon ring, called a benzene ring). Crude oil from different

sources contains different oil components and some variation occurs in the composition of petroleum even from the same production well. During the refining of crude oil, the various hydrocarbon products, such as natural gas, gasoline, kerosene, middle distillates (including heating oil, and jet and rocket fuels), wide-cut gas oil (lubricating oils, waxes, etc.) and residual fuel oil (bunker fuel for ships, and fuel for electrical utilities) are separated by fractional distillation (Freedman, 1989).

In an oil spill, light crude and refined oils are mainly responsible for the toxic effects due to their penetration into plant tissues (Baker, 1984; Alexander & Webb, 1985). Heavy crude oil has more of a smothering effect due to its physical characteristics (Boesch, 1974). In general, the toxicity increases in the order of paraffins, naphthenes, and aromatics; and within each series of hydrocarbons, the small molecules are more toxic than the large ones (Baker, 1970). Hydrocarbons with a boiling range between 150 - 275 °C (e.g. the naphtha and kerosene fractions) are very toxic to plants. Oils with a high boiling point may have molecules too large and viscous to penetrate plant tissue, whereas low boiling point volatile oils may evaporate before they have any effect on the plant (Baker, 1971c).

Besides the physical and chemical characteristics of an oil, the degree of change an oil undergoes as it weathers in the environment could also alter the toxicity of the oil to plants. Weathering processes mainly include evaporation, oxidation, dissolution, and biodegradation of oil (Boesch, 1974). Usually weathered oil is less toxic to plants than fresh oil (Cowell, 1969; Baker, 1970) due to a lower proportion of tissue-penetrating components (low molecular weight) in the weathered oil.

Concentration and Persistence of Oil

Undoubtedly, the concentration of oil will affect its impact on wetland vegetation. Alexander and Webb (1987) observed that growth of *Spartina alterniflora* was unaffected in light to moderately oiled sediments (< 5 mg oil g^{-1} sediment) but was significantly reduced in sediments with a high oil content (5 to 51 mg g^{-1}) 18 months

after 6,720 gallons of a light crude oil were released from pipeline blowouts into Dickinson Bayou in Galveston Bay, Texas. Krebs and Tanner (1981) reported that when the concentration of *No.* 6 fuel oil in the sediment was below 2 mg g^{-1}, *S. alterniflora* was not affected or showed slight increases in stem height, density, and aboveground biomass during the first growing season, but all of these parameters decreased as hydrocarbon concentrations rose above 2 mg g^{-1}. In a greenhouse microcosm experiment, Li *et al.* (1990) showed that above- and below-ground biomass of *S. alterniflora* was significantly increased by a chronic hydrocarbon dosage of 3.33 g C m^{-2} day^{-1} for 10 months, but biomass was decreased by a 33.3 g C m^{-2} day^{-1} dosage rate. Delaune *et al.* (1979) noted little damage to *S. alterniflora* following the application of up to 8 l m^{-2} of Louisiana crude oil to *in situ* plots with residue oil concentrations in the soil up to 53 mg g^{-1} 16 months after oil application. Mendelssohn *et al.* (1990) reported ca. 0.28 l m^{-2} of crude oil caused a 64% reduction in live vegetation cover of *S. patens* three months after an oil spill from a pipeline blowout. Therefore, oil concentration does influence the impact of oil on wetland vegetation, although variation exists from case to case.

The persistence of spilled oil is also important for controlling the impact of oil on the wetland. As soon as oil penetrates into the anaerobic substrate, its rate of degradation decreases because the absence of oxygen inhibits aerobic oil degradation, thus allowing the oil in the soil to have much longer-term effects (Getter *et al.*, 1984; Alexander & Webb, 1987; Baker *et al.*, 1993). *Spartina alterniflora* was significantly reduced in growth and was killed in sediments with high oil content; thus, the erosion of the shoreline was evident due to the persistence of oil in the soil and its impact on the vegetation throughout the 36 month study (Alexander & Webb, 1987). Baker *et al.* (1993) observed that 17 years after an oil spill in salt marshes in the Strait of Magellan, Chile, no vegetation recovered, and the oil beneath a surface skin was still relatively unweathered.

Plant Species

Plant species exhibit a variety of responses to oil pollution (Cowell & Baker, 1969; Delby, 1969; Baker, 1971b; Burk, 1977; Ferrell *et al.*, 1984). Usually annual species are more susceptible to oil than perennials because they have smaller belowground reserves for regrowth after aboveground tissue has been killed by the oil (Burk, 1977; Bake, 1979). In a four-year study of an oil spill in a freshwater marsh at the mouth of Mill River in Northampton, Massachusetts, Burk (1977) observed that all of the twenty-three species that were relatively unaffected or more abundant following the spillage were perennial. In an experimental oil spraying to above-ground plant tissue in a salt marsh near Pembroke, S. W. Wales, Baker (1971b) reported that there was little long-term vegetative damage to most perennial species, but the annual species were severely affected. Delby (1969) also observed that perennial species with large rhizomes survived much better than annuals, but that annuals were returning because they are opportunistic species biologically adapted to disturbed sites that are often ephemeral in nature. However, even within perennial species, there is also a large variation in responses to oil (Burk, 1977).

Soil Composition

Soil composition might also influence the effect of oil on wetland vegetation. Most petroleum chemicals are non-ionic and therefore associate more readily with organic than with mineral particles in the soil (Testa & Winegardner, 1991). A soil higher in organic content can absorb more oil, thus plants grown in a high organic soil might be affected more severely by oil. Besides the soil organic content, the soil texture also affects the residual oil in soil. Usually oil penetrates coarse soil (e.g., sand) more readily than fine soil (Getter *et al.*, 1984). Ferrell *et al.* (1984) showed that the regrowth potential of *Spartina alterniflora* in an oiled fine-textured marsh substratum was much greater than that in an oiled sand substratum. A dense clay substrate helped

prevent oil from penetrating the sediment, thus minimizing the acute toxic effect from oil exposure to the marsh plant rootstock (Hoff *et al.* 1993).

Season and Meteorological conditions

Season of year influences the impacts of oil on vegetation (Ranwell & Hewett, 1964; Baker, 1971b; Getter *et al.*, 1984, Scholten & Leendertse, 1991). Ranwell & Hewett (1964) observed that during the period of senescence, even relatively fresh oil did not cause significant mortality in salt marsh vegetation. This suggests that the plant growth and development stages might alter the oil's effect on plants. Usually plants grow vigorously during the growing season (e.g., spring and summer), and they senesce in late fall and winter. Oil may most severely damage plants during the active growing season, but may have little impact during the period of senescence (Ranwell & Hewett, 1964; Hershner & Moore, 1977; Scholten & Leendertse, 1991). Baker (1971b) indicated that a marked reduction of flowering can occur if plants are oiled when flower buds are developing; and flowers, if oiled, rarely produce seeds. Additionally, oiling of seeds may reduce germination. Alexander & Webb (1985) reported that *No.* 2 fuel oil applied to the sediment and the entire *Spartina alterniflora* shoot at a rate of 2 l m^{-2} caused a greater reduction in live biomass in May (during the growth season) than in November (at the end of the growth season), and they believed that long-term effects were the result of initial adverse effects on the roots, which slowed plant recovery.

Besides the annual growth cycle of the plant itself, meteorological conditions, such as sunlight and humidity may additionally influence the impact of oil on plants. Guille & Blanchet (1958) reported that if oil was sprayed on young maize plants in the light when the stomata were open, the plants were killed. If the oil was sprayed in the dark when the stomata were closed, the plants were not harmed. During very sunny weather the risk of oil impact is greater. It appears that oil penetrates more easily into a leaf through the open stomata under light conditions, allowing the penetrated oil to

affect mesophyll cells. Prendeville & Warren (1977) indicated that light was necessary for oil-based herbicides, such as oxyfluorfen and paraquat, to increase bean and soybean leaf-cell membrane permeability; the oily herbicides resulted in symptoms of injury including increased electrolyte and amino acid leakage, and appearance of necrotic areas on leaf disks. In the case of humidity, Kelly (1930) stated that high humidity favors oil injury because this condition favors stomatal opening, which aids oil penetration.

Biodegradation of Oil in the Soil

Microbes play an important role in oil degradation in soil. The rate of oil degradation depends on the chemical composition of the oil, the kinds and numbers of microbes present, and several interrelated environmental parameters which include: aerobic conditions (free or dissolved O_2 being essential, with 3-4 mg of O_2 required per gram of hydrocarbon for complete oxidation to CO_2 and H_2O), dispersed oil (rendering it more susceptible to enzymatic attack), moderate temperatures (microbes most active in the range of 20-35 °C), appropriate salinity (most species fitting their original environment salinity), organic matter (some organic compounds may promote the growth of hydrocarbon oxidizers), concentration of oil (the toxicity of the oil is usually greater at higher concentrations), and essential mineral nutrients (microbial activity is limited by specific nutrients) (ZoBell, 1973; Hambrick *et al.*, 1980; Rasiah *et al.*, 1991; Lindstrom *et al.*, 1991; Grosser *et al.*, 1991).

Grosser *et al.* (1991) indicated that mineralization of hydrocarbons can be significantly enhanced by reintroducing isolated hydrocarbon-degrading bacteria. Experimental application of fertilizer at the Exxon Valdez oil spill increased the numbers of hydrocarbon degraders and natural hydrocarbon biodegradation was enhanced (Lindstrom *et al.*, 1991). Carbon mineralization rate and cumulative carbon mineralization increased with increasing levels of fertilizer N added. The greatest enhancement of oily waste-C mineralization occurred when the ratio of waste-C to

fertilizer-N was in the range 18 to 22:1 (Rasiah *et al.*, 1991). The rate of microbial oil degradation decreases in the order of alkane, isoalkanes, and aromatics, and utilization rates were inversely related to chain length or molecular weight (Blumer & Sass, 1972; Kator, 1973; Mechalas *et al.*, 1973).

Additionally, the soil oxidation status plays an important role in oil degradation. Wetland soils that are saturated with water most of the time are usually under anaerobic conditions, as indicated by a low redox potential. The degradation of hydrocarbons decreases with decreasing (more reduced) soil redox potential (Hambrick *et al*, 1980). Marsh vegetation may transport oxygen through plant air-space tissue (aerenchyma) to the root rhizosphere (Teal & Kanwisher, 1966; Armstrong, 1978; Smirnoff & Crawford, 1983; Justin & Armstrong, 1983; Seliskar, 1985), and this may also increase oil degradation. Walton & Anderson (1990) reported that microbial activity is greater in rhizosphere soil, and trichloroethylene degradation occurred faster in the rhizosphere soil than in the edaphosphere (nonvegetated soil). This might be in part due to the release of enzymes and exudation of nutrients such as amino acids, simple sugars and complex carbohydrates by plant roots that create a more favorable microhabitat for microbes. Several other studies (Hsu & Bartha, 1979; Reddy & Sethunathan, 1983; Sandmann & Loos, 1984) indicated that the persistence of several oil-based herbicides and insecticides was reduced in soil with a rhizosphere. Oil degradation is generally slow as long as the oil penetrates into the soil substrate. Oil residue was measurable and still visible in sediments 7 years after a spill in Florida (Teal *et al.*, 1978) and 22 years after an oil spill in Milford Haven, Wales (Baker *et al.*, 1993).

THE OVERALL GOALS OF THIS STUDY

The overall goals of this study were to determine the responses of dominant marsh plant species to petroleum hydrocarbon pollution, to closely examine the main factors controlling the impacts of south Louisiana crude oil on marsh plants, to provide more detailed information for responding to oil pollution in different marsh types, and

to identify wetland plants with high resistance to oil for restoration and enhanced recovery of oiled marshes after spills.

Chapter 1 includes a comparative study of the effects of south Louisiana crude oil on the vegetation of fresh, brackish and salt marshes. Photosynthetic rate, stem density, biomass, and regrowth were monitored to determine the responses of dominant marsh plants to oiling. The relative sensitivity of marsh types to oil were compared.

In Chapter 2, the influence of the oiling position and soil composition on the impact of south Louisiana crude oil to marsh vegetation was studied. In these experiments, the three dominant marsh plant species were grown in the same soil substrate, so that oil sensitivity for the three species could be compared. The influence of oiling position, i.e. oiling the aerial portion, substrate or both, as well as soil composition (e.g. organic matter and soil particle size) were investigated.

In Chapter 3, the influence of season, sunlight and leaf surface structure on the response of plants to oil impacts was investigated. Responses of plants to oil application in summer and late fall were compared to test the hypothesis that the plants would experience more severe injury from oiling during the active growing season of summer than during senescence in fall or winter. Plant leaf surface structure was also examined, and the possible relationship between surface structure and sensitivity to oil coverage was examined.

Chapter 4 explored the possibility of revegetating heavily oiled soil in which plants had been killed. Simultaneously, a study of the influence of fertilization on revegetation and oil degradation was accomplished. The maximum oil concentration in the soil that still allows for successful plant transplantation and establishment was determined.

CHAPTER 1

A COMPARATIVE INVESTIGATION OF THE EFFECTS OF SOUTH LOUISIANA CRUDE OIL ON THE VEGETATION OF FRESH, BRACKISH, AND SALT MARSHES

INTRODUCTION

Knowledge of effects of an oil spill on the dominant coast marshes are important due to the fact that high productivity, habitat value, and other functions of estuaries (Turner, 1976; Mitsch & Gosselink 1986; Dahl, 1990) largely depend on the presence of vegetation. Since coastal wetlands of south Louisiana are sites of intensive petroleum oil production and transportation (Mendelssohn *et al.*, 1990), the risk of an oil spill affecting these important ecosystems is high, not only in coastal Louisiana, but throughout the world, wherever production and transportation of oil occurs.

A number of studies have investigated petroleum hydrocarbon impact on wetland vegetation. Most of these studies were conducted in salt marshes, especially those dominated by *Spartina alterniflora*, a common plant species along the Atlantic and Gulf of Mexico coastlines of the United States (Delaune *et al.*, 1979; Ferrell *et al.*, 1984; Alexander & Webb, 1985 & 1987; Mendelssohn *et al.*, 1990; Li *et al.*, 1990). Some studies showed no impact of oil to *S. alterniflora*. For example, *S. alterniflora* was unaffected by the application of up to 8 L m^{-2} of Louisiana crude oil to field plots with residual oil concentration in the soil up to 50 mg g^{-1} (Delaune *et al.*, 1979). In other cases, petroleum hydrocarbons stimulated the growth of *S. alterniflora* (Hershner & Moore 1977; Li *et al.*, 1990). Alexander & Webb (1987), however, reported that oil concentrations in the soil greater than 10.5 mg g^{-1} caused decreased live stem density of *S. alterniflora* and led to long-term impacts. Compared with *S. alterniflora*, less research has been conducted concerning the effect of oil on brackish marsh species, such as *S. patens*. Mendelssohn *et al.* (1990) reported that about 0.28 L m^{-2} of crude

oil, which coated the aboveground vegetation, caused a 64% reduction in live vegetation cover of *S. patens* three months after an oil spill from a pipeline blowout in southeast Louisiana. There is little information is available on the effects of oil on freshwater marsh species. Burk (1977) showed that *Sagittaria latifolia* increased in relative abundance following an oil spill, however, the auther did not provide information on oil concentration in this study.

The impact of oil on a marsh depends upon a number of abiotic and biotic factors including the type and amount of oil, the plant species and extent of oil coverage, the season of the spill, the weather conditions at the time of spillage, and soil composition (Burk, 1977; Hershner & Moore, 1977; Alexander & Webb; 1985 &1987; Mendelssohn *et al.,* 1990). Variation in these factors have resulted in conflicting results, even for the same species. Presently, there is insufficient information available for determining the relative impact of oil spills on major coastal marsh types. The primary objectives of this study were to (1) compare the impact of south Louisiana crude oil on the vegetation of three types of coastal marshes in Louisiana: salt marsh dominated by *Spartina alterniflora*, brackish marsh dominated by *S. patens*, and fresh marsh dominated by *Sagittaria lancifolia*, and (2) determine the possible causes for any differential oil impact to these three marshes. The following hypothesis was tested: Marshes with organic soils are more sensitive to oil spills than for mineral marshes due to the potential for greater oil penetration, and salt marsh vegetation will be more resistant to oil impacts because of their generally greater stress tolerance compared to fresh marsh species.

MATERIALS AND METHODS

Sod Collection and Experimental Design

Soil sods with intact plants of salt, brackish, and freshwater marshes were collected from marshes in South Louisiana and placed into plastic buckets (20 cm in diameter and 22 cm in height). The salt [6.0 (0.8) ppt of salinity, mean (SE)] and

brackish marsh sods [6.1 (1.2) ppt, mean (SE)] were collected near Cocodrie, LA and were dominated by *Spartina alterniflora* and *S. patens*. The fresh marsh sods [1.1 (0.1) ppt, mean (SE)], collected from the north shore of Lake Pontchartrain, LA, were dominated by *Sagittaria lancifolia*. The soil sods and associated vegetation were allowed to recover from any transplant shock for three weeks in the greenhouse prior to oil application.

The experiment was conducted in the greenhouse with 18-23°C (night temperature) and 28-35 °C (day temperature) with natural sunlight. Before oil application, the oil dosages, which would be used in the experiment, were tested with extra soil sods. The experimental design was a 5 X 3 factorial with 5 oil dosages applied to the above 3 marsh types. The treatment-level combination was replicated 4 times. In late July, unweathered south Louisiana crude oil provided by Exxon USA was added at rates of 0, 4, 8, 16, and 24 L m^{-2} to the marsh sods which were overlain by 1 cm of water; the oil did not come in contact with the leaves of the plants. At days 1, 30, and 60, the water over the marsh surface was drained through the soil column to allow the floating oil on the surface to contact and contaminate the marsh soil. The drained water was collected and then returned to the sod after ca. 8 hours of drainage.

Oil Composition

The composition of south Louisiana crude oil used in this experiment was analyzed by gas chromatography / mass spectrometry (GC/MS). Approximately 0.050 g of south Louisiana crude oil was diluted with 4 ml of reagent grade hexane. The HP 5890 Series II Gas Chromatograph was configured with a J&W DB-5 30 meter high resolution capillary column, directly interfaced to an HP 5971 quadrupole mass spectrometer. The GC was temperature programmed from 55 °C to 300 °C incorporating an initial three minutes isothermal stage, a temperature ramp program between 55 °C and 280 °C at 5 °C/minute and a final ramp from 280 °C to 300 °C at 0.5 °C/minute. The MS was operated in Scan mode for complete mid range petroleum

assessment and in Selective Monitoring mode (SIM) to maximize the detection of the selected polycyclic aromatic hydrocarbons (PAH) constituents in crude oil. In both Scan and SIM analyses the MS was operated between 4 and 76 minutes. The purpose of the SIM analysis was to characterize the more toxic PAH components within south Louisiana crude oil.

The total ion chromatographic profile for the oil used in this experiment over the temperature range of 55 °C to 300 °C was determined. which indicated higher concentrations of lighter range petroleum hydrocarbons (4 to 15 minutes) (Fig. 1.1). The experimental oil was high in napthalenes, containing roughly 1.2% by weight (Table 1.1), and is typical south Louisiana crude oil, which is a light crude relatively low in sulfur (only 0.13% dibenzothiophenes). The hydrocarbon composition of south Louisiana crude as identified by two separate sources (National Research Council 1985 and Bobra 1989) is as follow: 56.3 - 65.1% saturates, 16.5 - 26.3% aromatics , 8.4% polars and 0.2% asphaltenes by weight.

Plant Responses Measured

The photosynthetic rates of leaves of *S. alterniflora*, *S. patens* and *S. lancifolia* were measured at 1, 2, and 3 months after the application of oil using a portable photosynthesis system, including an infrared gas analyzer (ADC model LCA-2), an ADC air flow control unit, and an ADC Parkinson leaf chamber. Sample air, taken 5 m aboveground to obtain relatively stable CO_2 concentrations, was led through an ADC air flow control unit at a flow rate of 5 ml s^{-1} during photosynthetic rate measurements. Measurements were conducted at a quantum flux density of 2000 mol m^{-2} s^{-1} provided by a Kodak projector lamp. An intact, attached and fully expanded young leaf was enclosed in the leaf chamber and the difference in CO_2 and air humidity concentration between inlet and outlet air were measured. Photosynthetic rate (CO_2 exchange) and transpiration rate were calculated according to von Caemmerer & Farquhar (1981).

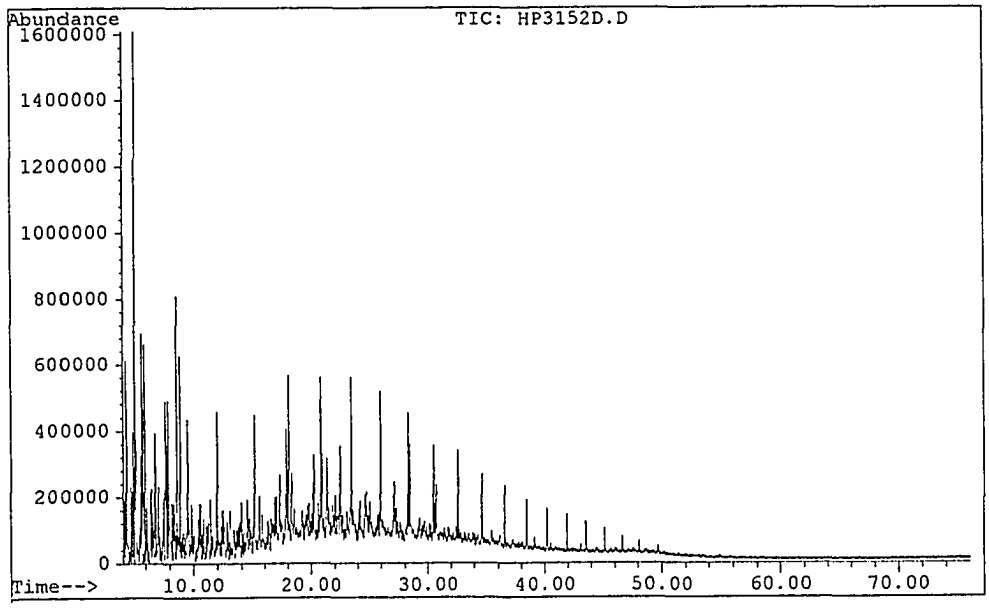

Fig. 1.1 The total ion chromatographic profile over the temperature range of 55 °C to 300 °C for the south Louisiana crude oil used in this experiment.

Table 1.1 Percent of target PAH's in south Louisiana crude oil by weight

Compounds	% by weight
Naphthalenes (C0-C4)*	1.2000
Fluorenes (C0-C3)*	0.1500
Dibenzothiophenes (C0-C3)*	0.1300
Phenanthrenes (C0-C3)*	0.3000
Anthracene	0.0000
Naphthobenthiophenes (C0-C3)*	0.0000
Fluoranthene	0.0008
Pyrenes (C0-C2)*	0.0190
Benzo(a)anthracenes	0.0007
Chrysenes (C0-C2)*	0.0340
Benzo(b,k)fluoranthenes**	0.0007
Benzo(e)pyrene	0.0005
Benzo(a)pyrene	0.0000
Perylene	0.0000
Ind.(1,2,3-cd)pyrene	0.0000
Benz.(g,h,i)perylene	0.0000
Dibenz.(a,h)anthracene	0.0000
TOTAL TARGET PAH's:	1.8000

* Denoted parent compound and homologues.
** Denotes two compounds summed.

Stem density was determined by counting the number of stems of each species at 0, 1, 2 and 4 months after the application of oil to determine new tiller growth from below-ground rhizomes.

The above-ground biomass was harvested four months after oil application (December, 1991) and live and dead plant tissues were separated and dried at 65 °C to a constant weight. The ratio of live/dead biomass was used as an indicator of oil impact to the vegetation.

Soil redox potential was measured 1 and 3 months after oiling at 10 cm depth with bright platinum electrodes, a calomel reference electrode and a pH/mV meter to examine if the oil changed the soil reduction-oxidation conditions. The reading was adjusted by the addition of a correction factor of 244 mV based on a standard hydrogen electrode to calculate soil Eh.

Analysis of Regrowth in the Year Following Oil Application

In January of 1992 (ca. 5 months after oil application and 1 month after the initial harvest of aboveground biomass), the oil floating on the soil surface was removed from each pot to prevent the contact of the regrowing plant tissue with the floating oil. In April 1992, the above-ground biomass which regrew from the oiled sods was harvested as an indicator of the long-term impact of oil contamination and the ability of these marsh types to recover from oiling.

Soil cores (4.8 cm in diameter and 10 cm in height) were collected from each pot immediately after harvesting vegetation regrowth and stored in a freezer in the dark until analysis. The concentration of residual oil in the soil cores was determined with a modification of EPA method 9071 (U.S. EPA 1986). Residual oil was analyzed gravimetrically by vigorously mixing with a stainless steel spatulas 20 ml hexane with 3 g of soil from the top 5 cm of the core in a glass vial . During the 24-hour extraction period, the vial with sample was vortexed at high speed 3 times for 2-minute periods each. After the extraction, 5 ml of clear extract was transferred to an evaporation dish

for hexane evaporation. The unevaporated oil remaining in the dish was weighed and total hydrocarbons in the soil were calculated.

After oil extraction, the dry soil (65 °C to constant weight) was combusted in a furnace at 550 °C (EPA method 160.4, U. S. EPA 1979) to remove soil organic matter. The difference in weight before and after combustion was used to calculate the percent soil organic matter.

Statistical Analysis

Statistical Analysis was conducted with the Statistical Analysis System (SAS Institute, 1985). Plant and soil variables were analyzed with analysis of variance (ANOVA) as a 5 X 3 factorial arrangement of treatments (5 oil levels and 3 marsh types) in a completely randomized design (n=4). Duncan's multiple range test was used to evaluate statistical differences among each marsh type and oil level. Significant differences were reported at the 0.05 probability level unless otherwise stated.

RESULTS

Photosynthetic Rates

Compared with the control (no oil), the relative photosynthetic rate of *S. patens* significantly decreased with increasing oil level one (Fig. 1.2A) and two (Fig. 1.2B) months after application of the oil; the photosynthetic rate of *S. patens* at an oil level of 24 L m^{-2} was 25% of the control ($p < 0.0001$). However, photosynthetic rates of the other two species, *S. alterniflora* and *S. lancifolia*, showed no significant differences among the oil treatments one and two months after oiling. After three months of oil application, the photosynthetic rate of *S. alterniflora* was significantly reduced; the photosynthetic rate at an oil level of 24 L m^{-2} was about 50% of the control (Fig. 1.2C). Even after three months of oiling, the photosynthetic rate of *S. lancifolia* was not significantly affected by the oil application. The photosynthetic rate of *S. patens* three months after oiling was not measured because the plant health was too poor to choose a suitable leaf for measurement.

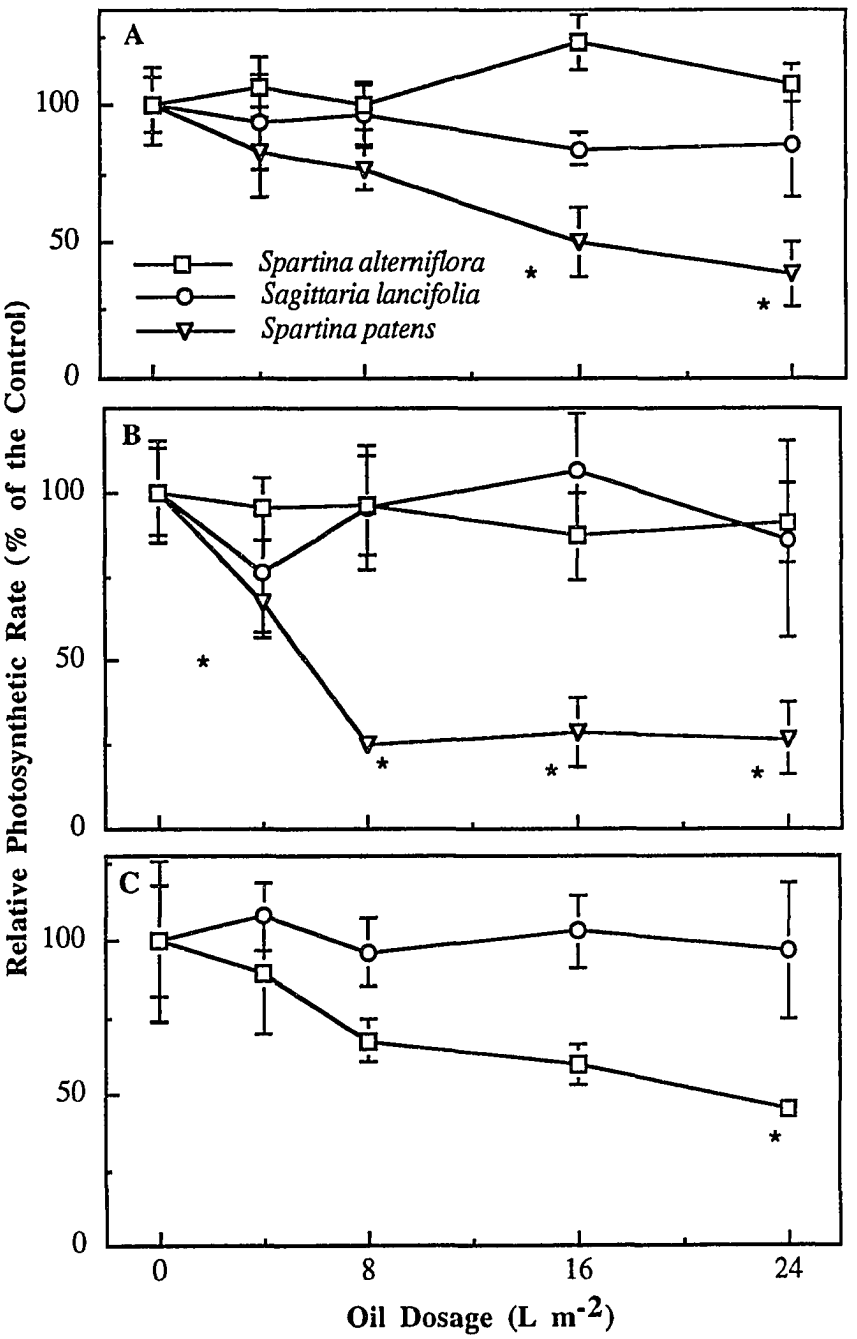

Fig. 1.2 Effect of crude oil on photosynthetic rate of *Spartina alterniflora, S. patens* and *Sagittaria lancifolia*. A: 1-month, B: 2-months, and C: 3-months following oil application; Photosynthetic rates were expressed as percentage of the control (means ± S.E.) with the control of each species equaling 100%.
* significantly different from the control (No oil).

Plant Stem Density

The increase in plant stem density (number of stems per pot) of *S. patens* over time was significantly impaired ($p < 0.001$) two and four months after oiling (Fig. 1.3A) compared to the control. Tiller survival ceased at oil levels above 8 L m^{-2}. We observed that the production and survival of new shoots of *S. patens* were completely inhibited as long as a continuous oil layer on the water surface existed. Three months after oiling, the thickness of the oil layer decreased and the oil layer became discontinuous; this was most likely due to oil evaporation, oxidation, biodegradation, and penetration into the soil. Few new young shoots of *S. patens* emerged at dosages above 8 L m^{-2} of oil.

Compared with the control, the increase in stem density of *S. alterniflora* was significantly affected by oil four months after oiling (Fig. 1.3B) . The continuous floating oil layer completely killed the newly emerging shoots of *S. alterniflora* similar to that observed for *S. patens* due to the sensitivity of their leaves to oil coating.

The stem density of *S. lancifolia* was not adversely affected by oil (Fig. 1.3C). The new shoots of *S. lancifolia* were able to penetrate through the thick floating oil layer and survived even at an oil level of 16 L m^{-2}. Two and four months after oiling, the stem densities of *S. lancifolia* at an oil dosage of 16 L m^{-2} were significantly ($p < 0.01$) higher than those of the control.

Above-ground Biomass in the Year of Oil Application

The live above-ground biomass of *S. patens* was significantly ($p < 0.01$) lower while the dead biomass component was significantly ($p < 0.01$) higher with higher oil dosage (Fig. 1.4A) compared with the control. The total aboveground biomass of *S. patens* was also significantly lower with higher oil dosage (Fig. 1.4A). The ratio of live/dead aboveground biomass shows the impact of the oil treatments ($p < 0.001$). The ratios at 16 and 24 L m^{-2} of oil were only about 20% of the ratio of the control (Fig. 1.4B).

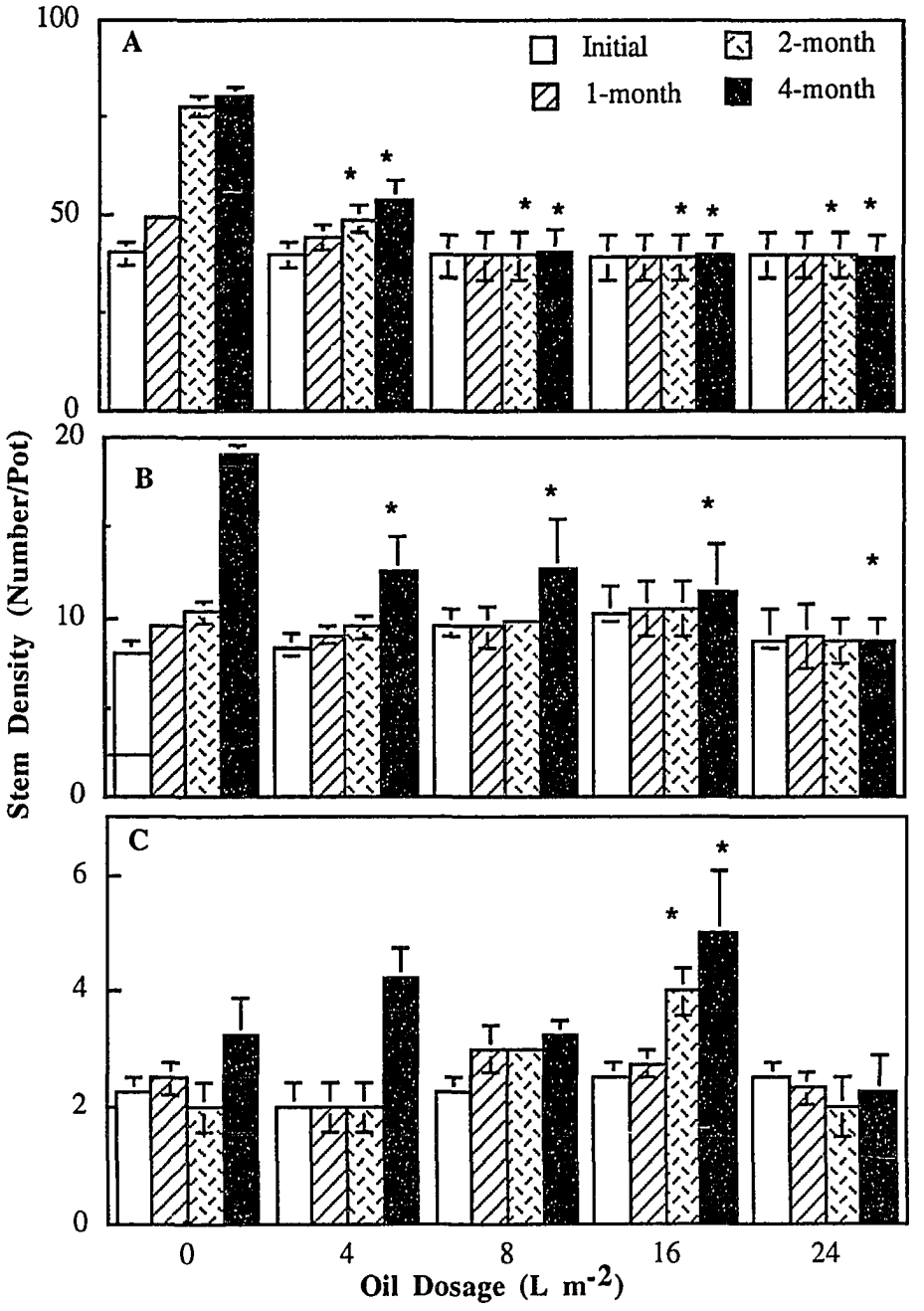

Fig. 1.3 Effect of crude oil on plant stem density (means ± S.E.). A: *Spartina patens*, B: *S. alterniflora*, and C: *Sagittaria lancifolia.* * significantly different from the control.

The total aboveground biomass of *S. alterniflora* did not significantly change with different oil levels (Fig. 1.5A), although live biomass tended to decrease and dead biomass tended to increase as oil levels increased. However, the ratio of live/dead aboveground biomass, which was a more sensitive indicator of oil impact, was significantly lower with higher oil dosage compared with the control, especially for oil levels of 8 L m^{-2} and higher (Fig. 1.5B).

Unlike the *Spartina* species, the total aboveground biomass of *S. lancifolia* was significantly higher with higher oil dosage ($p < 0.0001$); at the 24 L m^{-2} oil level, total aboveground biomass was two times that of the control (Fig. 1.6). The biomass of *S. lancifolia* was expressed only as total aboveground biomass because, at plant harvest in December, this species was senescing which made it difficult to distinguish the dead tissue caused by oiling versus natural senescence.

Soil Redox Potential

Oil application did not cause the soil to become more reduced compared to the controls. Soil redox potentials (Eh) one and three months after oiling were not significantly affected by oil application (Table 1.2).

Vegetation Regrowth in the Year Following Oil Application

Four and half months following the initial harvest of aboveground tissue (9 months following the oil application), the *Spartina* species showed little to no regrowth of biomass at oil levels of 8 L m^{-2} and higher while *S. lancifolia* exhibited enhanced regrowth of biomass compared to the control (Table 1.3). The aboveground biomass of *S. alterniflora* and *S. patens* after 4.5 months of regrowth was significantly ($P < 0.0001$) lower with oil dosage. In contrast, the regrowth of aboveground biomass from the fresh marsh sods dominated by *S. lancifolia* was higher with increasing oil dosage (Table 1.2). When considering only the dominant species, *S. lancifolia*, the aboveground biomass increased significantly with as much as 2.2 times the biomass at 24 L m^{-2} compared to the control. The fresh marsh sods, however, contained the

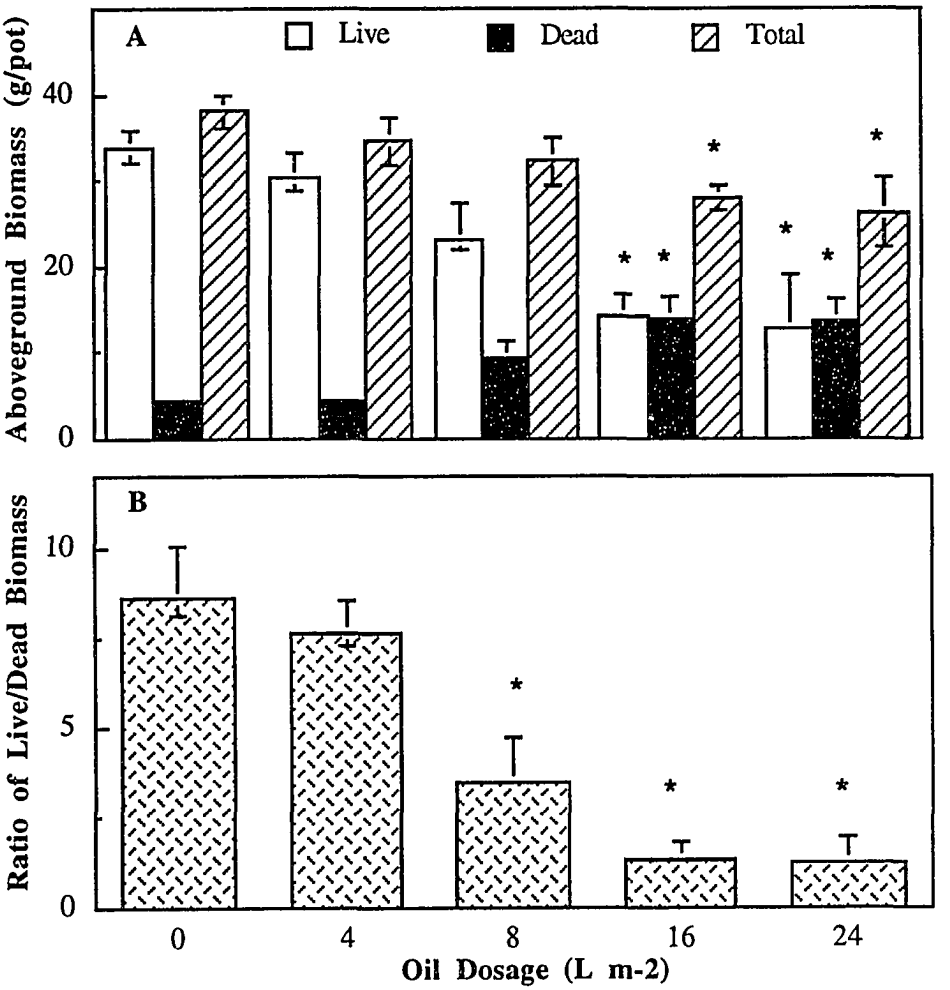

Fig. 1.4 Effect of crude oil on the aboveground biomass and ratio of live/dead biomass (means ± S.E.) of *Spartina patens* four months following oil application. A: aboveground biomass; B: ratio of live/dead aboveground biomass.
* significantly different from the control.

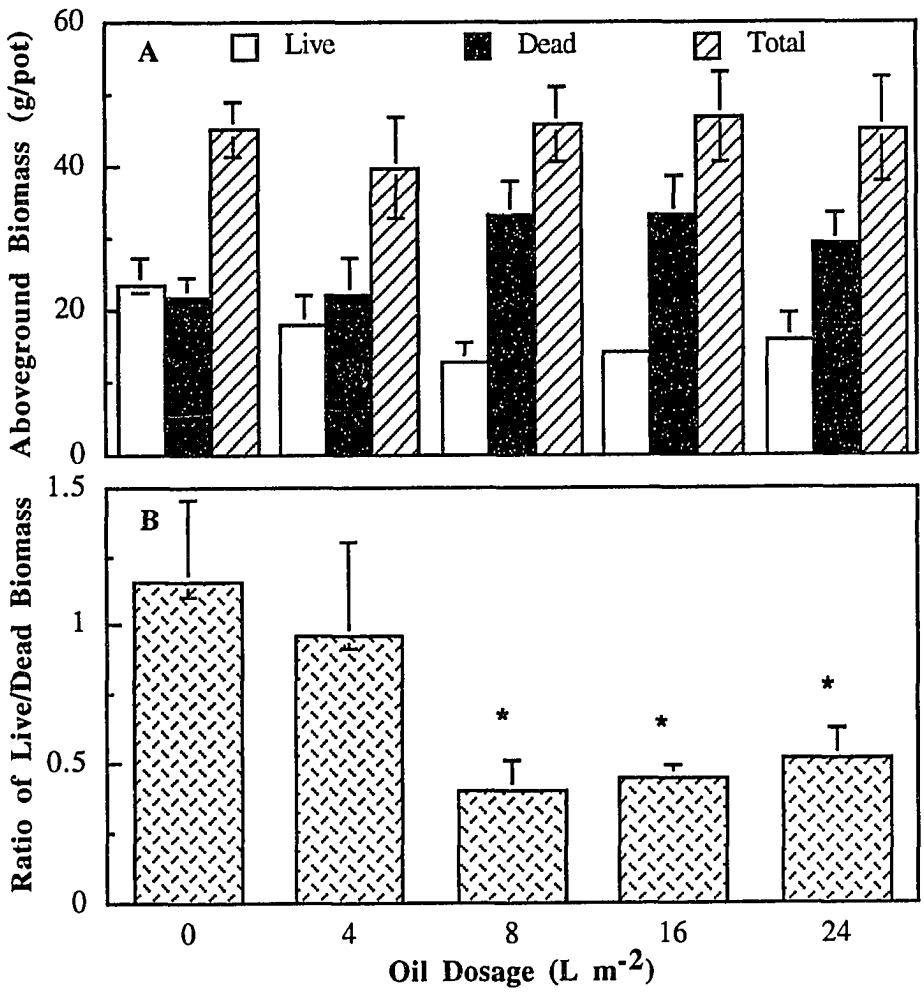

Fig. 1.5 Effect of crude oil on the aboveground biomass and ratio of live/dead biomass (means ± S.E.) of *Spartina alterniflora* four months following oil application. A: aboveground biomass; B: ratio of live/dead aboveground biomass. * significantly different from the control.

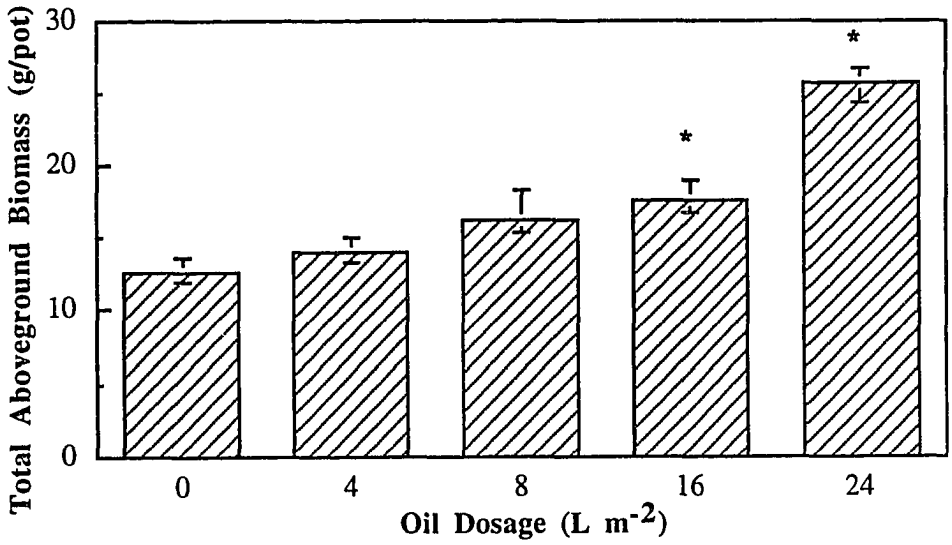

Fig. 1.6 Effect of crude oil on the aboveground biomass (means ± S.E.) of *Sagittaria lancifolia* four months following oil application. * indicates significantly different from the control.

Table 1.2 Effect of oil on soil redox potential (mV) one and three months after oiling. Values are means with standard errors in parentheses.

Months after oiling	*Sagittaria lancifolia*		*Spartina patens*		*Spartina alterniflora*	
	oil	no oil	oil	no oil	oil	no oil
1	-68 (30)	-60 (11)	-68 (26)	-99 (12)	-179 (10)	-126 (7)
3	-97 (20)	-37 (34)	-143 (15)	-112 (33)	-99 (10)	-70 (21)

species other than *S. lancifolia*: *Eleocharis quadrangulata*, *Cyperus ordoratus*, and *Ammania teres*. Even when the total biomass of these four species was considered, biomass still tended to be higher with higher oil dosage, but this increase was not significant. Therefore, the greater biomass in the fresh sods with higher oil dosage was primarily contributed by *S. lancifolia* (Table 1.3). *Sagittaria lancifolia* formed monospecific communities at 16 and 24 L m^{-2} of oil.

The Relationship between Oil Residue and Soil Organic Content

The residual oil concentration in the soil significantly increased with higher oil dosage in all three types of marsh soils (Fig. 1.7). Also, the residual oil concentration in the soil was significantly different among marsh types with highest residual oil concentration at all oil levels in *S. patens* sods and the lowest residual oil concentrations in *S. alterniflora* sods; the oil content of *S. patens* sods was more than 6 times higher than that of *S. alterniflora* sods at 24 L m^{-2} even though original oil dosage was the same. Residual oil in the *Sagittaria* soil was intermediate to that in the *Spartina* sods. In addition, the residual oil in the *S. alterniflora* soil plateaued at oil levels over 4 L m^{-2} (Fig. 1.7).

The soil organic matter content of the sods followed the same trend as the residue oil. The highest soil organic matter content was 42 % ± 3% (mean ± standard error) for *S. patens* soil; the lowest soil organic matter content was 19 % ± 0.6% for *S. alterniflora* soil, and 30 % ± 8% for *S. lancifolia* ranked intermediate. The coefficients of determination (r^2) between residual oil and soil organic content were relatively high and ranged from 0.69 to 0.89 (Fig. 1.8), thus, 69 - 89 % of the variation in oil holding capacity of the soil could be explained by the soil organic content. Figure 1.8, which combines the data from the three marsh types, demonstrates the positive relationship between soil organic matter content and residual oil concentration in the soil.

Table 1.3 Effect of oil on plant growth nine months after the application of oil. Values are means with standard errors in parentheses.

Oil Dosage ($l\ m^{-2}$)	Aboveground Biomass in Each Marsh type (g / pot)						
	S. patens	*S. alterniflora*	*Sagittaria*				
			Sagittaria lancifolia	*Eleocharis quadrangulata*	*Cyperus ordoratus*	*Ammania teres*	Total Biomass
0	18.75(2.3) a*	16.7(1.0) a*	4.6(0.9) a*	2.7(1.1) a*	0.3(0.06) a*	0.6(0.4) a*	8.2(1.7) a*
4	11.2(1.2) b*	11.6(4.1) a*	7.3(1.0) ab*	1.0(0.6) ab*	0 b*	0 a*	8.4(1.1) a*
8	1.45(1.4) c*	0.9(0.5) b*	7.3(0.7) ab*	0.35(0.2) b*	0 b*	0 a*	7.7(1.1) a*
16	0 c*	0 b*	9.5 (1.0) b*	0 b*	0 b*	0 a*	9.5(1.0) a*
24	0 c*	0 b*	10 (1.8) b*	0 b*	0 b*	0 a*	10(1.8) a*

* Means with the same letter within a column are not significantly different.

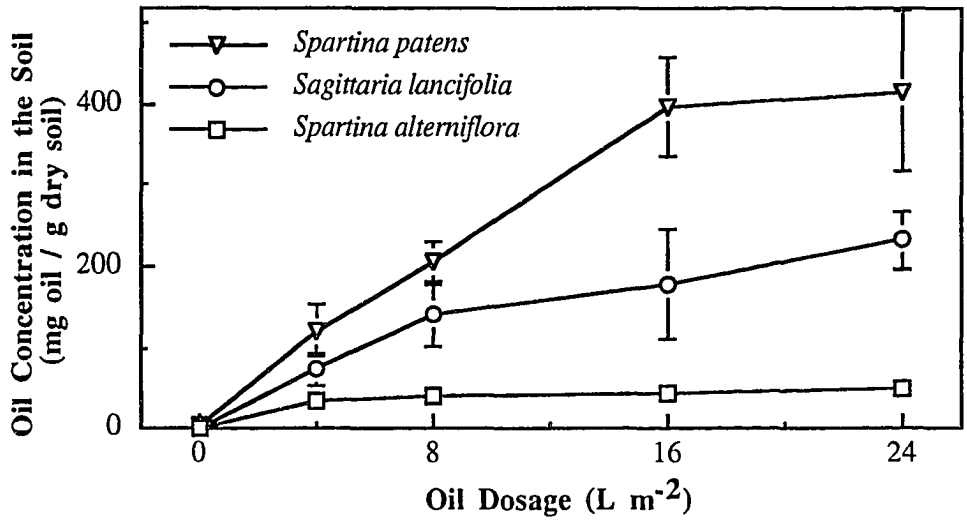

Fig. 1.7 Residual oil concentration in the soil (means ± S.E.) nine months following oil application.

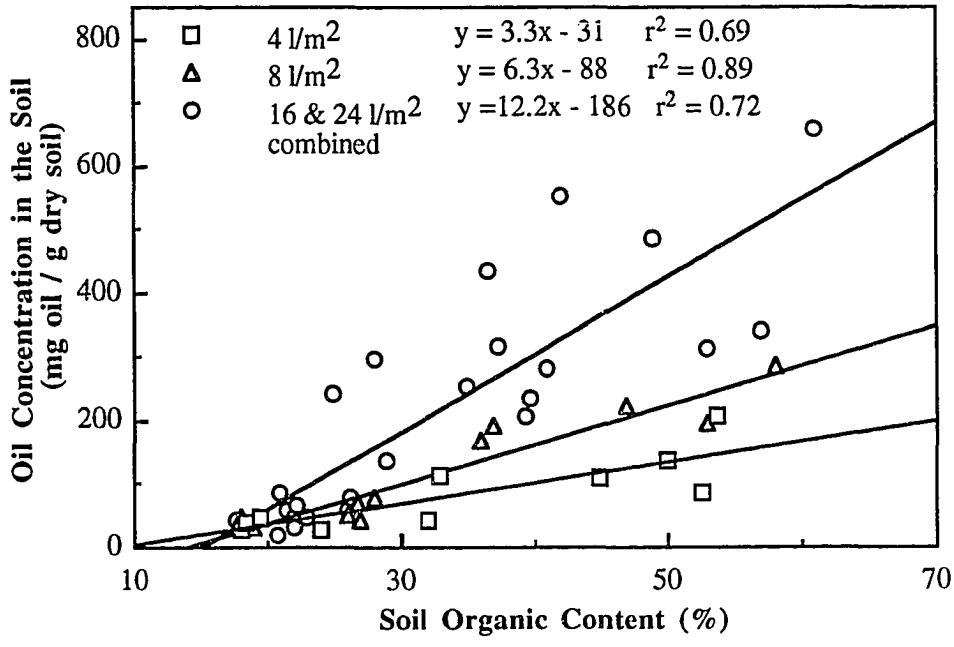

Fig. 1.8 Relationship of the soil residue oil concentration and soil organic matter content of three marsh types at various oil dosages (this relationship was not significantly different between 16 and 24 L m^{-2} and, therefore, the regression lines were combined for these oil dosages).

DISCUSSION

South Louisiana crude oil, when applied to the soil, significantly impaired the two *Spartina* species, while not adversely impacting *Sagittaria lancifolia*. Petroleum hydrocarbons may affect plants in several ways: (1) Disruption of plant-water relationships (McCown & Deneke, 1972), (2) Direct impacts to plant metabolism, for instance, nutrient uptake (McCown & Deneke, 1972), (3) Toxicity to living cells, for example, the lipid component of the protoplasm or the chloroplast membrane may be affected by lipophilic hydrocarbons (Prendevolle & Warren 1977), and (4) Reduced oxygen exchange between the atmosphere and the soil, affecting root function (Stebbing 1970). The present study, however, showed no significant decrease in soil redox potential (Eh) induced by the oil. Delaune (1979) found no change in soil Eh in response to crude oil addition to sediment suspensions. Under flooded conditions, the primary route by which atmospheric oxygen moves to the roots and surrounding rhizosphere of wetland plants is via plant aerenchyma (air space) tissue (Teal & Kanwisher, 1966; Armstrong, 1978; Smirnoff & Crawford, 1983; Justin & Armstrong, 1983; Seliskar, 1985). It would appear that movement of oxygen to the rhizosphere through aerenchyma was not prevented by the oil additions, otherwise the impairment of oxygen exchange between the atmosphere and the soil by the floating oil layer would have reduced Eh in the oiled sods.

The impact of the oil on *S. patens* was expressed in reduced photosynthesis, plant stem density, and plant biomass. In this study, photosynthesis of *S. patens* was affected during a period of vigorous growth (in August). The decrease in photosynthetic rate was associated with a reduction in biomass harvested 3 months later; the reduction in biomass was most likely due to a substantial reduction in photosynthate during this period. In addition, the biomass reduction of *S. patens* with higher oil dosage was also associated with a reduction in stem density because of either the lethal

effect of the floating oil layer on new tillers or the effect of the penetrated oil in the soil on the generation of new tillers from rhizomes. However, the large decrease in the ratio of live/dead biomass (Fig. 1.4B) was also indicative of the reduced survival of existing plants. The belowground tissue was likely affected by the oil penetrating into the rhizosphere and thus affecting plant survival both in the year of oil application (Figs. 1.3A and 1.3B) and the year following oil application (Table 1.2). The penetration of oil into the soil caused the high initial mortality at greater oil dosages and the long-term effects on plant regrowth in the following year.

The general trend of lower photosynthetic rate and biomass with higher oil dosage for *S. alterniflora* was similar to that found for *S. patens*, except that the impacts were delayed. Compared with *S. patens*, a measurable oil effect on *S. alterniflora* was not detected until 3 months (in October) after oil application. In late October, the *S. alterniflora* aboveground biomass was near maximum for this experiment. The photosynthate produced from late October to early December (harvest) probably did not contribute significantly to increasing above-ground biomass since the photosynthetic rate was low (about 7 *u*mol CO_2 m^{-2} s^{-1}) during this period compared to August and September (about 20 *u*mol CO_2 m^{-2} s^{-1}). This might explain the non-significantly effect of higher oil dosage on the total aboveground biomass (live and dead) of *S. alterniflora* at harvest. However, the decreases in the photosynthetic rate, plant stem density, and the ratio of live/dead biomass with increasing oil dosage indicated an adverse effect of oil on *S. alterniflora*. This effect was most likely due to oil penetrating the soil and affecting belowground processes, which in turn, impacted photosynthesis since oil never coated the plant leaves or shoots in this study. The adverse effect was also likely due to the lethal effect of the oil layer on new tiller production, preventing an increase in stem density of *S. alterniflora* over time. The regrowth of *S. alterniflora* in the following year was reduced with increasing oil dosages and completely inhibited by

oil levels of 16 and 24 L m^{-2}. This suggests a lethal effect of the penetrated oil on belowground processes as found for *S. patens* at high oil dosages.

Unlike the two *Spartina* species in this study, the photosynthetic rate of *Sagittaria lancifolia* was not adversely affected by the oil treatment (Fig. 1.2A, B, C). The aboveground biomass of *S. lancifolia* increased with increasing oil dosage even at the highest dosage of 24 L m^{-2}. The high resistance of *S. lancifolia* to oil was probably due to both large belowground rhizomes that provide carbohydrates for vegetative generation of new shoots and oil-resistant shoots that can penetrate surface oil without injury. Oil-induced increase of plant growth has been reported in other studies. Hershner and Moore (1977) observed a significant increase in live standing crop in a *S. alterniflora* marsh oiled by No. 6 fuel oil. Li *et al* (1990) reported that chronic low level hydrocarbon additions enhanced *S. alterniflora* growth, but high levels of oil led to reduced growth. The shoot lengths of *Festuca rubra* and the biomass of *Puccinellia maritima* were also increased by oil (Baker, 1971). In those studies, however, the concentration of oil that increased growth was much lower than the concentration of oil that enhanced the growth of *S. lancifolia* found in the present study. The mechanisms by which oil stimulates the growth of some plants is not clearly understood. It may be (1) indirectly, through an increase of microbial N-fixation, (2) directly, by the addition to the soil of some plant growth stimulatory analogues in the petroleum or its metabolites, and (3) shifting competitive interactions (Baker 1971, Boesch *et al.*, 1974, Thomson & Webb 1984; Li *et al.*, 1990; Scholten & Leendertse, 1991). In addition, plant resistance to oil varies greatly with species. In this study, the four fresh marsh plant species, *Sagittaria lancifolia*, *Eleocharis quadrangulata*, *Cyperus ordoratus*, and *Ammania teres*, occurred in the sods of the *Sagittaria* marsh receiving no oil, but *C. ordoratus* and *A. teres* did not appear in any of the oil treated sods. *Eleocharis quadrangulata* could only exist at oil levels up to 8 L m^{-2} but experienced reduced biomass with increasing oil dosage up to that level. *Sagittaria lancifolia* occurred at all

oil dosages and formed monospecific communities at 16 and 24 L m^{-2} of oil, exhibiting a high oil tolerance and even an enhancement in growth with higher oil dosage.

The oil concentrations in the soil were strongly associated with the soil organic matter content (Fig. 1.8). Generally, most petroleum components are nonionic and therefore associate more readily with organic than with mineral particles in soil. A small percentage of organic carbon can have a larger adsorptive capacity than the total of the mineral components (Testa and Winegardner 1991). The *S. patens* sods had the highest oil residue which was correlated with its high soil organic matter (42 %). The lowest oil residue concentration was found in the *S. alterniflora* sods, which had the lowest soil organic matter (19 %). We measured the concentration of oil that penetrated the soil in digested and undigested marsh soil. The concentration of oil was 41 to 279 times greater in the undigested soil, compared to the digested soil, where organic matter was removed. Therefore, the organic matter in the soil must be the primary control of the oil content in the soil.

In addition to soil organic matter, the soil texture could also affect the residual oil concentration. Usually, oil penetrates a coarse soil (e.g. sand) more readily than fine soil. In this study, however, the texture of the mineral component of the marsh soil was very fine (more than 90% < 0.05 mm), and, therefore, if it were not for the organic matter in the soil, south Louisiana crude oil could not readily penetrate these fine, water-saturated sediments.

The marsh vegetation could enhance the penetration of oil into soil. The interface between plant stems or roots and the soil could act as a conduit and accelerate the penetration of oil into the soil. The penetrated oil could subsequently be sorbed by organic matter in the soil. The higher oil residue in the *S. patens* soil could also be, in part, due to a lesser ability of *S. patens* to transport O_2 to its rhizosphere than *S. alterniflora* (Gleason & Zieman, 1981). This may have resulted in lower rates of aerobic biodegradation of the oil in the *S. patens* sods compared to the sods of *S.*

alterniflora. In this study, the rapid effect of the oil on the photosynthetic rate of *S. patens* was most likely due to high soil organic matter content in this marsh type, which readily sorbs oil into the rhizosphere and affects the plant through belowground plant processes.

SUMMARY

The impact of south Louisiana crude oil on the dominant vegetation, *Spartina alterniflora, Spartina patens*, and *Sagittaria lancifolia*, of three types of coastal wetlands, salt, brackish, and freshwater marshes, respectively, was studied. In the greenhouse, south Louisiana crude oil was applied to natural marsh sods at rates of 0, 4, 8, 16, and 24 L m^{-2}. The photosynthetic rate of *S. patens* significantly decreased with increasing oil dosage after one month of oil application, while the photosynthetic rate of *S. alterniflora* was not significantly reduced by oil application until three months. The photosynthetic rate of *S. lancifolia* was not detrimentally affected by the oil treatments. Four months after oil application, live aboveground biomass production was significantly reduced at higher oil dosages for *S. patens*, not affected in *S. alterniflora*, while *S. lancifolia* exhibited significantly higher total biomass at 24 L m^{-2} compared to the controls. The year following oil application, no regrowth of *S. patens* and *S. alterniflora* occurred at oil levels above 8 L m^{-2}. In contrast, the regrowth of aboveground biomass of *S. lancifolia* increased with increasing oil dosage and resulted in monospecific communities at 16 and 24 L m^{-2}. Furthermore, the residual oil in the soil greatly differed among the three marsh types with the highest concentrations in the *S. patens* soil sods and the lowest concentrations in *S. alterniflora* soil sods. This difference in oil residue among the marsh types was mainly due to differences in soil organic content. The sensitivity of these marsh types to south Louisiana crude oil increased in the following order: *S. lancifolia, S. alterniflora*, and *S. patens*.

CHAPTER 2

THE INFLUENCE OF OILING POSITION AND SOIL COMPOSITION ON THE IMPACT OF SOUTH LOUISIANA CRUDE OIL TO MARSH VEGETATION

INTRODUCTION

Oil spills may cause widespread damage to plant communities. The degree of oil damage to vegetation may depend upon a number of factors, such as the plant species, the extent of oil coverage, the type and amount of oil, the season of the spill, and the soil composition (Baker, 1971b; Burk, 1977; Hershner & Moore, 1977; Alexander & Webb, 1985 &1987; Mendelssohn *et al.*, 1990).

Plant species vary greatly in response to oil pollution (Cowell & Baker, 1969; Delby, 1969; Burk, 1977; Ferrell *et al.*, 1984;). Usually annual species are more susceptible to oil impact than perennials (Delby 1969; Burk; 1977; Baker, 1971b), probably due to their small belowground reserves for regrowth after the aboveground tissue is killed. In a four-year study of an oil spill in a freshwater marsh at the mouth of Mill River in Northampton, Massachusetts, Burk (1977) observed that all of the twenty-three species that were relatively unaffected or more abundant following the spill were perennials. In an experimental oil spraying to plant aboveground tissue in a salt marsh near Pembroke, S. W. Wales, Baker (1971b) reported that there was little long-term vegetative damage to most perennial species, but the annual species were severely damaged. However, even among perennials, large variation in response to oil spills was observed (Burk, 1977).

Oil coverage on aerial portions may impair plants. Short-term effects of oil coating on plants have been reported in a number of studies (Ferrell *et al.*, 1984; Smith *et al.*, 1981; Webb *et al.*, 1981; Mendelssohn *et al.*, 1990). Coating the entire leaf surface of *S. alterniflora* with oil resulted in the cessation of photosynthesis (Pezeshki

& Delaune, 1993). In addition, oil coverage of the plant reduced the stem density and aboveground biomass (Ferrell *et al.*, 1984), and killed the aboveground portion (Webb *et al.*, 1981) of *S. alterniflora*. However, oil coverage of aerial portions of plants appears to have no long-term effect, and recovery of salt marsh vegetation from oil coverage is generally good (Baker, 1973; Webb *et al.*, 1981; Alexander & Webb, 1985).

Oil incorporation into soil could cause acute and chronic damage to plants, reducing the stem height, stem density and aboveground biomass, increasing mortality, impacting growth, and even inhibiting aerial regrowth of *Spartina alterniflora* by killing roots and rhizomes at high oil concentration (Ferrell *et al.*, 1984; Alexander & Webb, 1987; Krebs & Tanner, 1981).

Soil composition may also influence oil incorporation into the soil, thus affecting oil impact to plants. Oil associates more readily with organic matter than mineral matter (Testa & Vinegardner, 1991), thus, potentially affecting plants grown in high organic soils more severely. Aside from the soil organic content, soil texture may influence the degree of penetration of oil into the soil. Usually, oil penetrates coarse soil (e.g., sand) more readily than fine-textured soil (Getter *et al.*, 1984). Thus, soil containing both coarser particles and higher organic matter could allow for greater penetration and adsorption of oil and more severe impacts to the vegetation.

In order to better understand how plant species, soil type, and oiling position affect the intensity of plant response to oil, a series of greenhouse experiments were conducted with the following objectives: (1) Determine if oiling position (oil applied to soil or to lower aerial portion of a plant) is important in influencing oil effect on plants, (2) Determine if there is a differential response to oil treatment impact among three dominant marsh plants in the same soil type, and (3) Determine the effect of different soil substrates on the impact of oil to plants.

MATERIALS AND METHODS

Plant and Substrate Materials

Three dominant marsh macrophytes (*Spartina alterniflora*, *S. patens*, and *Sagittaria lancifolia*) were each transplanted to both an organic soil (Jiffy-Mix, a commercial potting soil by Jiffy Products of America, Inc., West Chicago, Illinois) and mineral/organic soil (2:1 mixture by volume of freshwater marsh sediment and Jiffy-Mix). The organic content in the organic soil was higher, with 50% organic matter compared with 32.7% organic matter in the mineral/organic soil. Furthermore, the texture of the organic soil was much coarser, with 1-3 mm diameter vermiculite particles compared to the fine deltaic sediment (< 0.05 mm) in the mineral/organic soil. Into each experimental unit, either four stems of *S. patens*, two stems of *S. alterniflora*, or one stem for *Sagittaria lancifolia* were transplanted.

Experimental Design

Influence of oiling position on plant response to oiling

Oil application

To assess the effect of oiling position on the plants, the three dominant plant species were transplanted to the organic soil (described above) and allowed to grow for a 6-week period prior to exposure to the oil treatments. Weekly applications of 150 ml of full strength Hoagland's nutrient solution were made to each pot after transplantation and continued for the duration of the experiment. The experimental design was completely randomized with a 2 X 2 factorial arrangement of the following treatments: (1) oil added directly to the soil (hereafter referred to as OAS) (0 and 8 L m^{-2} of unweathered south Louisiana crude oil) and (2) oil added to the plant (hereafter referred to as OAP) (no oil and oil sprayed on the lower 30 cm of the aerial portions of each plant). For each species, therefore, there were four treatment-level combinations with 5 replicates each (n=5).

Measurement of plant response to the oiling position

After oil application, photosynthetic rates of the plants were measured at two, four, seven and nine weeks, and plant leaf elongation rates were measured at one, two, three, five and six weeks to determine the short-term impacts of the oil on the plants. Live, dead, and total aboveground biomass were harvested and analyzed 2.5 months after the application of the oil to assess the comprehensive effect of the oil on plant growth. The regrowth of biomass was determined one year following the oil application to quantify the long-term oil impact on the plants. Methods were detailed in Chapter 1.

Leaf elongation rate was measured as an index of plant growth status. The youngest leaf was chosen for measurement. At the beginning of the measurement period, a vertical distance between the leaf tip and a specific reference point (near soil surface) was recorded. This distance was measured again 2 days later after plant leaf growth. Leaf elongation was expressed in centimeters per day.

Influence of soil composition on plant response to oiling

Oil application

To determine the influence of the soil composition on plant response to oiling, the three dominant plant species were transplanted individually to both the organic soil and the mineral/organic soil as described above, and allowed to grow for a 6 weeks prior to applying the oil to the soil surface. At days 1, 7, 14, 21 and 28, the oil floating on the surface was drained through the soil column to maximize the soil-contact and the oil effect. The experimental design was completely randomized with a 2 x 2 factorial arrangement of treatments with 4 replicates [2 soil substrate types (organic soil and mineral/organic soil) and 2 oiling levels (0 and 8 l/m^2)] for 3 species.

Analysis of influence of soil composition on plant response to oiling

Photosynthetic rates were measured to assess the short-term effect of the oil on the plants. Aboveground biomass was harvested and analyzed 2.5 months after oil

application to determine the comprehensive effect of the oil on plant growth. Soil cores were taken after the first harvest, and oil concentration in the soil was analyzed. Regrowth biomass was analyzed one year following oil application to compare the long-term effect of the oil in the two soil types (methods detailed in Chapter 1).

Statistical Analysis

Statistical analysis was conducted using the Statistical Analysis System (SAS Institute, 1985). Plant variables were analyzed with repeated measures analysis of variance. Duncan's multiple range test was used to evaluate statistical differences among each oiling position and oil level. Significant differences were reported at the 0.05 probability level unless otherwise stated.

RESULTS

The Influence of Oiling Position on Plant Responses

Photosynthetic rates

The photosynthetic rates of the three marsh plants greatly decreased with the application of south Louisiana crude oil to the soil substrate (Figs. 2.1-2.3). The photosynthetic rate of *Sagittaria lancifolia* was significantly ($p<0.0001$) reduced compared to the control two weeks after oil applied to the soil (OAS treatment) and continued to decline and was significantly different from controls at 4, 7 and 9 weeks (Fig. 2.1). The OAP treatment (oil added to the lower aerial portion of the plant), however, did not significantly affect the photosynthetic rates of *Sagittaria lancifolia*. Since the interaction between the OAP and OAS treatments was not significant ($p> 0.23$), the mean photosynthetic rates were averaged over the OAP treatment ($n=10$, Fig. 2.1).

The photosynthetic rate of *Spartina alterniflora* was significantly ($p < 0.01$ - 0.0001) lower than controls at 2, 4, 7 and 9 weeks after the application of the oil to the soil substrate (Fig. 2.2). As occurred for *S. lancifolia*, the OAP treatment of *Spartina*

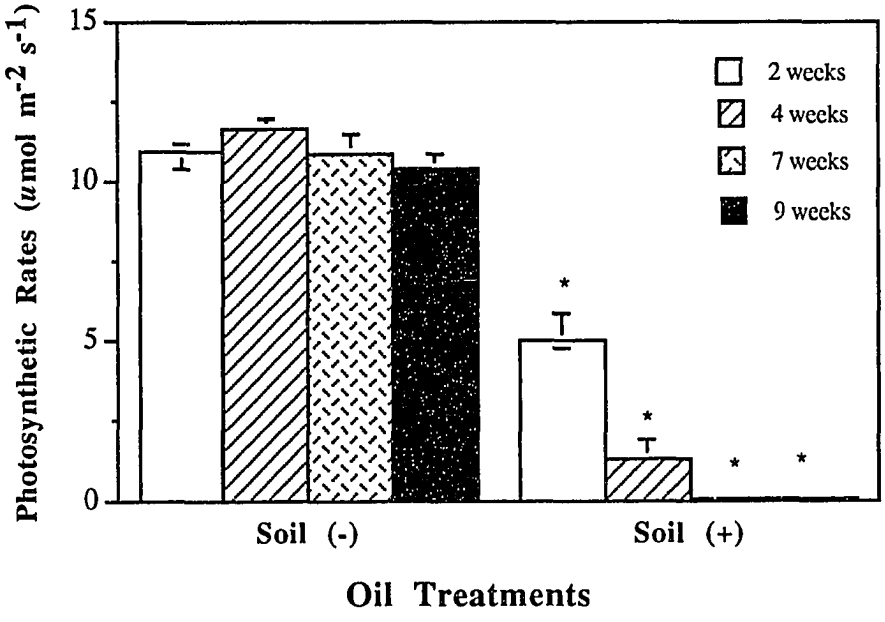

Fig. 2.1 Photosynthetic rates of *Sagittaria lancifolia* after application of south Louisiana crude oil to the soil (n=10). * indicates significant difference from the control (no oil). Soil (-): no oil applied to the soil; Soil (+): 8 L m^{-2} of oil applied to the soil.

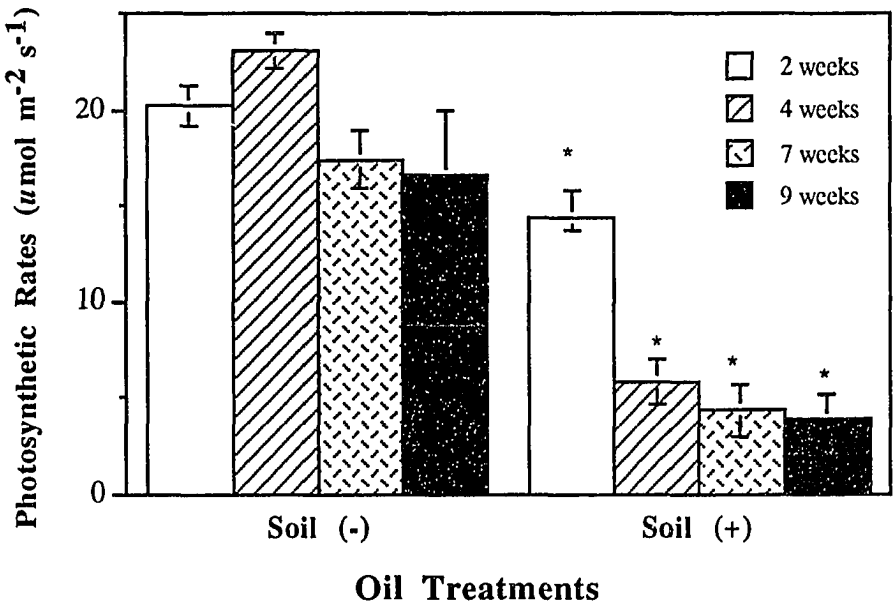

Fig. 2.2 Photosynthetic rates of *Spartina alterniflora* after application of south Louisiana crude oil to the soil (n=10). * indicates significant difference from the control (no oil). Soil (-): no oil applied to the soil; Soil (+): 8 L m^{-2} of oil applied to the soil.

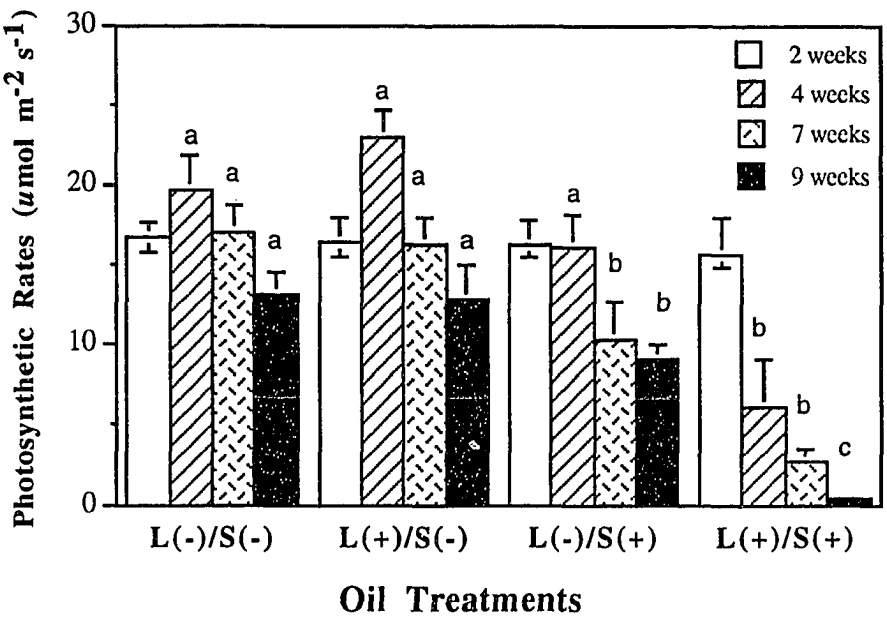

Fig. 2.3 Photosynthetic rates of *Spartina patens* after application of south Louisiana crude oil to the lower aerial portion of the plant (L) and to the soil (S); - and + represent no oil and oil (8 L m^{-2}), respectively. Different letters indicate significant differences within a time period between treatment-level combinations (n=5).

alterniflora did not significantly affect the photosynthetic rates. Means were averaged over the two levels of the OAP treatment (no significant interaction between OAS and OAP treatments, Fig. 2.2).

The photosynthetic rate of *Spartina patens* was significantly ($p < 0.0005$) lower than controls at 4, 7 and 9 weeks after the application of oil to soil substrate (Fig. 2.3). However, the effect on plant photosynthetic rate was greater when the oil was also applied to the lower stem (significant interaction between OAS and OAP treatments). For example, the photosynthetic rate at 7 and 9 weeks were significantly lower in the combined OAP and OAS treatments compared to just the OAS treatment (Fig. 2.3).

Leaf elongation rates

Oil added to the soil impaired leaf elongation rates of all three marsh plants (Figs. 2.4 - 2.6). The leaf elongation of *Sagittaria lancifolia* significantly ($p < 0.005$) differed from the control 1, 2 and 3 weeks after application of the oil to the soil (Fig. 2.4). The leaf elongation rates of *Spartina alterniflora* and *S. patens* were significantly ($p < 0.01$ to 0.0001) reduced at 2, 3, 5 and 6 weeks after application of oil (Figs. 2.5 and 2.6). However, the OAP treatment did not significantly affect the leaf elongation rates of *Sagittaria lancifolia*, *Spartina alterniflora* and *S. patens*. Means were averaged over the OAP treatment (no significant interaction between OAS and OAP treatments, Figs 2.4 - 2.6).

Aboveground biomass production 2.5 months after oiling

The application of south Louisiana crude oil to the soil substrates detrimentally affected the aboveground biomass of all three marsh plant species (Figs. 2.7 - 2.9). The live and total aboveground biomass of *Sagittaria lancifolia* was significantly ($p<0.0001$) suppressed by application of the oil to the soil, but not by the OAP treatment (Fig. 2.7 a). Means were averaged over the OAP treatment (no significant interaction between OAP and OAS treatments, Fig. 2.7). The dead aboveground biomass of *Sagittaria lancifolia* was not significantly affected by the oil treatments.

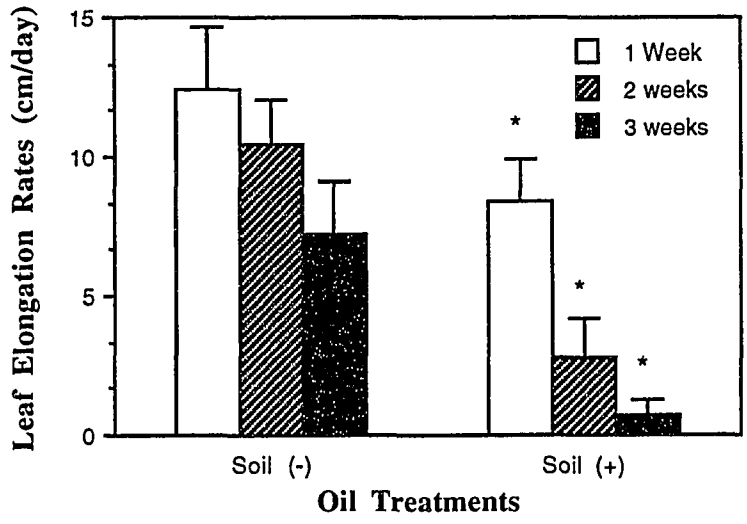

Fig. 2.4 Leaf elongation rates of *S. lancifolia* after application of the oil to the soil; - and + represent no oil and oil (8 L m^{-2}), respectively (n=10). * indicates significant difference within weeks.

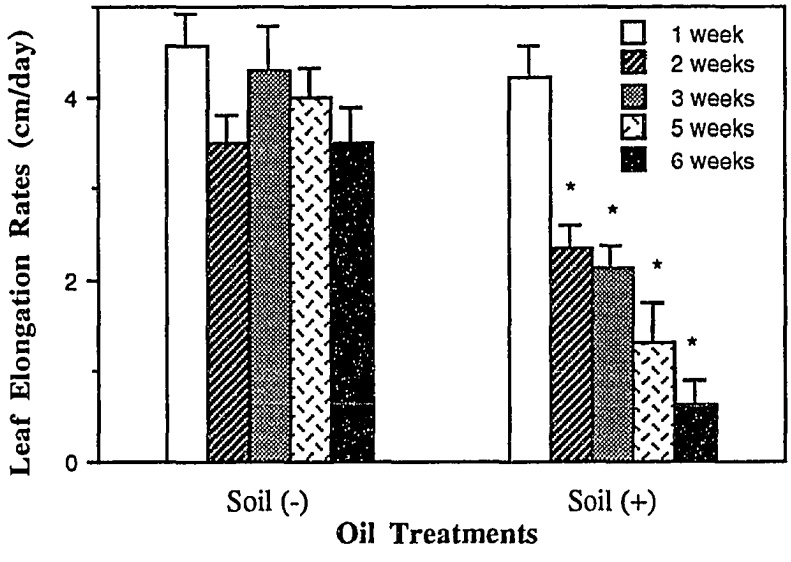

Fig. 2.5 Leaf elongation rates of *S. alterniflora* after application of the oil to the soil; - and + represent no oil and oil (8 L m^{-2}), respectively (n=10). * indicates significant differences within weeks.

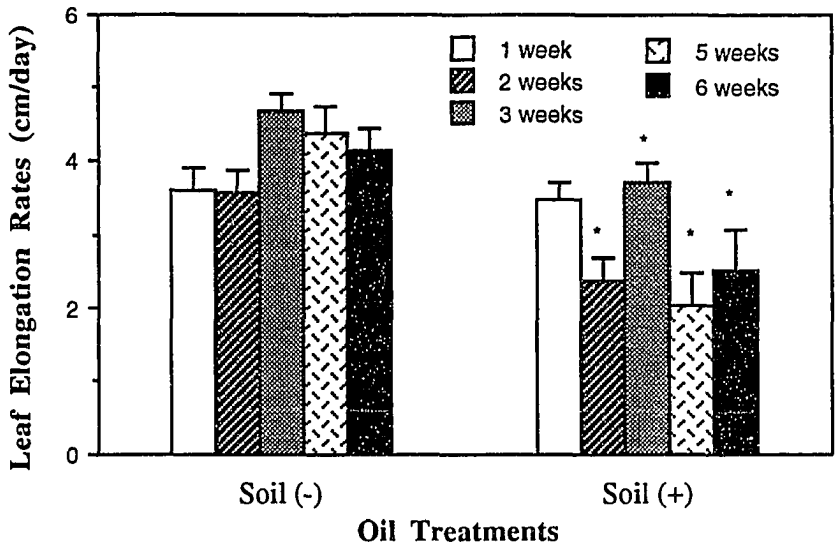

Fig. 2.6 Leaf elongation rates of *S. patens* after application of the oil to the soil; - and + represent no oil and oil (8 L m^{-2}), respectively (n=10). * indicates significant differences within weeks.

Plant stem density showed a similar trend as the aboveground biomass with a significant suppression of live and total stem densities plus a substantial increase of dead stem density by oiling the soil (Fig. 2.7 b). The OAP treatment for *Sagittaria lancifolia* did not significantly affect stem density.

The application of south Louisiana crude oil adversely affected the aboveground biomass of *Spartina alterniflora* (Fig. 2.8 a). Live and total aboveground biomass significantly ($p<0.0001$) decreased and dead aboveground biomass significantly increased by application of the oil to the soil. In addition, the OAP treatment for *Spartina alterniflora* significantly ($p<0.005$) decreased the live and total aboveground biomass and significantly increased dead biomass (Fig. 2.8 b). The effect of the oil on the stem density of *Spartina alterniflora* was similar to that on biomass. Live and total stem densities significantly decreased with the OAS or OAP treatments for *Spartina alterniflora* (Fig. 2.8 b). The OAP treatment, when applied with the OAS treatment, did not impact the live and total biomass and density more than the OAS treatment along (Fig. 2.8).

The aboveground biomass of *Spartina patens* was detrimentally affected by the application of south Louisiana crude oil. Application of the oil to the soil significantly ($p < 0.0001$) decreased live and total aboveground biomass (Fig. 2.9 a), and the OAP treatment significantly ($p < 0.001$) reduced total aboveground biomass (Fig. 2.9 b). Dead aboveground biomass was not significantly affected by either OAS or OAP treatments. Live and total stem densities of *Spartina patens* significantly ($p<0.0001$) decreased and dead stem density significantly ($p < 0.0001$) increased with application of the oil to the soil (Fig. 2.10 a). Furthermore, live stem density of *Spartina patens* was significantly ($p < 0.005$) decreased and dead stem density was significantly ($p < 0.005$) increased by the OAP treatment for *Spartina patens* (Fig. 2.10 b). Means were averaged over the OAP and OAS treatment (no significant interaction between OAP and OAS treatments, n=10, Fig. 2.9 and 2.10)

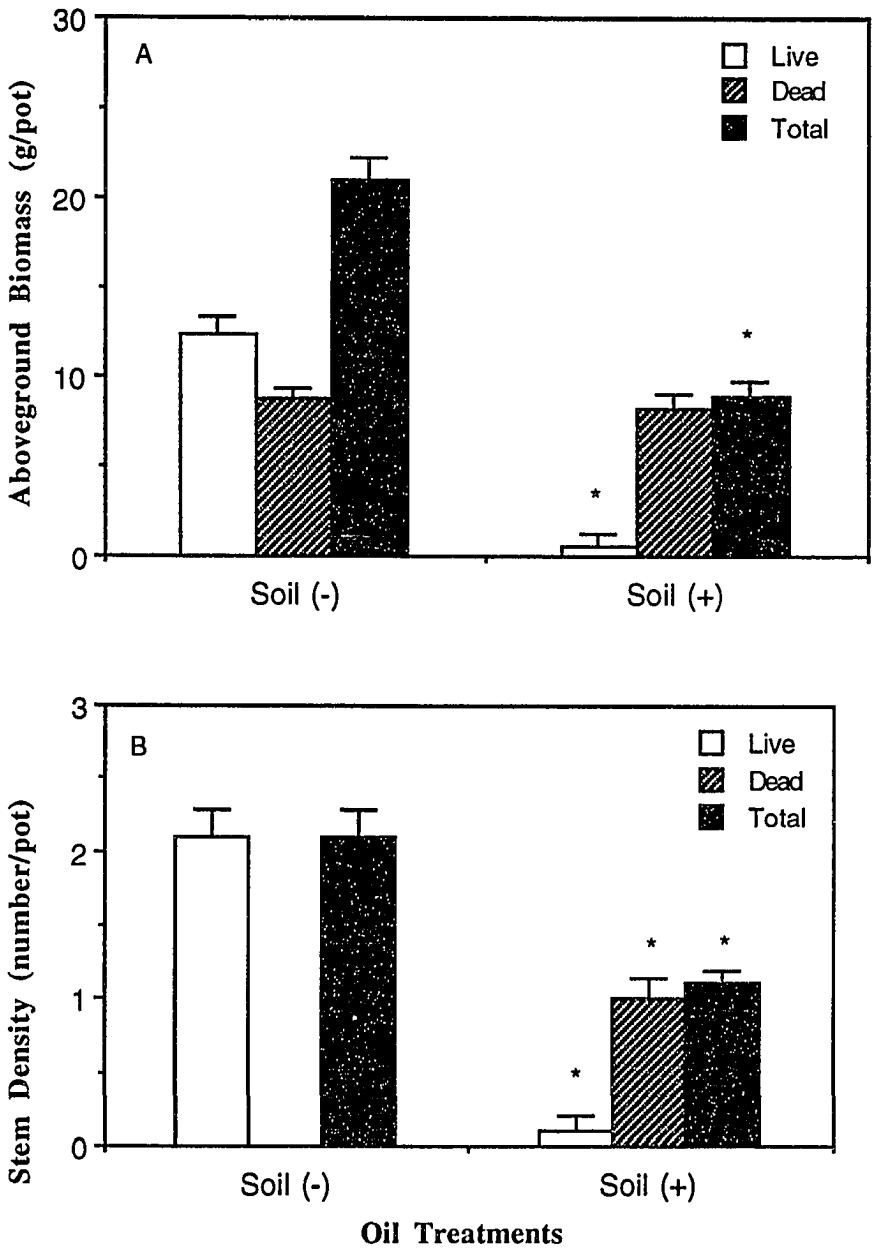

Fig. 2.7 Aboveground biomass (A) and stem density (B) of *S. lancifolia* 2.5 months after application of the oil to the soil (n=10). * indicates significant differences within plant components.

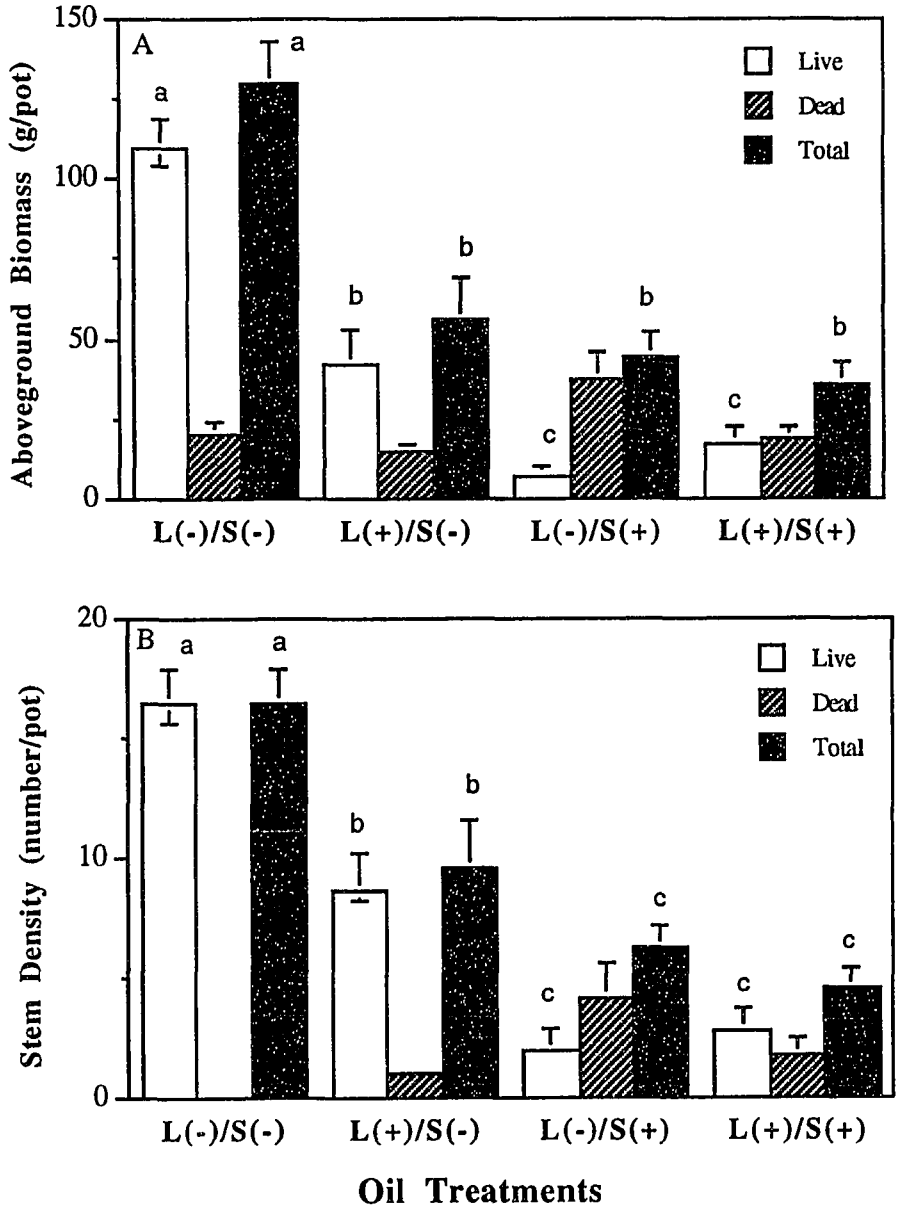

Fig. 2.8 Aboveground biomass (A) and stem density (B) of *S. alterniflora* 2.5 months after application of the oil (n=5). Different letters indicates a significant difference within plant component.

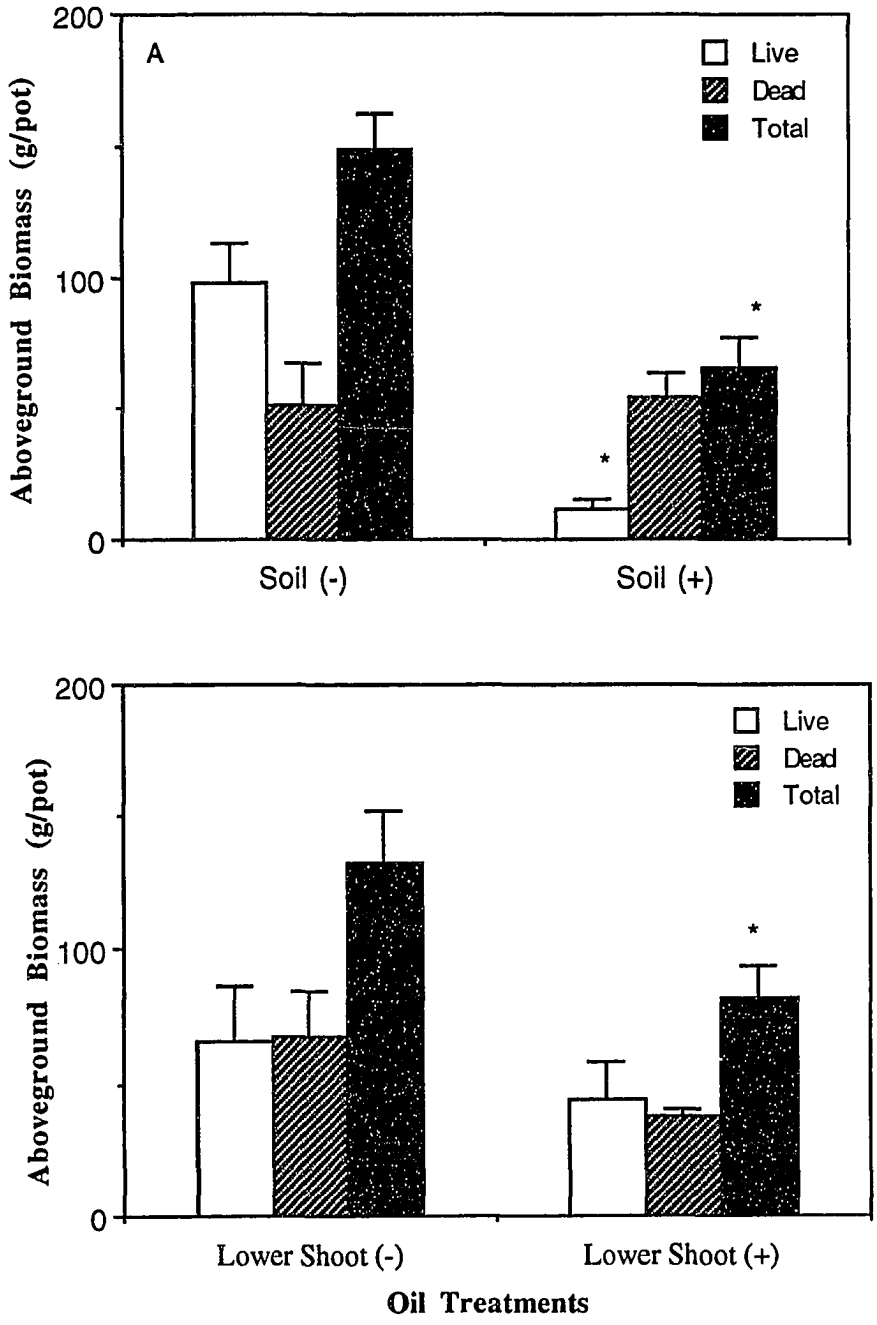

Fig. 2.9 Effects of the oil applied to the soil (A) and to lower aerial portion (B) on aboveground biomass of *S. patens* 2.5 months after application of the oil (n=10). * indicates a significant difference within biomass component.

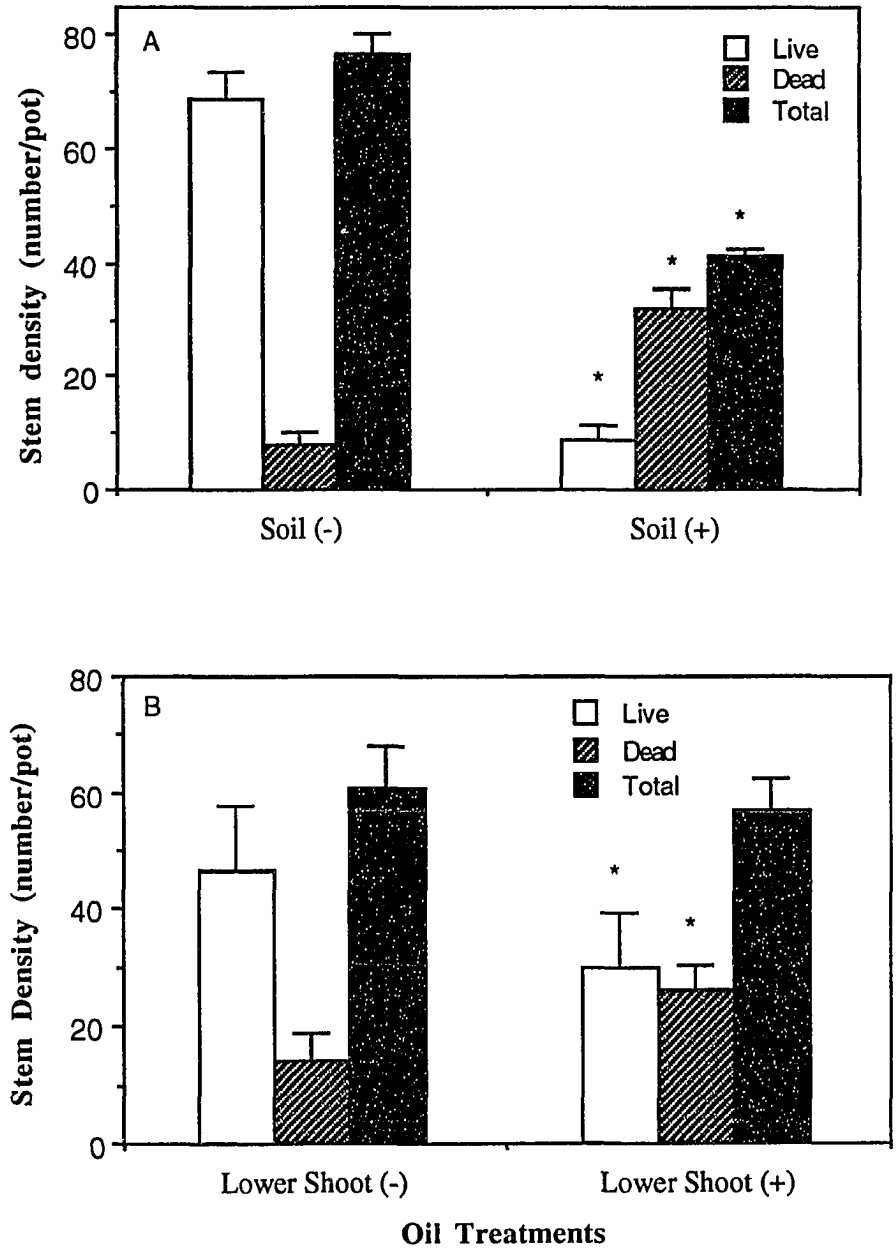

Fig. 2.10 Effects of the oil applied to the soil (A) and to lower aerial portion (B) on stem density of *S. patens* 2.5 months after application of the oil (n=10).
* indicates a significant difference within stem density component.

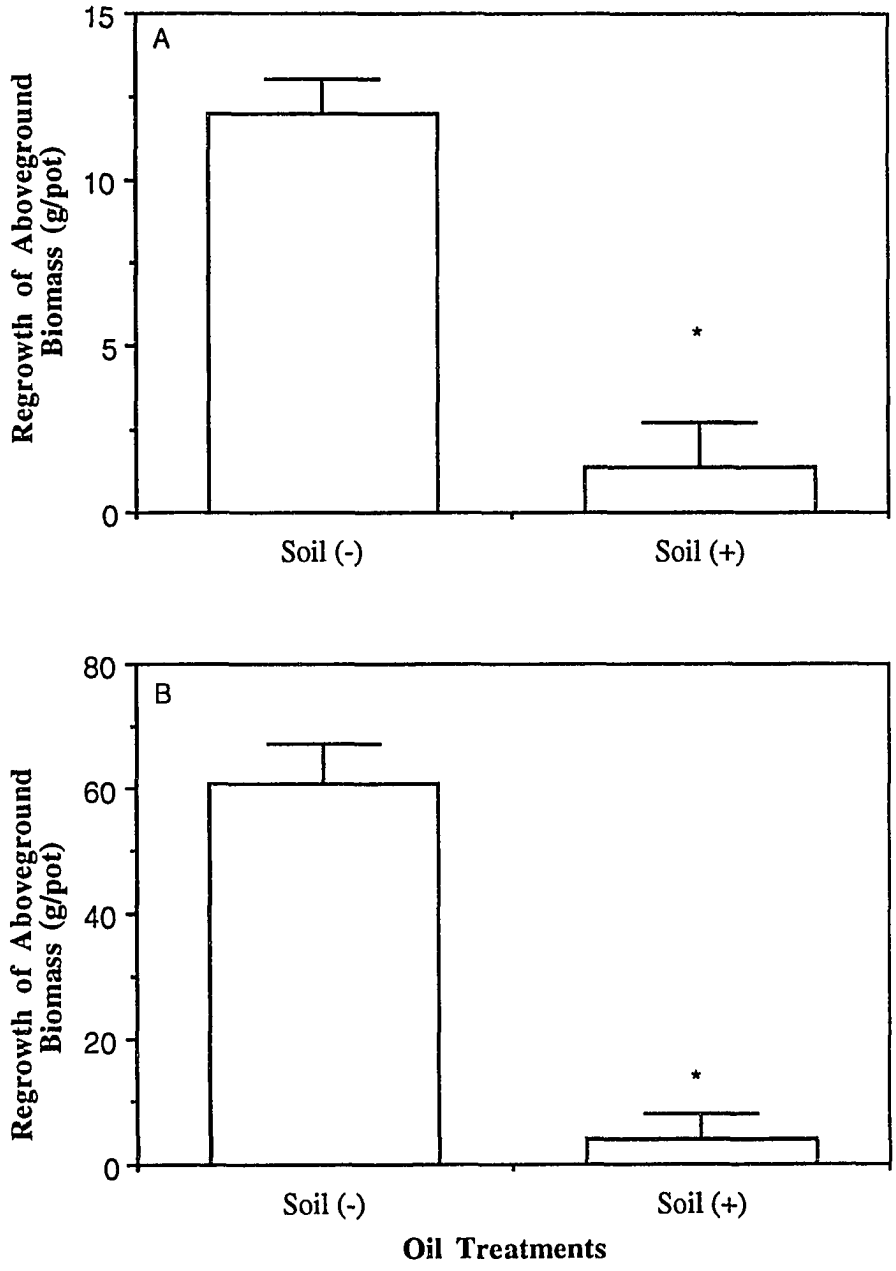

Fig. 2.11 Regrowth biomass of *S. lancifolia* (A) and *S. alterniflora* (B) one year after the oil application (n=10). * indicates significant difference from the control (no oil).

Regrowth the year following the oil application

Regrowth of biomass one year after the oil application was measured to determine the long-term effect of the oil on plant growth response. Application of the oil to the soil killed substantial proportion of plants; nine out of ten experimental units of *Spartina alterniflora* and *Sagittaria lancifolia* were killed by oiling the soil. Regrowth of aboveground biomass was significantly suppressed by the oil applied to the soil, with only 11% and 7% of total biomass of the reference (no oil in the soil) for *Sagittaria lancifolia* and *Spartina alterniflora*, respectively (Fig. 2.11). The OAP treatment, however, did not significantly affect the aboveground biomass regrowth for *Sagittaria lancifolia* and *Spartina alterniflora*. Unfortunately, no regrowth of *S. patens* occurred in either the oil treatment or the control due to unknown reasons.

The Influence of the Soil Composition on Responses of Plants to Oiling

Photosynthetic rates

The composition of soil substrate greatly affected the oil impact on the vegetation. In the mineral/organic soil substrate, the impact of the oil was less severe than that in the organic soil. The photosynthetic rates were less affected in the mineral/organic soil substrate than in the organic soil, although the oil significantly decreased the photosynthetic rates of *S. alterniflora* in both substrates (Fig. 2.12). The photosynthetic rate of *S. alterniflora* in the mineral/organic soil was 78 % of the control compared to only 20 % of the control in the organic soil 50 days after oil application (Fig. 2.12). The photosynthetic rate of *Sagittaria lancifolia* was completely suppressed by oiling of the organic soil compared to about 50% of the photosynthetic rate of the control in the mineral/organic soil 50 days after oil application (Fig. 2.12). The photosynthetic rates of *S. patens* in two soil substrates were not significant difference, both at about 60% of their respective controls (Fig. 2.12)

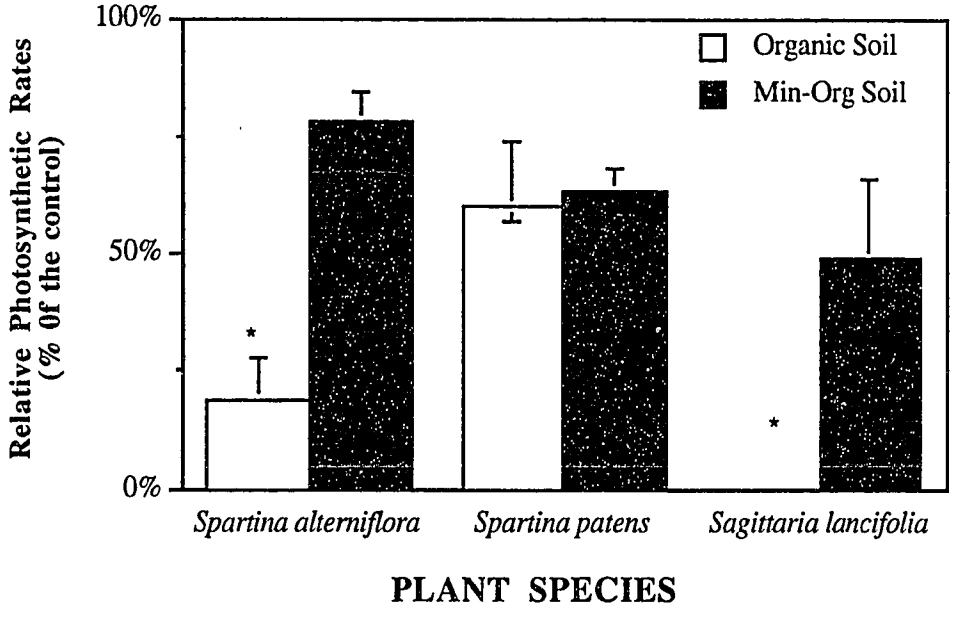

Fig. 2.12 Influence of soil composition on the effect of the oil on photosynthetic rates 50 days after oil application (n=4). Photosynthetic rates were 17.7 *u*mol m^{-2} s^{-1} for *Spartina alterniflora*, 17.1 *u*mol m^{-2} s^{-1} for *Spartina patens*, and 10.5 *u*mol m^{-2} s^{-1} for *Sagittaria lancifolia.* * indicates a significant difference within plant species.

Aboveground biomass

Impact of application of the oil to the soil on biomass production was also greatly influenced by the soil substrate composition. The effect of the oil on aboveground biomass was much more severe for all three species in the organic soil than in the mineral/organic soil (Fig. 2.13). The live aboveground biomass production in the organic soil was much lower (0-20% of the controls) compared to the mineral/organic soil (50-107% of the controls) for the three marsh plants (Fig. 2.13 A). Similarly, the total aboveground biomass was much lower in the organic soil, with 35% - 45% of the controls compared to 60% - 110% of the controls for the mineral/organic soil (Fig. 2.13 B).

The regrowth of biomass indicates the long-term effect of oil on the plants. The small amount of regrowth in the organic soil substrate one year after oiling demonstrated the intense stress of this treatment on the vegetation (Fig. 2.14). However, the 37% - 80% regrowth biomass in the mineral/organic soil (Fig. 2.14) suggested a less severe oil impact on regrowth compared with that in the organic soil. Application of the oil did not significantly affect the regrowth of biomass of *Sagittaria lancifolia* in the mineral/organic soil compared to its control.

Oil concentration in the soil

It was observed that the oil applied was incorporated almost immediately into the organic soil compared to 1-3 weeks for incorporation into the mineral/organic soil. The oil concentration in these two soil substrates, however, was not significantly different although oil in the organic soil tended to be higher (Table 2.1).

DISCUSSION

The present study showed that oil applied to the lower aerial portions of these plant species caused less impact on the plants than oil applied to the soil. Photosynthesis, leaf elongation, aboveground biomass and regrowth of *Sagittaria lancifolia* were not adversely affected by oiling the lower aerial portion of the plant.

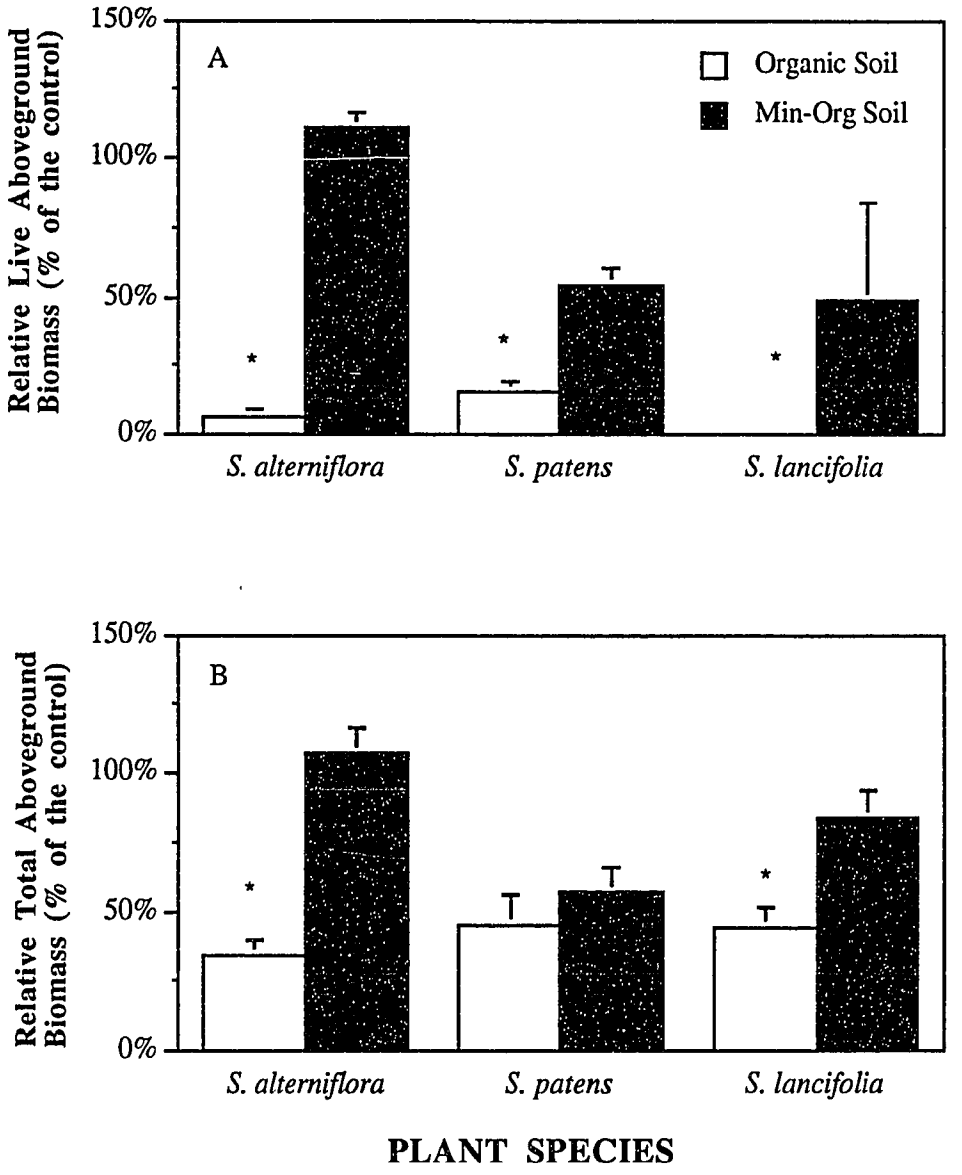

Fig. 2.13 Influence of soil composition on the effect of the oil on relative live (A) and total (B) aboveground biomass after oil application (n=4). * indicates a significant difference within plant species.

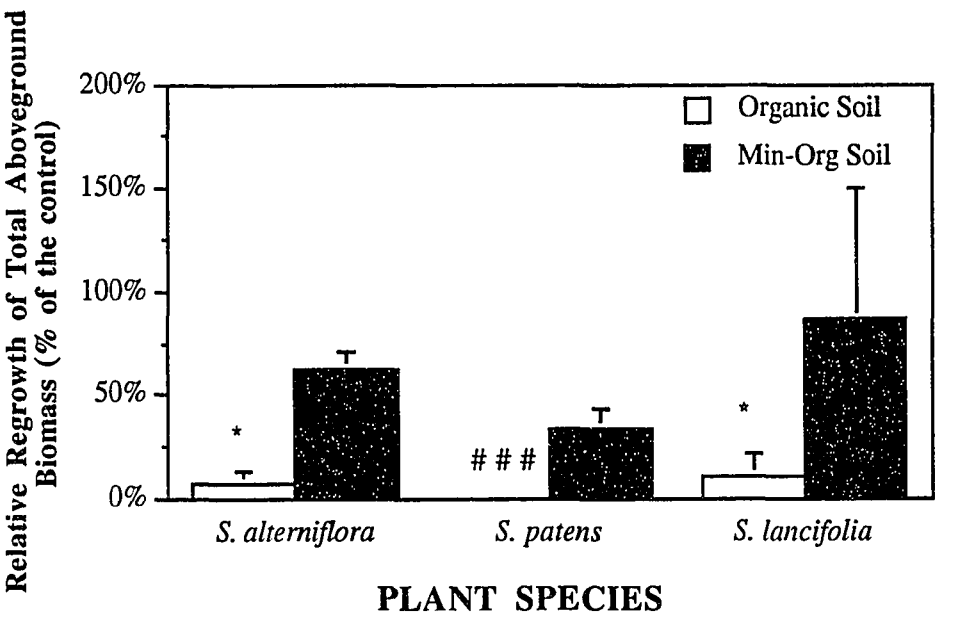

Fig. 2.14 Influence of soil composition on the effect of the oil on regrowth of total aboveground biomass one year after the oil application (n=4). * indicates a significant difference within plant species. ### For unknown reasons, *Spartina patens* did not regrow on either control or oil treated pots after the initial harvest.

Table 2.1 Oil Concentration (mg g^{-1} dry soil) in the soil 2.5 months after oil application. Values are means and standard errors in parentheses.

Soil layer	Organic soil	Mineral/organic soil
Top 5 cm	122.5 (19.3)	101.5 (16.4)
Bottom 5 cm	73.7 (11.1)	49.5 (10.3)

For this species, oil applied to the lower aerial portion, in fact, did not coat the leaf blade since the leaf blade of *Sagittaria lancifolia* is supported by a long, erect petiole (> 30 cm). Thus, the primary photosynthetic surface of this species was not coated with oil, and growth and biomass were not affected.

The effect of oil coverage on the plants was species-specific. Unlike *Sagittaria lancifolia,* oiling the lower aerial plant portions detrimentally affected live and total aboveground biomass of the two *Spartina* species 2.5 months after oiling. However, the photosynthetic rates of unoiled leaves were unaffected by oiling the lower aerial portions of these plants. Furthermore, in this experiment, the OAP treatment actually coated the lower located leaf blades and sheaths, and thus, the photosynthetic rates of these lower located leaves were completely inhibited for the two *Spartina* species (data not shown). The lower leaves of *Spartina* coated by the oil changed color from green to yellow and died within 2-4 weeks, thus, the dead biomass of the two *Spartina* species exposed to the OAP treatment were greater than that of the control. Therefore, the total leaf area for active photosynthesis was less in the OAP treatment compared to the no oil control. This reduced the total accumulation of photosynthate, and, hence, live and total aboveground biomass of the OAP treatment were lower than for the control for the two *Spartina* species.

A number of studies have reported the short-term effect of oil coating on plants (Ferrell *et al.*, 1984; Smith *et al.*, 1981; Webb *et al.*, 1981; Mendelssohn *et al.*, 1990). Coating the entire leaf of *S. alterniflora* with oil resulted in cessation of photosynthesis (Pezeshki & Delaune, 1993) and killed the aboveground tissue (Webb *et al.*, 1981). Also, oiling the shoot reduced the stem density and aboveground biomass of *S. alterniflora* (Ferrell *et al.*, 1984). A 64% reduction in live vegetation cover of *S. patens* occurred three months after an oil spill in a southeast Louisiana marsh when ca. 0.28 L m^{-2} of crude oil coated the aboveground vegetation (Mendelssohn *et al.*, 1990).

Oil coverage on the aerial portions of the plant, however, appears to have no long-term effect. The regrowth of both *Spartina alterniflora* and *Sagittaria lancifolia* in this experiment was not significantly affected by the OAP treatment. A number of studies have shown that plants can recover from tissue oil coating. Baker (1973) indicated that recovery of salt marsh vegetation from up to four oil sprays was generally good. Alexander and Webb (1985) showed that live biomass of *Spartina alterniflora* was not reduced 5 and 12 months after spraying crude oil on the lower part of the plant. Field data of a *No.* 6 fuel oil spill near Bolivar Peninsula in Texas showed that *Spartina alterniflora*, regardless of the extent of oil coverage, produced new growth in the following spring that appeared similar to other *Spartina alterniflora* communities of the area (Webb *et al.*, 1981).

In contrast to the short-term effect of oil coating, the incorporation of crude oil into the soil had more severe long-term impacts. Photosynthesis, leaf elongation, stem density, biomass and regrowth the following year were considerably impaired by addition of oil to the soil in the present study. Ferrell *et al.* (1984) reported that incorporation of oil into the soil significantly reduced stem density and aboveground biomass, and increased the mortality of *S. alterniflora* 30 and 60 days after exposure to oil in a greenhouse experiment; regrowth of stem density and aboveground biomass was also significantly impacted by the oil in the soil. A crude oil spill in Dickinson Bayou (Galveston Bay, Texas) indicated that the growth of *S. alterniflora* was significantly reduced in sediments with high oil content (5-51 mg g^{-1}) after 18 months (Alexander & Webb, 1987). Krebs & Tanner (1981) found that 5-17 mg g^{-1} of *No.* 6 fuel oil in the soil reduced stem height, stem density and biomass through the first growing season, and the highest oil concentration inhibited the aerial regrowth by killing roots and rhizomes of *Spartina alterniflora.* Heavy oiling inhibited the regrowth of *S. alterniflora* (Thomas, 1973; DeLaune *et al.*, 1979). The impact of oil on vegetation in all these studies seemed to be related to a high concentration of oil

incorporated into the soil. In the present study, a considerable amount of oil was sorbed into the soil substrate. Furthermore, the oil added was incorporated into the soil substrate very quickly, therefore, the more volatile oil components entered the substrate before they could evaporate. Fresh oil is more toxic to marsh vegetation than weathered oil (Cowell, 1969; Baker, 1971c), therefore, sufficient fresh oil could have incorporated into the soil and substantially impacted all three plant species in the present study.

Sensitivity of a plant to fresh oil in the soil appears somewhat related to its transpiration rate. In the present study, *Sagittaria lancifolia* exhibited the highest transpiration rate with 12,500 umol m^{-2} s^{-1}, compared with the two *Spartina* species with transpiration rates between 7,000-8,500 umol m^{-2} s^{-1}. The leaves of young beans and peas that were grown in oil-treated sand had a higher oil content than plants which were grown in non-oiled soil (Knight *et al.*, 1929), possibly due to oil absorbed by the roots and moving upwards through the plant via the transpiration stream (Baker, 1970). In the present study, *Sagittaria lancifolia* was affected by oil addition to the organic soil within one to two weeks, with the youngest leaves losing turgor and developing necrotic spots on the leaves and dying. The effect of the oil then spread to the older leaves. I assumed that the oil components, at least the water-soluble hydrocarbons, entered the plants through the roots and moved upward with the transpiration stream to the leaves. The higher the transpiration rate, the more oil a plant could accumulate. Oil may also accumulate more in the roots when transpiration rate is higher, and affect the function of the roots. The roots of *Sagittaria lancifolia* in the OAS treatment were dark in color, not turgid, and less branched compared to the roots of the control (white color, turgid and more branched), suggesting that the oil damaged the roots and may have affected root function. The assumption that species with high transpiration rates may be more sensitive to oil, however, needs further study.

Soil composition may also influence the degree of oil incorporation into the soil, and thus the intensity of impact on the vegetation. Most petroleum chemicals are nonionic and therefore associate more readily with organic than with mineral particles in soils (Testa & Winegardner, 1991). In addition, sediment characteristics, including particle size distribution and drainage efficiency, considerably affect oil penetration (Getter *et al.*, 1984). Sands (large particles) tend to be more freely draining and more easily penetrated by oil than fine-textured, waterlogged mud. The regrowth potential of *S. alterniflora* grown in oiled substrata was greater in a fine-textured marsh soil than in a sand substrate (Ferrell *et al.*, 1984). A fine-textured clay substrate helped prevent oil from penetrating the sediment, thus minimizing the oil's toxic effect on the marsh plant rootstock (Hoff *et al.*, 1993).

In the present study, all three plants were affected more severely by the oil in the organic soil than in the mineral/organic soil. The differential responses of the plants in these two substrates were most likely due to the amount of soil organic matter and the soil texture. The organic soil had a soil organic matter content of 50% compared with 32.7% in the mixed mineral/organic soil. Furthermore, the texture of the potting soil was much coarser, with 1-3 mm diameter vermiculite particles compared with the fine deltaic sediment (< 0.05 mm) in the mixed mineral/organic soil. The oil applied to the organic soil penetrated and was sorbed almost immediately compared to the mixed mineral/organic soil, where 1-3 weeks were necessary for complete absorption. Thus, more unweathered, volatile hydrocarbons could have incorporated into the organic soil than into the mineral/organic soil before these hydrocarbon components evaporated from the surface. Unweathered oil, highly refined petroleum products and volatile oil components are more toxic to plants (Cowell, 1969; Baker, 1971c). In a comparison of two spills of the same type of oil in England, Cowell (1969) found that the damage to marsh vegetation was more severe from oil that reached the marsh within minutes of the spill than from oil that was at sea for 8 days. The evaporation of volatile hydrocarbons

during the weathering process resulted in a decreased toxicity of weathered oil since the volatile components are most toxic to plants (Baker, 1971c; Boesch *et al.*, 1974). In the present study, the oil applied to the mixed mineral/organic substrate floated on the water surface longer than that on the organic soil, and the more volatile oil components could have evaporated before they were incorporated into the soil.

Sagittaria lancifolia showed no resistance to the oil applied to the organic soil. However, in the mineral/organic substrate with relatively lower organic matter and finer texture (compared to the organic soil), *Sagittaria lancifolia* showed greater oil resistance with no significant difference in regrowth biomass between the oil and no-oil treatments. Furthermore, *Sagittaria lancifolia* showed the greatest resistance to the oil in natural soil sods, with an enhancement of biomass by oil levels up to 24 l m^{-2} (Table 3, Chapter 1). The differential response of this species to oil was most likely because the oil was incorporated into the fine-textured natural marsh soil more slowly, thus allowed the more toxic, volatile oil components to evaporate before being sorbed. Oil floating on the marsh surface had little impact on this species. Therefore, the overall resistance of *Sagittaria lancifolia* to oil is high. However, the two *Spartina* species were sensitive not only to oil in the soil, but also to floating oil (Ferrell *et al.*, 1984; as well as observed in Chapter 1), suggesting that the oil impact on the *Spartina* species can result from both oil in the soil and oil floating on the marsh surface and coming in contact with aboveground tissues

SUMMARY

The effect of oil coverage on plants is species-specific. *Sagittaria lancifolia* was relatively resistant to oil coverage, and showed no detectable effects of oil coverage on their aerial portions. The OAP treatment did not affect photosynthesis and leaf elongation of any of the three marsh species. The two *Spartina* species exhibited some sensitivity to oil contact with their aerial portions with decreased live and total biomass

production and stem density. However, oil coverage did not cause long-term damage in any of the species studied, with regrowth after oil coverage similar to that of the control.

In contrast, oil incorporation into the substrate caused both short- and long-term severe damage to the plant species with a large reduction in photosynthetic rate, biomass, stem density and regrowth biomass. The suppression of the regrowth most likely resulted from the initial impact of the oil in killing the rhizomes that initiate vegetative growth.

The soil substrate composition greatly influenced the impact of the oil on the vegetation. The effects of the oil on the *Sagittaria* and *Spartina* species growing in the coarse-texture organic soil were much more severe than those in the fine-texture mineral/organic soil. Photosynthetic rates, aboveground biomass and regrowth biomass for the marsh plants in the organic soil were much more reduced compared to those in the mineral/organic soil. This suggests that the effect of oil application to the high organic matter, coarse-textured soil was more severe for all three marsh plant species.

CHAPTER 3

THE INFLUENCES OF SEASON, SUNLIGHT AND LEAF SURFACE STRUCTURE ON THE RESPONSE OF PLANTS TO OIL IMPACT

INTRODUCTION

A number of factors may influence the impact of oil on vegetation. In addition to the type and amount of oil, soil composition and plant species (Baker, 1971c; Burk, 1977; Alexander & Webb, 1987; Mendelssohn *et al.*, 1990), the season and weather conditions during a spill may also influence the effect of oil on plants (Overbeek & Blondeau, 1953; Ranwell & Hewett, 1964; Baker, 1971b; Prendeville & Warren, 1977; Alexander & Webb, 1985).

The weather conditions, such as sunlight, may modulate plant injury from oil. Liquids with very low surface tension, such as low viscosity oils, can penetrate pores of the size of stomata (Fogg, 1948). Oil, which comes in contact with plant leaves when the stomata are open in the light, may enter the leaf and cause internal injury. However oil exposure during the night, when the stomata are closed, may not be able to reach internal tissue (Overbeek & Blondeau, 1953; Prendeville & Warren, 1977). Although cuticle penetration (interstomatal) of the epidermis is possible in some leaves, in plants with a tough continuous waxy cuticle, stomatal penetration is probably the main mechanism of oil access into the leaf (Overbeek & Blondeau, 1953).

The season of the year may also, in part, influence the sensitivity of plants to oil spills, e.g., vegetation may be severely impacted by an oil spill during the growing season (spring and summer), but exhibit little damage when plants are in senescence (fall and winter) (Ranwell & Hewett, 1964; Baker, 1971b; Getter *et al.*, 1984; Alexander & Webb, 1985; Scholten & Leendertse, 1991). However, no studies have successfully tested this hypothesis.

The objectives of this study were to determine (1) If plant leaves were injured more severely when oil was applied to leaves under sunny conditions than under shade conditions, (2) If leaf surface structure played an important role in leaf injury by oil coating, and (3) If plants were more sensitive to oil applied to the soil in summer than in fall.

MATERIALS AND METHODS

Factors Controlling Plant Response to Oil Coverage of Leaves

A number of questions were addressed in the experiments of leaf oil coverage. Does differential recovery from oil coverage occur among plant species? Does leaf surface structure lead to differences in responses to oil coverage? Under what conditions, sun or shade, would a plant recover better? To answer these questions, experiments were designed as follow:

Influence of sunlight on response of plants to oil coverage

To determine if there is differential recovery from oil coverage among plant species and under the light or shade, three dominant marsh species (*Spartina patens*, *S. alterniflora* and *Sagittaria lancifolia*) were treated with south Louisiana crude oil. The experimental design was completely randomized with a 2 x 2 factorial arrangement of treatments: (1) light conditions - shade (10% of full sunlight) or full sunlight light, and (2) leaf oiling - present or absent, with 4 replicates per treatment-level combination. Young but fully expanded leaves of each species were selected. One half of each leaf (left or right of the mid-vein for *S. lancifolia* and lower or upper half of the leaf blade for *S. alterniflora* and *S. patens*) was coated with south Louisiana crude oil in either the light or the shade. The unoiled half of the leaf served as the control. Measurements of photosynthetic rates were taken one, two and four weeks after oiling to assess the oil injury and leaf recovery from oiling, based on percent of the control. The advantage of using one half of the leaf for the oil treatment and the other half for the control was to insure the same physiological age for the two treatments.

Influence of leaf surface structure on the response of leaves to oil coverage

To aid in determining the causes for differential injury from oil coverage to the leaves of these three plant species, the leaf surface structures were examined under a dissecting microscope, and the possible relationship of the sensitivity of a leaf to oil and leaf surface structure was described. Due to differences in leaf surface structure on the adaxial and abaxial surface for *Spartina* species, an experiment was designed to determine whether the abaxial or adaxial leaf surface structure played an important role in the sensitivity of plant leaf to oil coverage. The experimental design was completely randomized with a 2 X 2 factorial arrangement of treatments: (1) surface of oiling - abaxial or adaxial and (2) oiling level - present or absent. Treatment-level combinations were replicated 4 times. The leaves of the *S. alterniflora* and *S. lancifolia* were coated with oil by using a cotton swab on the adaxial side alone, abaxial side alone, and both abaxial and adaxial sides, to compare with an uncoated leaf.

Photosynthetic and transpiration rates (detailed in Chapter 1) were measured 0.5, 1.5 and 5 weeks following the oil application to determine how the different leaf surfaces structure may relate to the sensitivity of the leaf to oil coverage.

Plant leaf chlorophyll content was measured 5 weeks after oiling with a dimethyl sulphoxide (DMSO) extraction (Hiscox & Israelstam, 1979). Fifty milligrams of fresh leaf tissue (cut in small fractions) were placed in a vial containing 5 ml DMSO, and chlorophyll was extracted at 65 °C for 40 minutes. An aliquot of the chlorophyll extract was transferred to a cuvette, and the OD values at 645 and 663 nm were read on a spectrophotometer against an DMSO blank. Chlorophyll content was calculated by the equation of Arnon (1949).

Influence of Season on the Response of Plants to Oil

To determine the influence of season on the response of plants to oil applied directly to the soil, marsh plants were transplanted into an organic soil substrate (Jiffy-

Mix, by Jiffy Products of America, Inc., West Chicago, Illinois) and allowed to grow for a 6-week period prior to oil exposure. Weekly applications of 150 ml of full strength Hoagland's nutrient solution were made to each pot after transplantation and during the experiment. The reason for using the organic soil as the substrate was that oil can readily penetrate and be sorbed by this substrate due to its porous nature and coarse texture. The experimental design was a completely randomized design with 2 x 2 factorial arrangement of treatments: (1) season - oil applied in early June and in late October (2) oil level - 0 l m^{-2} and 8 l m^{-2}. Treatment-level combinations were replicated 4 times. The experiment was conducted simultaneously for *Spartina* alterniflora and *Sagittaria lancifolia.*

Photosynthetic rates of plant leaves were measured 1 month after oil application. Aboveground biomass was harvested 2.5 months after oiling. Regrowth of aboveground biomass one year after oil application was analyzed to assess the long-term effect of oil on the regeneration potential of plants. Methods were detailed in Chapter 1.

Statistical Analysis

Statistical Analysis was conducted with the Statistical Analysis System (SAS Institute, 1985). Photosynthetic responses were expressed as percent of the control. Photosynthetic rates and biomass were analyzed by analysis of variance with time as a repeated measurement (n=4). Duncan's multiple range test was used to evaluate statistical differences among treatment-level combinations. Significant differences were reported at the 0.05 probability level unless otherwise stated.

RESULTS AND DISCUSSION

Effect of Plant Species and Sunlight on the Impact of Oil Coverage to Leaves

The sensitivity of plant leaves to oil coverage depended upon the plant species. Photosynthesis in the *Spartina* species was significantly inhibited by oil coating the

leaves (Fig. 3.1 a). No net photosynthesis was detected in either *S. alterniflora* or *S. patens* one to four weeks after oiling, and leaf transpiration rates in both *S. alterniflora* and *S. patens* were significantly reduced to between 5-10% of the controls by oiling (Fig. 3.1 b). *Sagittaria lancifolia* showed less impact from oil exposure than the *Spartina* species. However, the extent of recovery was dependent on light condition (significant interaction with oil application), with plant photosynthetic and transpiration rates affected less with shade exposure compared to light exposure for *Sagittaria lancifolia* (Fig. 3.2 a & b). Light had no significant effect on the response of the *Spartina* species to leaf oiling; the photosynthetic and transpiration rates of the two *Spartina* species were greatly reduced by oil in both the light and shade (Figs 3.1 a & b)

A number of studies have shown that the *Spartina* species are sensitive to oil coverage. Webb *et al.* (1981) observed that oil killed the aboveground portion of *S. alterniflora* when oil covered most of the plant. Ferrell *et al.* (1984) reported that the presence of an oil layer on the water surface completely inhibited regrowth of *S. alterniflora*. Baker (1971d) indicated that a thin oil film of refinery discharge which became stranded on *Spartina anglica* during high spring tides resulted in plant mortality. In contrast, *Sagittaria* species have exhibited relative resistance to oil. Leck & Simpson (1992) indicated that recruitment from the seed bank of *Sagittaria lantifolia* was enhanced after an oil spill.

Under sun conditions, generally the stomata of leaves are open. It was observed in the present study that the oil applied to a leaf surface of *Sagittaria lancifolia* in the sunlight penetrated through open stomata into the leaf immediately, and led to a darkening of the leaf color due to the oil. However, oiling in the shade showed no obvious oil penetration into the leaf for *Sagittaria lancifolia*. This could be the main reason that *S. lancifolia* leaves exposed to sunlight and oil were injured more severely than leaves oiled in the shade. Currier (1951) showed that in bright sunlight, benzene and methylated benzene entered the plant rapidly through the stomata and destroyed

chlorophyll. Prendeville & Warren (1977) reported that light was necessary for the oily herbicides, paraquat and oxyfluorfen, to increase cell membrane permeability, resulting in injury symptoms.

The differential response of the two *Spartina* species and *Sagittaria lancifolia* was most likely due to differences in leaf surface structure. Crude oil accumulated to a greater extent in the furrows on the adaxial surface of *Spartina* leaf than on its abaxial one (observation with dissecting microscope). Three weeks after oiling, the oil layer in the adaxial furrows was still thick and became more viscous due to evaporation of the lighter oil components. However, the oil coating of the abaxial and adaxial surfaces of *Sagittaria lancifolia* leaves was much thinner than that on the adaxial surface of *Spartina* leaves. One week after oiling, there was almost no liquid oil that could be detected on the leaves of *Sagittaria lancifolia* even though the oil was applied to completely cover the leaf surfaces of all three species.

For *Sagittaria lancifolia*, the effect on the transpiration rates was less than on photosynthetic rates, with transpiration rates of 65% and 100 % of the controls two weeks after oiling under sunlight and shaded conditions, respectively (Fig. 3.2 a & b). During the same time, however, photosynthesis of *S. lancifolia* was only to 35 % and 70% of the controls under the sunlight and the shade, respectively (Fig. 3.2 a). Both transpiration and photosynthesis share the same pathway, the stomata, for exchanging water vapor and carbon dioxide, respectively, with the atmosphere. Decreased transpiration at the early oiling period suggested at least, in part, physical blocking the stomata. The greater impact on photosynthesis, which is a synthetic process catalyzed by enzymes, suggested that some of the oil impact was a direct toxic effect of the oil. Oil was able to penetrate into the stomatal chamber through the open stomata when a leaf was exposed to the sunlight which allowed the oil to injure the mesophyll cells on the exposed surface in the stomatal chamber. In contrast, the two *Spartina* species showed no difference in impact to transpiration and photosynthesis. Impact to these species was

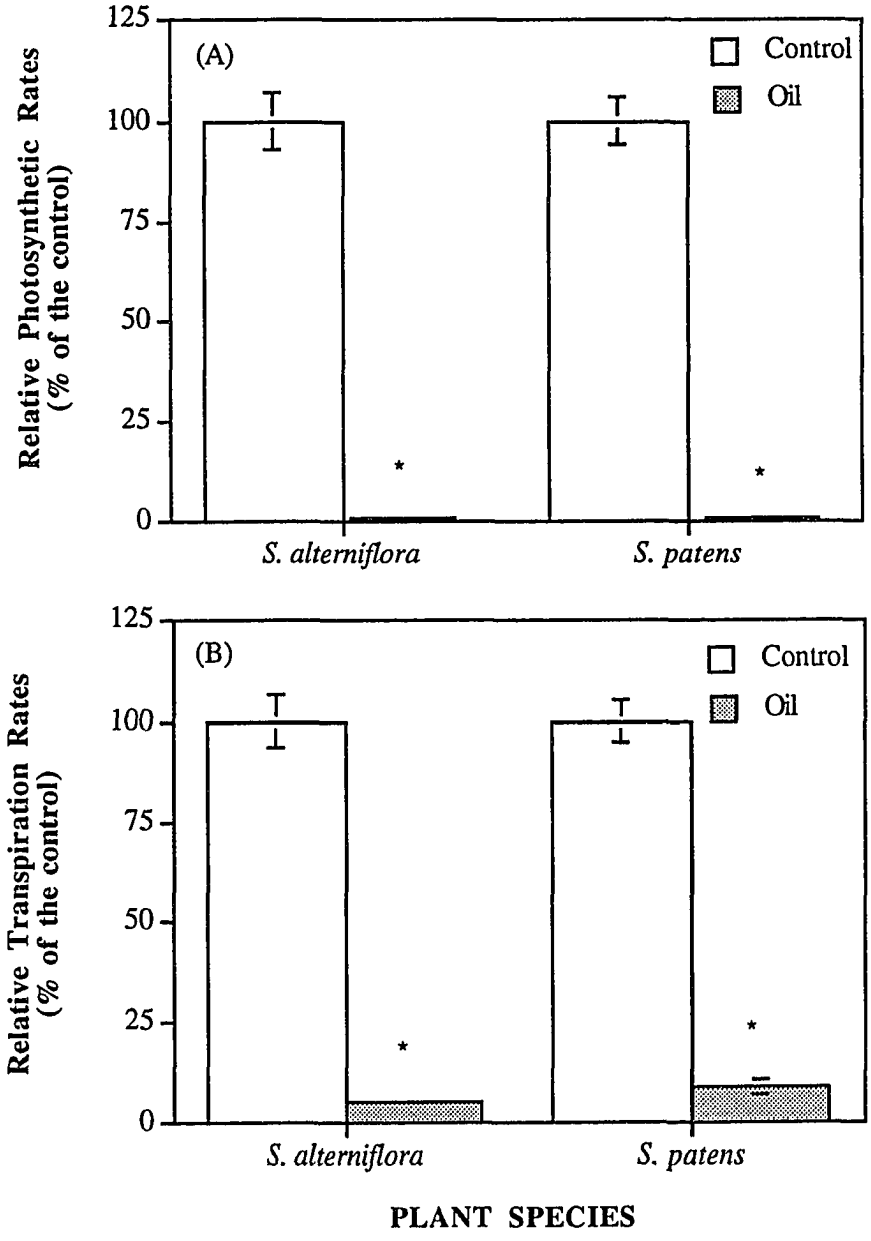

Fig. 3.1 Effects of the oil coverage on photosynthetic rates (A) and transpiration rates (B) of *Spartina alterniflora* and *Spartina patens*. Data were averaged over time (1, 2 and 4 weeks) and the sunlight treatment (n=24) (no significant interaction between sunlight and oil or with time). * indicates significantly difference within species.

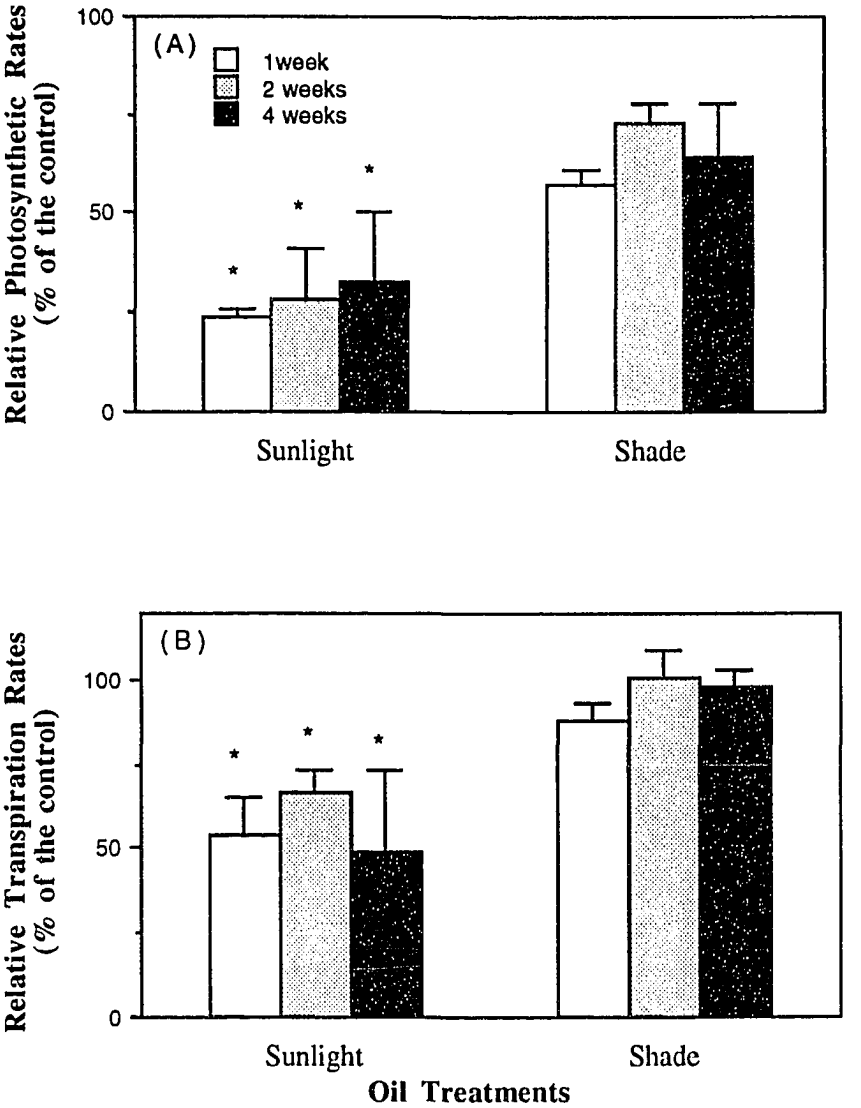

Fig. 3.2 Influence of sunlight on responses of photosynthetic rates (A) and transpiration rates (B) of *Sagittaria lancifolia* to the oil coverage (n=4). The mean photosynthetic rate was 9.1 μmol m^{-2} s^{-1} for the control
* indicates significantly difference within the time measurement.

at least partially due to a thick oil layer that accumulated in the adaxial furrows and physically blocked the air exchange pathway. In addition, the internal mesophyll cells were also been affected since no photosynthesis was detected after the oil application to the leaves, and the oiled leaves died eventually.

Influence of Leaf Surface Structure on Plant Injury Oil Coverage of Leaves

To determine the relationship of leaf surface structures and their oil resistance, the leaves of three species were first observed under a dissecting microscope and secondly treated with oil on a surface specific basis. Both adaxial and abaxial leaf surfaces of *Sagittaria lancifolia* were smooth and covered by a waxy cuticle (ca. one quarter the thickness of an epidermis cell). However, for the two *Spartina* species, although the abaxial leaf surface was smooth and covered with an even thicker cuticle (ca. one half of the thickness of an epidermis cell), the adaxial side of the leaf contained many deep furrows parallel to the main vein throughout the leaf. These furrows are as deep as one-half the thickness of the *S. alterniflora* leaf and two-thirds the thickness of the *S. patens* leaf. These adaxial furrows do not have an apparent cuticle.

The effect of the oil was completely different depending on the leaf surface structure. Photosynthetic rates were not significantly affected when the abaxial leaf surface of *S. alterniflora* was exposed to oil, but when the adaxial surface was exposed, photosynthesis was completely inhibited (Fig. 3.3a).

This results clearly demonstrate that the adaxial surface of the leaf plays a key role in the extreme sensitivity of the *S. alterniflora* leaf to oil. Many deep furrows were found on the adaxial compared with the abaxial leaf surface, the latter being smooth and covered with a thick cuticle. Oil accumulated within the adaxial furrows and resulted in a thick oil coating. The chlorophyll of the adaxial surface cell layers deteriorated and disintegrated three weeks after oiling, as determined from microscopic examination, and thereby, reduced the capacity for CO_2 fixation. The inhibition of photosynthesis was

also caused by the oil in the adaxial furrows physically blocking CO_2 exchange, as evidenced by low transpiration rates for these treated leaves (Fig. 3.3b). Since most stomata are on the adaxial leaf surface (Anderson, 1974), the oil impact was greater when this side of the leaf was exposed to oil. Little oil penetrated the abaxial surface as demonstrated by the absence of a significant difference in the chlorophyll content between abaxial-oiled leaves and abaxial non-oiled leaves. However, the chlorophyll content of the adaxial-oiled leaves was significantly lower than adaxial non-oiled leaves (Fig. 3.4).

When crude oil was applied on either the adaxial or abaxial sides of leaves of *Sagittaria lancifolia*, the photosynthetic rates were reduced by about 30 and 40%, respectively (Fig. 3.5 a). In contrast, the photosynthetic rate of the leaves coated with oil on both adaxial and abaxial surfaces was inhibited almost completely 0.5 weeks after oiling and recovered to about 35% of the control 1.5 and 5 weeks after oiling. Leaf transpiration rate showed the same trend as photosynthetic rate 0.5 weeks after oiling (Fig. 3.5 b), suggesting that the reduction of photosynthetic rate was primarily caused by the physical blocking of the gaseous exchange pathway (mainly stomata) soon after the initial oiling. The recovery of transpiration in all oil treatments suggested that the gas exchange pathway was not obstructed 5 weeks after oiling. Therefore, the reduction in photosynthesis for *S. lancifolia* could at least be partially due to an injury of the photosynthetic apparatus, as evidenced by the significantly lower chlorophyll content of leaves exposed to the oil (Fig. 3.6).

Influence of Season on the Response of Plants to Oil Impact

The time of the year that an oil spill occurs can be important in determining oil impact on plants. The effect of oil added to the soil on the plants was much more severe during the summer active growth period compared to the fall period of senescence. The photosynthetic rates were significantly reduced for both *Spartina alterniflora* and *Sagittaria lancifolia* oiled in the summer, however, the photosynthetic rates were not

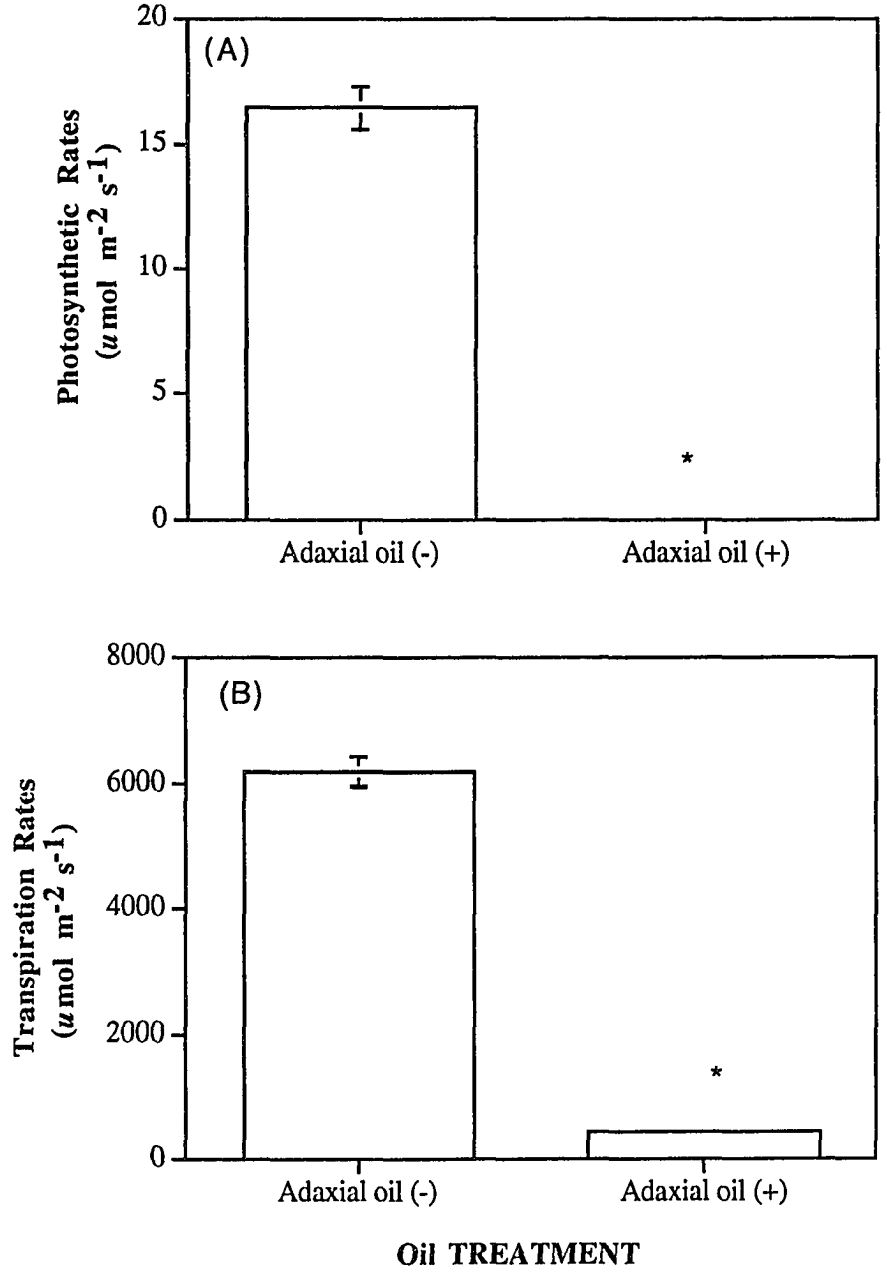

Fig. 3.3 Influence of the leaf surface on responses of photosynthetic rates (A) and transpiration rates (B) of *Spartina alterniflora* to the oil coverage. Data were averaged over time (0.5, 1.5, 3 and 5 weeks) and the leaf surface treatment (n=32) (no significant interaction between leaf surface and oil, as well as repeated time). * indicates significantly difference.

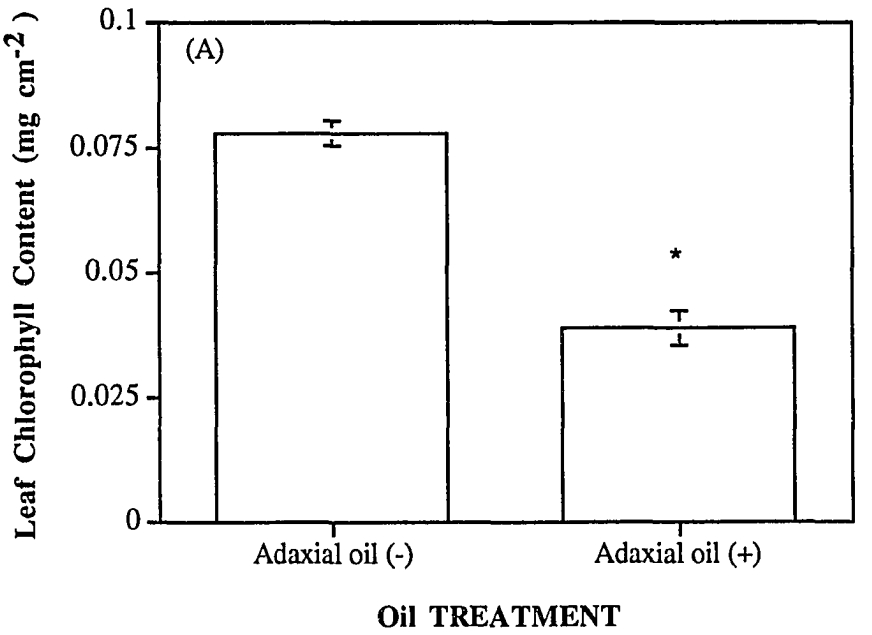

Fig. 3.4 Response of leaf chlorophyll content of *Spartina alterniflora* to the oil coverage on leaf surface. Data were averaged over oil treatment (n=8) (no significant interaction between adaxial and abaxial treatment). * indicate a significant difference.

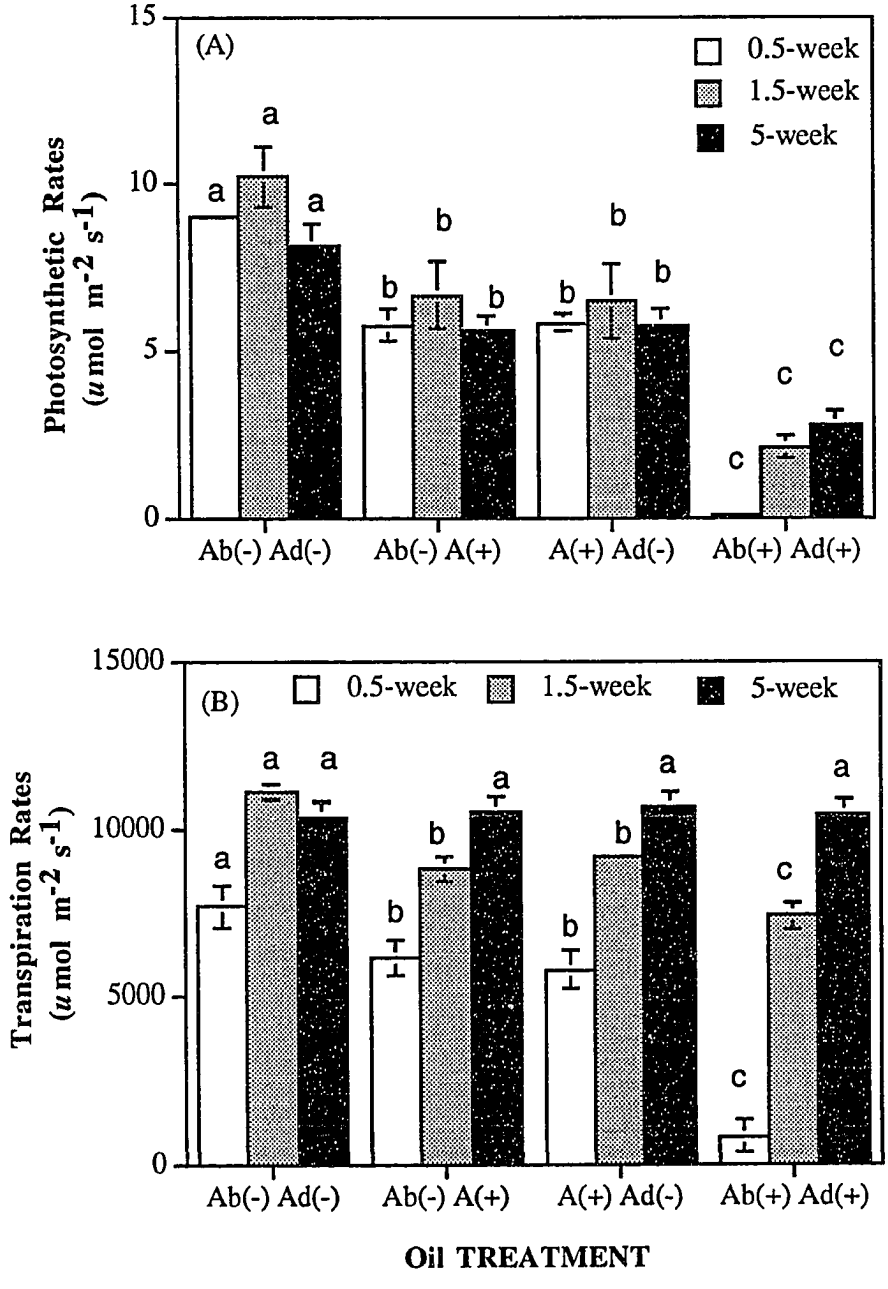

Fig. 3.5 Influence of the leaf surface on responses of photosynthetic rates (A) and transpiration rates (B) of *Sagittaria lancifolia* to the oil coverage (n=4). Different letters indicate a significant difference within a measurement time.

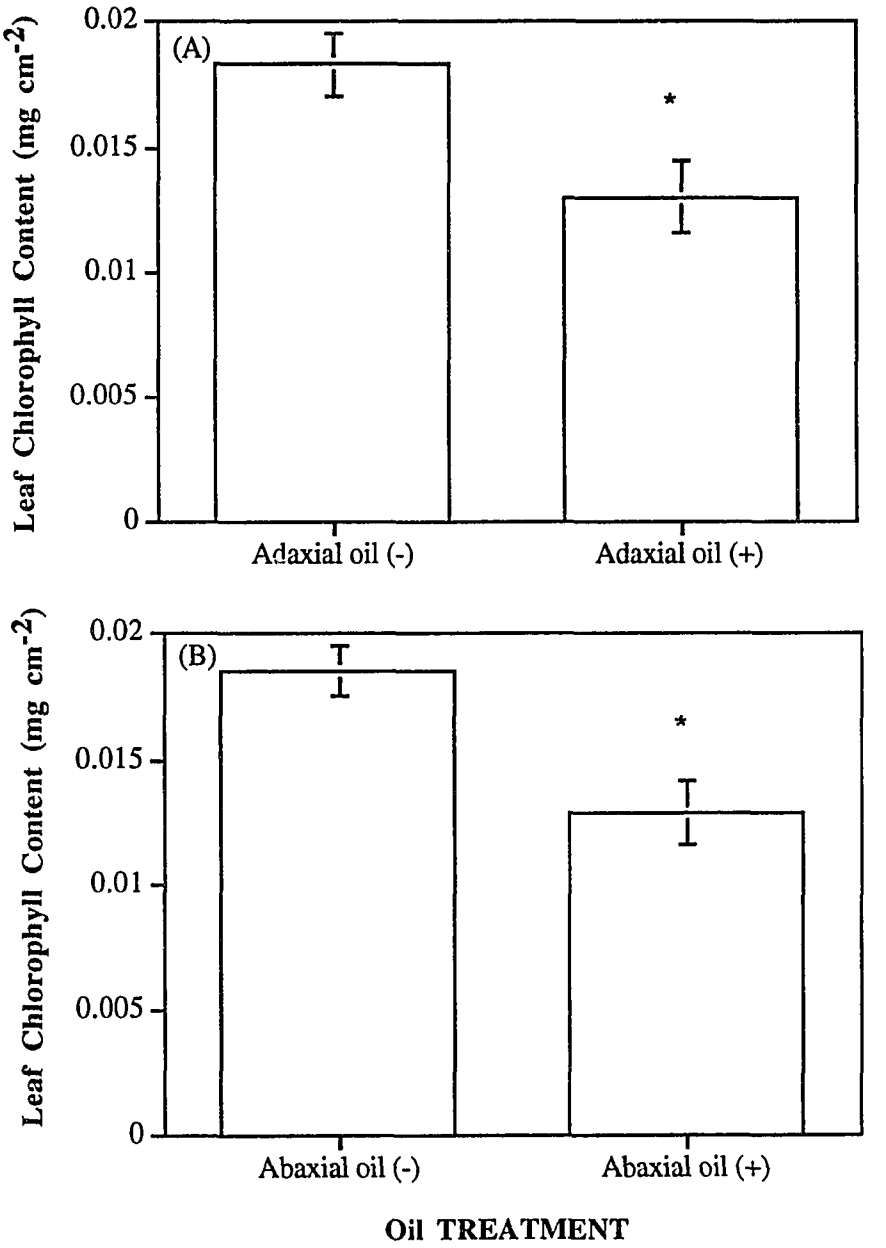

Fig. 3.6 Response of leaf chlorophyll content of *Sagittaria lancifolia* to the oil coverage on (A) the adaxial and (B) abaxial surface. Data were averaged over oil treatment (n=8) (no significant interaction between adaxial and abaxial treatment). * indicate a significant difference.

significantly affected when oil was applied in the fall (Fig. 3.7). Consistent with the photosynthesis data, the aboveground biomass of *S. alterniflora* and *S. lancifolia* 2.5 months after oiling was significantly reduced for the summer oil application, but not for the fall oiling (Fig. 3.8) Regrowth of aboveground biomass , an index of longer-term oil impact, was also significantly different between the seasons with limited regrowth of both species from a summer oiling as compared with the controls. However, regrowth of aboveground biomass production of these two species was not adversely affected by oiling in the fall (Fig. 3.9).

Few studies have attempted to relate season to the impact of oil on plants (Ranwell & Hewett, 1964; Baker, 1971b; Getter *et al.*, 1984; Scholten & Leendertse, 1991). Ranwell & Hewett (1964) observed that outside the growing season even relatively fresh oil did not cause significant mortality of some salt marsh vegetation. This suggested that plant growth and development stages might alter the effect of oil on plants. Usually plants grow vigorously in the spring and summer, and senesce in the late fall and winter. More severe damage of an oil spill to plants might occur during the vigorous growth season, with little damage during senescence or dormant season (Ranwell & Hewett, 1964; Hershner & Moore, 1977; Scholten & Leendertse, 1991). Baker (1971b) indicated that a marked reduction of flowering can occur if plants are sprayed with oil when flower buds are developing; flowers, if sprayed with oil, rarely produce seeds, and oiling of seeds may reduce germination.

In a study most relevant to the present study, Alexander & Webb (1985) showed that season did not influence the response of *Spartina alterniflora* to oil applied at a dosage of 1 L m^{-2} of crude oil to the sediment during a November and May application. The result of the present study, however, showed that season did influence the responses of *Spartina alterniflora* and *Sagittaria lancifolia* to oil impact. If we carefully examine the results of Alexander & Webb (1985), a lack of a seasonal response to the oil in that study was likely because the dosage was low (1 L m^{-2}), and the oil did not

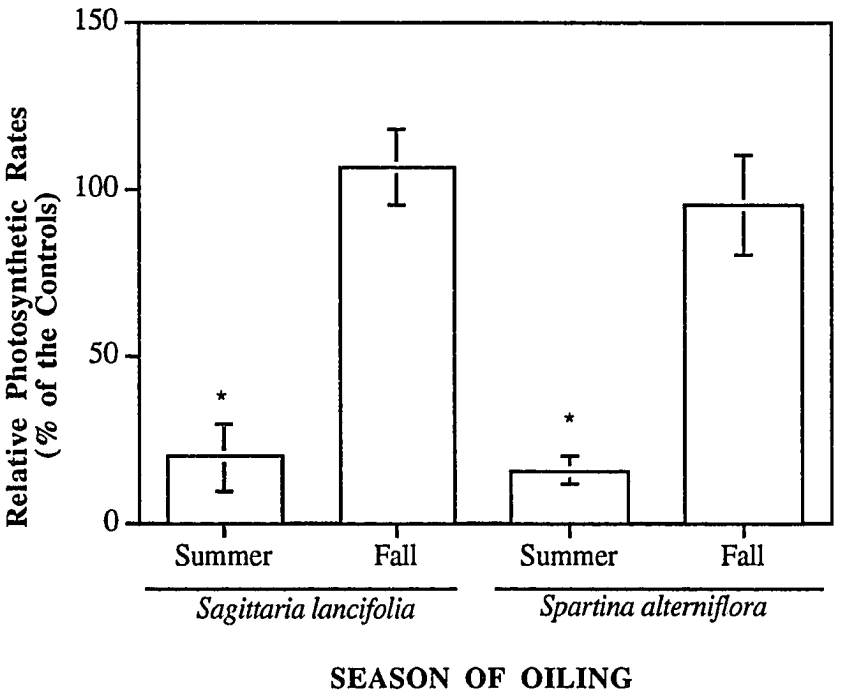

Fig. 3.7 Influence of season on responses of the photosynthetic rates of *Sagittaria lancifolia* and *Spartina alterniflora* to oil applied to the soil (n=4). The photosynthetic rates of the controls were 22.3 *u*mol m^{-2} s^{-1} for *Spartina alterniflora* and 11.6 *u*mol m^{-2} s^{-1} for *Sagittaria lancifolia.* * indicates a significant difference within plant species.

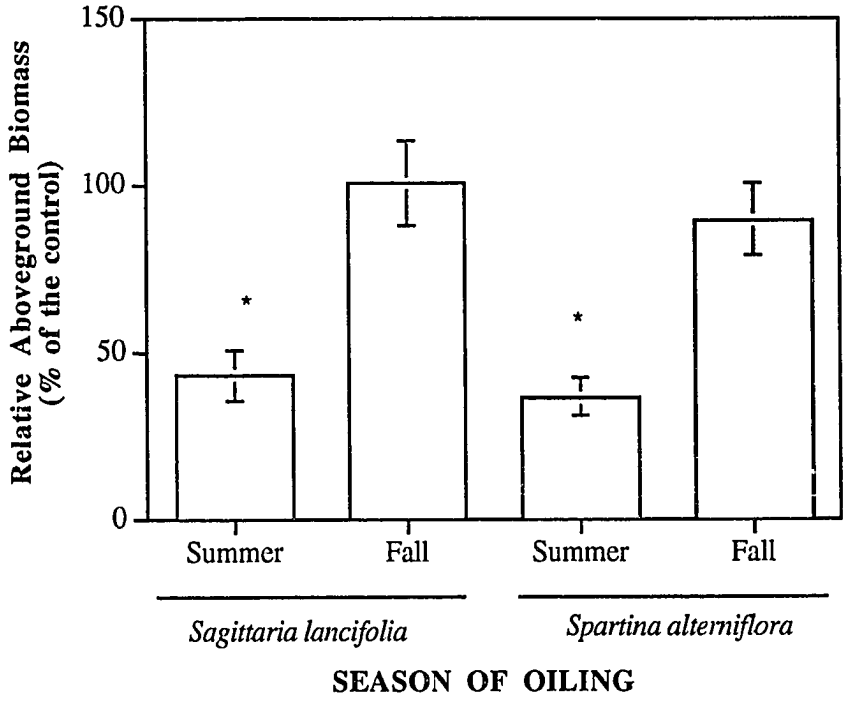

Fig. 3.8 Influence of season on responses of total aboveground biomass of *Sagittaria lancifolia* and *Spartina alterniflora* 2.5 months after the oil applied to the soil substrate (n=4). * indicates a significant difference within species.

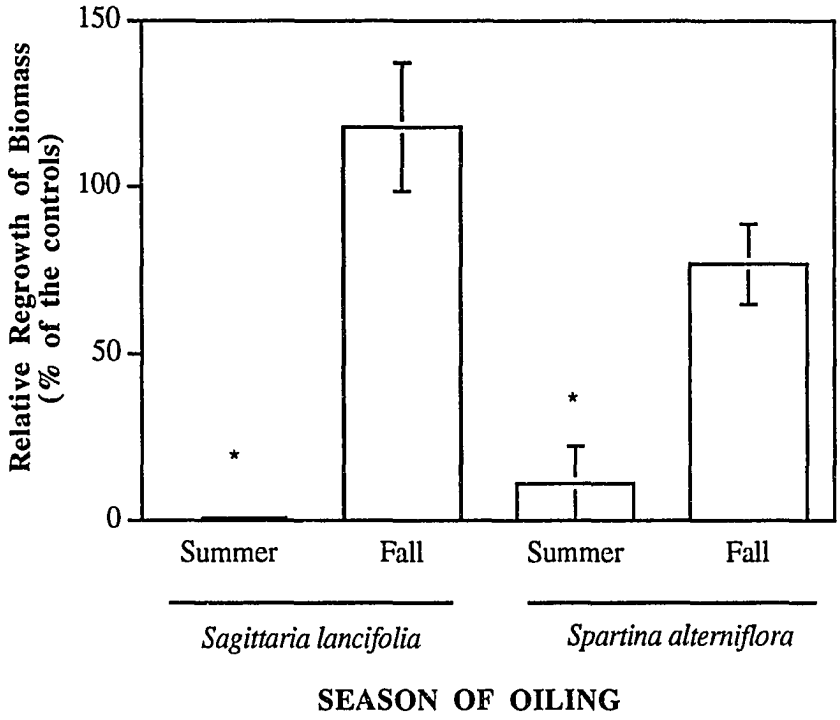

Fig. 3.9 Influence of season on regrowth of total aboveground biomass of *Sagittaria lancifolia* and *Spartina alterniflora* 1 year after the oil applied to the soil substrate (n=4). * indicates a significant difference within plant species.

incorporate much into the sediment (< 5 mg oil per g dry soil). The oil level was not high enough to show an effect in even the summer, thus, potential resistance of a plant to oil impact in the fall was not exhibited. In the present study, however, a large oil dosage (8 L/m^2) plus a porous soil substrate led to a high oil content in the soil. The large amount of oil incorporation into the soil maximized the oil effect and killed the plants during the summer application. Thus, in this case, the different responses to oil between summer and fall were fully expressed with a high plant resistance to oil impact during the slower growth period.

SUMMARY

Resistance to south Louisiana crude oil depended largely upon the plant species. *Sagittaria lancifolia* exhibited a greater relative resistance to oil coverage than the *Spartina* species. Photosynthetic rates recovered up to 70% of control values for *Sagittaria lancifolia* 2 to 4 weeks after oil coverage. Transpiration rates in *Sagittaria lancifolia* recovered more completely than did photosynthetic rates during the same period. *Spartina alterniflora* and *S. patens* were sensitive to oil coverage. Photosynthesis was completely inhibited and never recovered after oil applied to the leaves of the two *Spartina* species for oil application. Effect of oil coverage on plants in sunlight was more severe than in shade. Photosynthetic rates of *Sagittaria lancifolia* coated by oil under sunny condition recovered to 35% of the control compared with 75% recovery under shade condition. The effect of the oil on the *Spartina* species was not influenced by sunlight.

Plant leaf surface structure also plays an important role in the sensitivity of leaves to oil coverage. The smooth, waxy cuticle on both sides of the *Sagittaria lancifolia* leaf led to only a thin oil film being retained on the leaf surfaces. Photosynthesis could recover to some extent after most of the oil on the leaf surface evaporated. In contrast, many deep furrows occur on the adaxial sides of the leaves of the *Spartina* species, and most stomata are located in the furrows. Oil accumulated in

the furrows on the adaxial surface, and completely inhibited photosynthetic capability. A thick cuticle partially protects the abaxial surface.

The season when the oil was applied to soil also proved important in influencing the oil impact on plants. *Sagittaria lancifolia* and *Spartina alterniflora* showed resistance to the crude oil applied to the soil substrate in the fall, but little resistance in the summer. Regrowth biomass of *Sagittaria lancifolia* and *Spartina alterniflora* one year after application of 8 L m^{-2} oil in the fall was not significantly different from that of the unoiled controls.

CHAPTER 4

REVEGETATION OF OILED SOIL BY TRANSPLANTATION AND EFFECT OF FERTILIZER ON TRANSPLANTATION SUCCESS

INTRODUCTION

Coastal marshes are important ecosystems because of their high productivity and role as a nursery for fisheries, habitat for wildlife, flood mitigation, protection of shoreline against erosion, buffering the impact of coastal storms, and water quality enhancement (Gosselink *et al.*, 1974; Turner, 1976; Mitsch & Gosselink, 1986). These functions depend on the presence of vegetation. Coastal wetlands of south Louisiana are sites of intensive petroleum hydrocarbon production and transportation (Turner & Cahoon, 1987; Mendelssohn *et al.*, 1990); as a result, an oil spill could greatly affect these important ecosystems.

After a spill, oil may penetrate into the soil and cause acute and chronic damage to the plants, reducing stem height, stem density and aboveground biomass, increasing mortality, and inhibiting growth and regrowth of the vegetation (Krebs & Tanner, 1981; Ferrell *et al.*, 1984; Alexander & Webb, 1987). The elimination of the vegetative cover can accelerate shoreline erosion (Alexander & Webb, 1987), and reduce the value of these systems.

Following an oil spill, marshes are usually cleaned by low-pressure flushing and/or cutting and removal of the oiled aboveground vegetation in conjunction with removal of any free oil with absorbents and vacuum cleaning (Baker, 1971c; Westree, 1977; Mattson *et al.*, 1977; Krebs & Tanner, 1981b; Hoff *et al.*, 1993). In many instances, no cleanup activities are attempted to avoid physical damage to the wetlands (Mearns, 1993). The marshes are then left to recover through natural processes of vegetative regrowth which may take years (Alexander & Webb 1987; Baker *et al.*,

1993). Slow vegetative recovery from an oil spill may result in significant ecological consequences. The primary reasons for the inability of marsh vegetation to recover after a spill are the death of underground rhizomes caused by the heavy oil in the sediment (Krebs & Tanner, 1981) and negligible recruitment of reproductive propagules into the oiled area. Transplanting marsh vegetation into the affected marsh may be a way to accelerate marsh recovery to oil spills. Krebs & Tanner (1981) reported that *S. alterniflora* could be transplanted to an oiled marsh with up to 17 mg g^{-1} of oil. However, little or no information is available to test the applicability of this method of marsh restoration from oil spill impacts.

The objectives of this study were to determine (1) the possibility of revegetating oil contaminated soil in which the vegetation was previously killed by petroleum oil; (2) the effect of fertilizer (N, P, K) on transplant success; and (3) the effect of fertilization on oil degradation.

MATERIALS AND METHODS

Experiment Design

Marsh sods, collected as described in Chapter 1, of *Spartina patens* and *Spartina alterniflora* were treated with south Louisiana crude oil at rates of 0, 4, 8, 16, and 24 L m^{-2} in July 1991. The oil floating on the water surface in each pot was removed six months after oiling in January 1992. The dominant plant species, *Spartina patens* and *S. alterniflora*, were killed by oil levels above 8 l m^{-2} in 1992 due to the high oil content in the soil.

Two years after oil application (in May, 1993), the oil remaining in the top 5 cm of soil was analyzed (detailed in Chapter 1) to determine the concentration of the residual oil that still existed in the soil. Based on these concentrations, the oiled sods, regardless of species composition, were divided into 4 oil categories: no oil, medium oil (20 to 50 mg g^{-1}), heavy oil (50 to 150 mg g^{-1}) and very heavy oil (> 150 mg g^{-1}).

The vegetation, including rootstock, that was not killed by the original oiling was removed from the soil sods. Then, the two dominant marsh species, *Spartina patens* and *Spartina alterniflora*, were transplanted into each oiled sod. Two culms of *S. alterniflora* and 4 culms of *S. patens* were transplanted into each sod with each species on opposite sides (ca. 14 cm away from each other). To replenish water loss through evapotranspiration, the transplants were watered every day to bring the water level to the soil surface. One month following transplanting when the plants were well established, half of the sods in each oil category were fertilized with N-P-K at rates of 666 kg N/ha, (NH_4-N), 272 kg P/ha (P_2O_5) and 514 kg K/ha (K_2O) (2/3 the dosage used by Wilsey *et al.*, 1992). The remaining sods served as controls. Therefore, the experimental design was a completely randomized 4 x 2 factorial arrangement, with 4 oil levels (no oil, medium, heavy and very heavy oil) and 2 fertilization treatments (no fertilizer and fertilizer). The treatment-level combination replicated four or more.

Six months after transplanting in November 1993, the aboveground biomass was harvested and analyzed to determine the effect of the oil and fertilizer on transplant success. One month after the first harvest of transplants, the fertilizer was again applied at the same rates as before. The regrowth biomass was harvested and analyzed in May 1994 to determine the longer-term effect of the oil and fertilizer on the vegetation. The residual oil concentration in the top 5 cm of the soil was analyzed (detailed in Chapter 1) in May 1994, one year after transplanting, to assess oil degradation rate. The difference in oil concentration between May 1993 and May 1994 was calculated as oil degradation rate. The relative oil degradation rate was calculated as follows: percentage of degradation (% per year) =[(oil concentration in May 1993 - oil concentration in May 1994) / (oil concentration in May 1993)]*100%.

Statistical Analysis

Statistical Analysis was conducted with the Statistical Analysis System (SAS Institute, 1985). Plant and soil variables were analyzed with analysis of variance

(ANOVA) as a 4 X 2 factorial arrangement of treatments (4 oil levels and 2 fertilization levels) in a completely randomized design. Duncan's multiple range test was used to evaluate statistical differences among oil and fertilizer levels. Significant differences were reported at the 0.05 probability level unless otherwise stated.

RESULTS

Soil Oil Concentration and Transplant Biomass after One Growing Season

Immediately after transplanting in May 1993, the residual oil in the soil was still relatively high (Fig. 4.1) and was not significantly different from that in May of 1992 (Fig. 1.7, Chapter 1). The mean concentration of oil in the sods for the very heavy oil level was as high as 440 mg/g. This suggested that the oil incorporated into the soil was degrading slowly in the water saturated sediment.

The combined biomass of the *S. alterniflora* and *S. patens* transplants was not significantly different for medium and heavy oil levels, but was significantly reduced at the very heavy oil level in November 1993 compared to the control (no oil) (Fig. 4.2). Application of fertilizer significantly increased the combined biomass of *S. alterniflora* and *S. patens* (Fig. 4.2). The biomass of *S. patens* in the fertilized treatment was higher than the unfertilized except that of very high oil level. The biomass of *S. alterniflora* was higher in the medium to high oil with fertilizer in November, 1993 (Fig. 4.2). The stem density data of *S. alterniflora* and *S. patens* showed the similar trend to the biomass data (Fig. 4.3)

Regrowth of Transplant Biomass the Year Following Transplanting

The application of fertilizer significantly ($p < 0.002 - 0.0001$) increased the regrowth biomass production of transplants of *S. patens*, *S. alterniflora* and the combination of these two species (Fig. 4.4). Regrowth biomass production (Fig. 4.4) and stem density (Fig. 4.5) of *S. patens* transplants was significantly reduced at higher oil levels, while those of *S. alterniflora* transplants significantly increased at higher oil

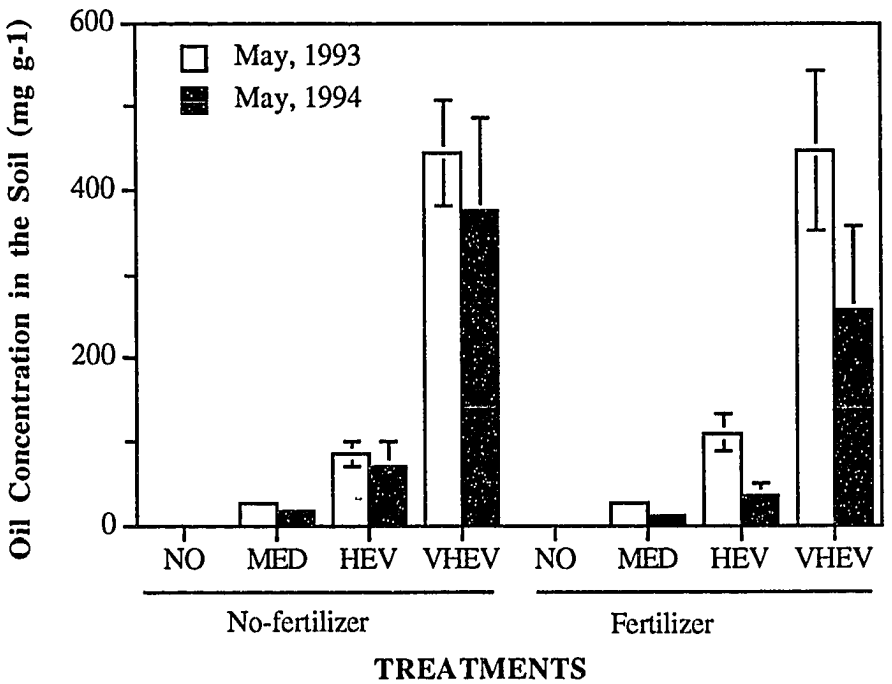

Fig. 4.1 Oil concentrations in the soil before transplanting (May 1993) and one year after transplanting (May 1994). NO: no oil; MED: medium oil; HEV: heavy oil; VHEV: very heavy oil.

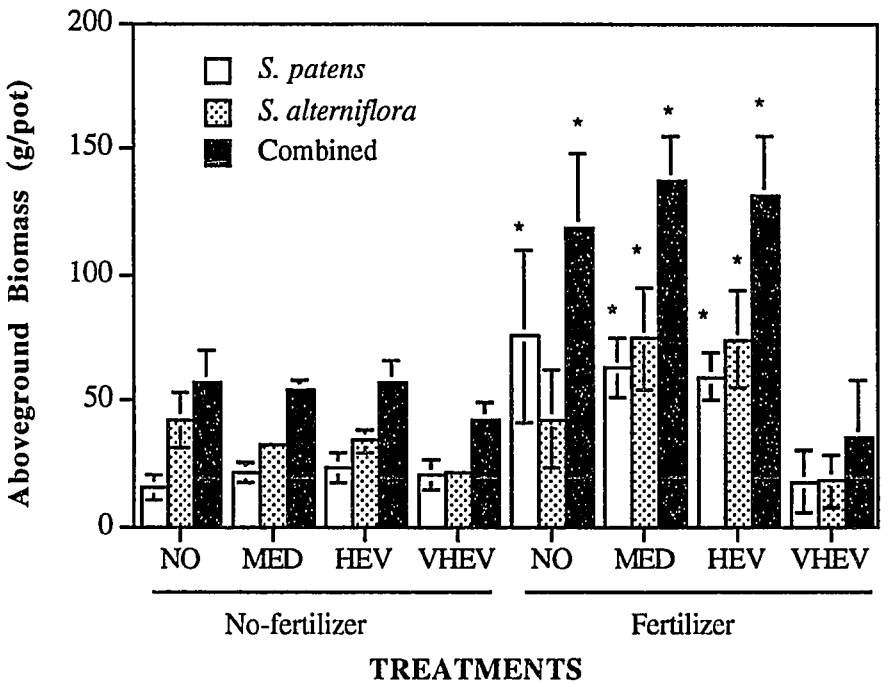

Fig. 4.2 Aboveground biomass of transplants 6 months after transplanting to the oiled marsh sods. * indicates a significant difference within plant species. Combined represents the combined biomass of *Spartina patens* and *Spartina alterniflora*. NO: no oil; MED: medium oil; HEV: heavy oil; VHEV: very heavy oil.

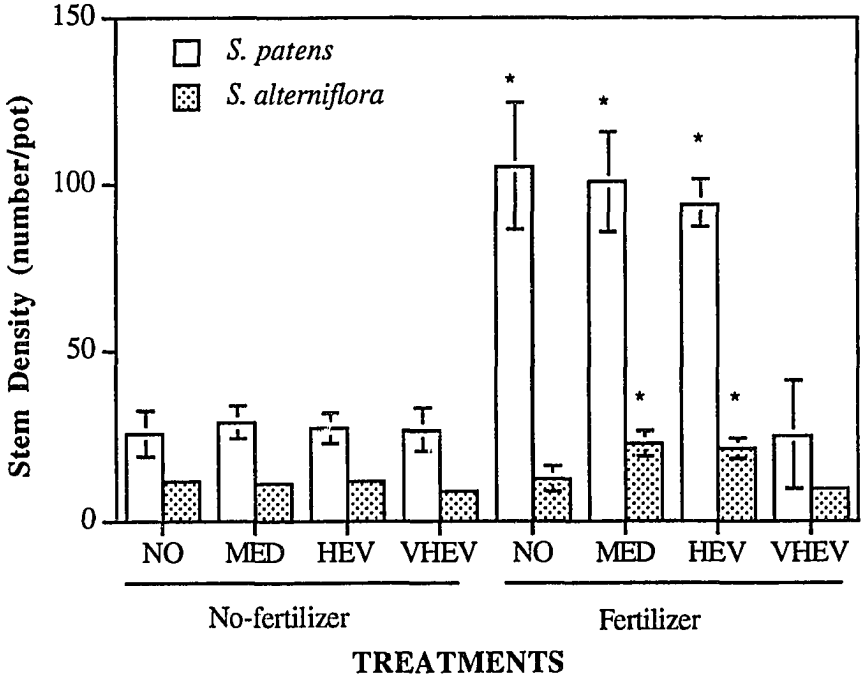

Fig. 4.3 Stem density of transplants 6 months after transplanting to the oiled marsh sods. * indicates a significant difference within plant species. NO: no oil; MED: medium oil; HEV: heavy oil; VHEV: very heavy oil.

levels (Figs. 4.4 & 4.5). The combined biomass of the two species was higher in medium oil, but was not significantly different between the control and the very heavy oil level (Fig. 4.4). *S. alterniflora* performed better than *S. patens* at high oil concentration and fertilization, showing increased biomass with oil when fertilizer was applied (Fig. 4.4), while the biomass of *S. alterniflora* slightly decreased with oil when no fertilizer was applied (significant interaction between oil level and fertilizer application).

Residual Oil Concentration the Year Following Transplanting

Oil concentration in the soil one year after transplanting in May 1994 compared with that in may 1993 was lower, especially at the high and very high oil levels, with the application of fertilizer (Fig. 4.1). The relative oil degradation rate was significantly increased by the application of fertilizer (Fig. 4.6).

DISCUSSION

After oil penetrates an anaerobic substrate, the oil may persist for a long time since oil degradation is much slower in absence of oxygen than in oxidizing conditions (Getter *et al.*, 1984). In this study, the residual oil in the soil two years after oiling (May 1993) was not significantly different from that nine months after oiling (May 1992). The floating oil was removed five months after oiling (January 1992), so no more oil could be sorbed into the sediment to maintain a high oil concentration in the soil. This finding suggests that oil degradation in the water saturated soil was slow. Teal *et al.* (1978) reported that oil was still measurable in marsh sediments 7 years after an oil spill in Florida. Baker *et al.* (1993) observed that an oil layer was still clearly visible 22 years after an oil spill on a marsh in Wales, and mousse deposits at the marsh surface in Chile were still apparent 17 years after an oil spill.

Oil degradation was accelerated by fertilization. The oil degradation between May 1993 and May 1994 was greater with N-P-K fertilization compared to no fertilization. The increased degradation rate was likely due to both increased microbial

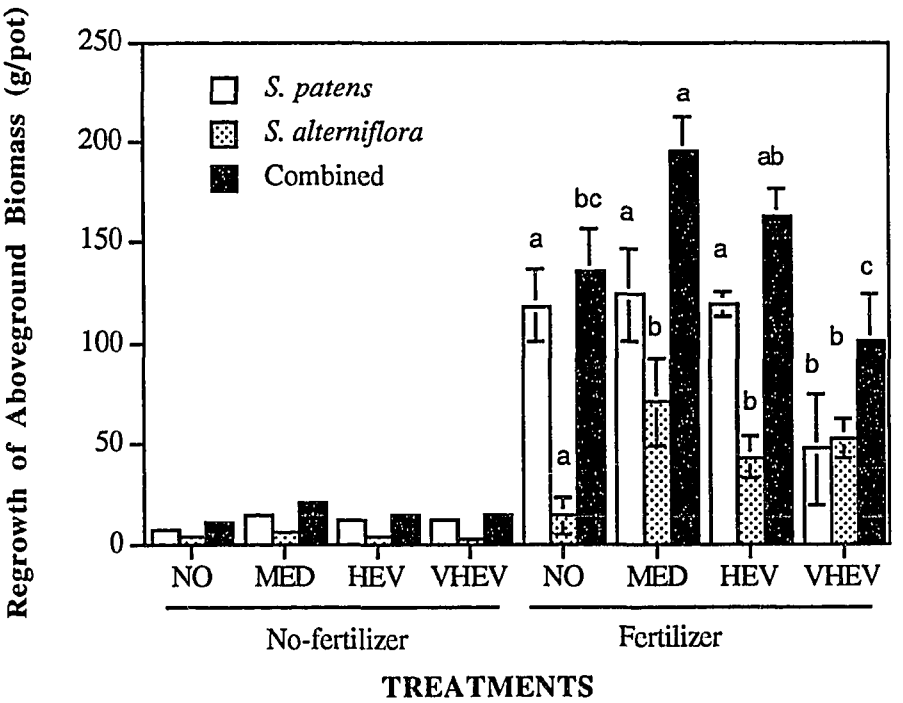

Fig. 4.4 Regrowth aboveground biomass of transplants in the year following transplanting to the oiled marsh sods. Different letters indicate the significant difference within plant species. Combined represents the combined biomass of *Spartina patens* and *Spartina alterniflora.* NO: no oil; MED: medium oil; HEV: heavy oil; VHEV: very heavy oil.

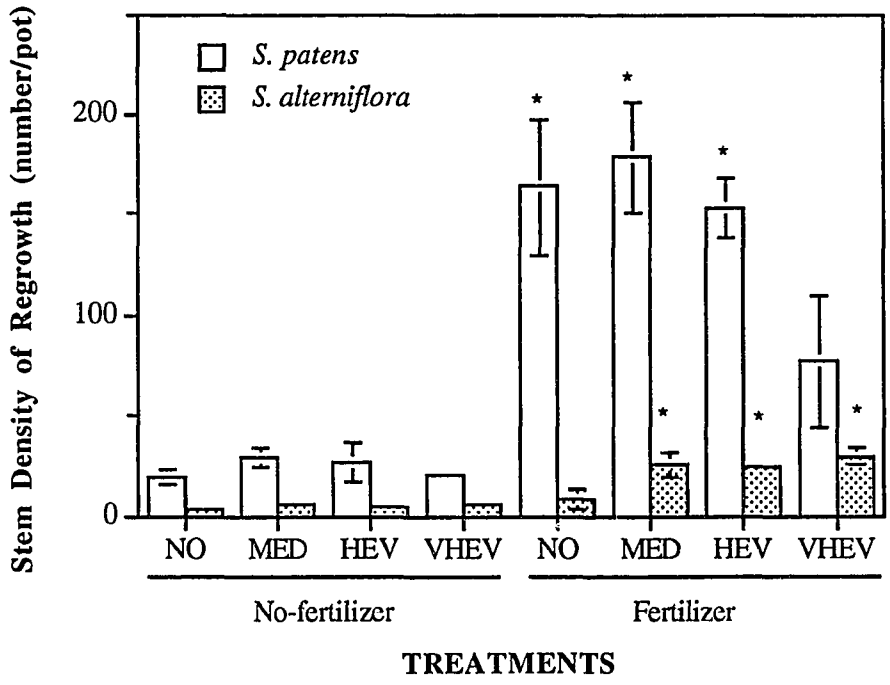

Fig. 4.5 Regrowth stem density of transplants in the year following transplanting to the oiled marsh sods. * indicate the significant different within the plant species. NO: no oil; MED: medium oil; HEV: heavy oil; VHEV: very heavy oil.

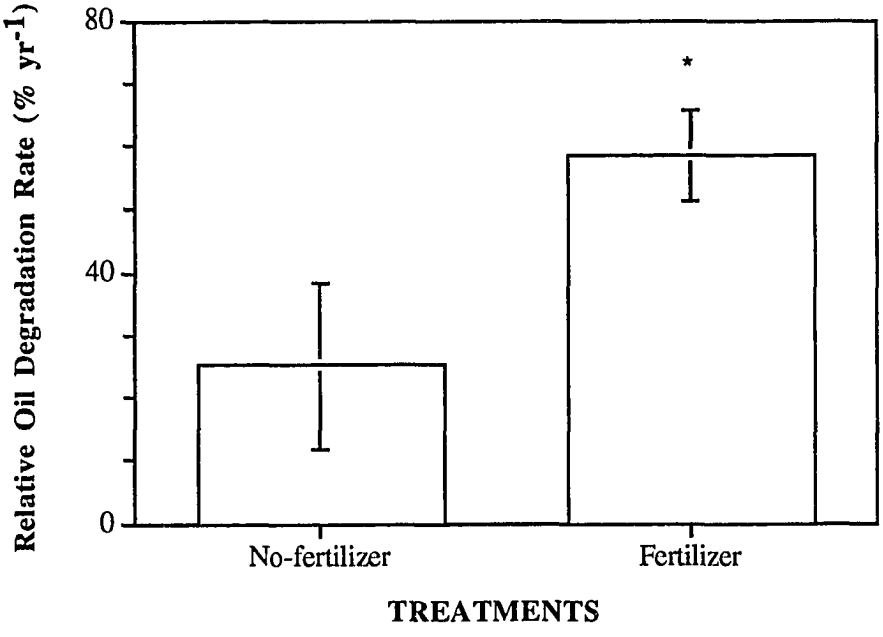

Fig. 4.6 The effect of the application of fertilizer on relative oil degradation rates. Data were average over the oil categories (n=16) (no significant difference between oil levels). * indicates a significant difference.

activity and increased plant biomass by the fertilizer. Fertilizer has been shown to directly increase the number and activity of microbial populations. The experimental application of fertilizer at the Exxon Valdez oil spill increased the numbers of hydrocarbon degraders, and natural hydrocarbon biodegradation was enhanced (Lindstrom *et al.*, 1991). Carbon mineralization rate and cumulative carbon mineralization increased with increasing levels of fertilizer N (Rasiah, 1991).

The fertilizer-induced increase in vegetative biomass could indirectly increase oil degradation. Wetland soil, which is usually saturated with water, is largely under anaerobic conditions, and the hydrocarbon degradation is lower under these conditions (Hambrick *et al.*, 1980). Marsh vegetation can transport oxygen through plant air-space tissue (aerenchyma) to the soil rhizosphere (Teal & Kanwisher, 1966; Armstrong, 1978; Smirnoff & Crawford, 1983; Justin & Armstrong, 1983; Seliskar, 1985), providing oxygen for aerobic oil degradation. In addition, it was observed that in the present study the fertilizer application resulted in the water table falling below the soil surface in between watering periods, probably due to greater transpirational water loss with greater vegetative biomass. This allowed air to enter the soil and increased oxidized conditions. Thus transpiration of vegetation may be important in maintaining an aerobic soil condition for oil degradation, especially during low tide.

Furthermore, the presence of vegetation might reduce the oil content in the soil via uptake of oil into the plants. Salt marsh plants are able to take up hydrocarbons from oil contaminated sediment and increase the hydrocarbon or total lipid fraction of the aerial portion of plants (Lytle & Lytle, 1987). In the present study, the increased biomass by fertilization could have resulted in increased uptake of the oil from the soil, thus reducing the oil content in the soil.

The higher plant biomass might also result in more organic leachates added to the rhizosphere, thus enhancing microbial degradation in the present study. Microbial activity was found to be greater in rhizosphere soil than the bulk soil, and the

degradation of the oily insecticides and herbicides occurred faster in the rhizosphere than in the edaphosphere (nonvegetated soil) (Hsu & Bartha, 1979; Reddy & Sethunathan, 1983; Sandmann & Loos, 1984; Walton & Anderson, 1990). This might be partially due to release of enzymes, exudation of nutrients and carbon sources such as amino acids, simple sugars and complex carbohydrates by plant roots, creating more favorable microhabitats for microbes.

Therefore, the reduced the oil content in the soil in this study could relate directly to a higher microbial number and activity induced by the fertilizer, as well as indirectly to the more favorable aerobic condition, more nutrients in rhizosphere, even increased hydrocarbon uptake and metabolism by a substantially increased plant biomass with fertilizer.

The toxicity of petroleum oil to vegetation appears largely related to the degree of freshness of a crude oil with weathered oil usually less toxic to plants than fresh oil (Cowell, 1969; Baker, 1970). The weathering process, mainly including evaporation, dissolution, oxidation, and biodegradation (Boesch, 1974), could alter the bulk oil components. As the weathering process continues, small molecular components, which are more toxic than large ones (Baker, 1970), are evaporated or degraded more readily, so that the toxicity of the weathered oil is reduced. In this study, regrowth of *S. alterniflora* and *S. patens* was inhibited by the relatively fresh oil in the year after oiling (1992) at oil levels above 8 l m^{-2} (Chapter 1, Table 1.3), while these two species could grow in about same concentration of the more weathered oil when transplanted to previously oiled sods, supporting the conclusion that the weathered oil is less toxic to plants. Baker *et al.* (1993) also observed that no vegetation recovered where oil beneath the surface skin of thick deposits was quite fresh, while where oil was well weathered, vegetation recovered in an oil-affected salt marsh.

Oil pollution may change species competitive interactions. In this study, without oil application, fertilization increased the biomass of *S. patens* which apparently

inhibited the growth of *S. alterniflora.* However, with increasing oil concentration in the soil, the oil-sensitive *S. patens* decreased in biomass at the very high oil level, even when fertilized, allowing the more oil tolerant *S. alterniflora* to take advantage of the fertilization and less competitive habitat and showed an increase in biomass with the application of oil, suggesting that *S. alterniflora* might dominate in the recovery following a spill of oil on a mixed marsh of *S. patens* and *S. alterniflora.* At the medium oil level, the total combined biomass of *S. patens* and *S. alterniflora* was significantly higher than the control with fertilizer application, suggesting the medium oil (15 mg g^{-1} in this study) plus fertilizer enhanced the plant growth. Mendelssohn *et al.* (1993) observed that *S. alterniflora* displayed the greatest increase in live percent cover in an oil impacted brackish marsh in south Louisiana dominated by *S. alterniflora*, *S. patens* and *Distichlis spicata* four years after an oil spill from a pipeline blowout. Scholten & Leendertse (1991) indicated that under unpolluted conditions *Halimione portulacoides* had a competitive advantage over *Puccinellia maritima.* This advantage progressively declined with increasing oil content in the sediment, because of a decreasing impact of *Halimione* on *Puccinellia* as a result of the reduced growth.

Undoubtedly, oil concentration can affect the intensity of impact to marsh vegetation. It was observed that growth, stem height, density, and aboveground biomass of *Spartina alterniflora* (Krebs & Tanner, 1981; Alexander & Webb, 1987; Li *et al.*, 1990) and live percent cover of *S. patens* (Mendelssohn *et al.*, 1990) were reduced with high oil level. In the present study, high oil concentration (440 mg g^{-1} in very heavy oil), even with some degree of weathering, inhibited plant growth (Fig. 4.2) even if fertilizer was applied. However, decreased oil concentration with time (Fig. 4.1) allowed better plant growth, with no significant difference in the transplant regrowth between no oil and very high oil in the fertilizer treatments, suggesting that the plants, especially *S. alterniflora,* could be successfully transplanted to oiled soil and grow well at a relatively high soil oil content of up to 250 mg g^{-1}. Furthermore,

fertilization increased the biomass production of transplants, showing that fertilizer is important for establishing transplants in oil contaminated wetlands.

SUMMARY

Spartina alterniflora and *S. patens* were killed at high oil dosages six months after application. Two years after application of south Louisiana crude oil to the soil sods, these two *Spartina* species were transplanted to the oiled and unoiled sods. Fertilizer containing N, P and K was applied 1 and 7 months after transplanting. Compared with the control (no oil), the total combined biomass of transplants for *S. alterniflora* and *S. patens* was not significantly different for medium and heavy oil levels, but was significantly reduced at the very heavy oil level in November 1993 (6 months after transplanting). The combined biomass of *S. alterniflora* and *S. patens* in each pot with the fertilizer was significantly higher than that of the control (no fertilization) in the year of transplantation. One year after transplanting, regrowth biomass production of transplants for *S. patens*, *S. alterniflora* and the combination these two species was significantly increased by application of fertilizer. Regrowth biomass production of transplants was significantly reduced by very heavy oil for *S. patens*, while, significantly increased for *S. alterniflora*. The combined regrowth biomass of *Spartina alterniflora* was not significantly affected by oil as high as 250 mg g^{-1}. The oil degradation rate in the sediment was enhanced by application of the fertilizer. The results demonstrate that it is possible to revegetate oil contaminated marshes by transplanting and that fertilization accelerates oil degradation and plant growth.

OVERALL CONCLUSIONS

Oil pollution can cause wide spread impacts to coastal wetlands. A number of biotic and abiotic factors might influence and change the impact of petroleum hydrocarbons on wetland vegetation. These factors mainly include the type of petroleum hydrocarbon, degree of oil weathering, amount and persistence of oil, extent of oil coverage on plants, soil substrate composition such as organic content and texture, plant species, season and meteorological conditions when plants are oiled, etc. The interaction of these factors makes it difficult to accurately predict the effects of an oil spill on wetland vegetation.

Different marsh types respond differently to oil impacts. The vegetative sensitivity of the selected coastal marsh types to south Louisiana crude oil increased in the order of *Sagittaria lancifolia*, *Spartina alterniflora*, and *S. patens*. The sensitivity is based on the effect of oil on photosynthetic rate, live and dead biomass as well as the ratio of live/dead biomass, and the plant regrowth in the year following oil application. The differential oil sensitivity was partially due to differences in soil organic matter among the marsh types. The soil organic matter played an important role in accelerating penetration and sorption of the oil into the soil. Organic matter content may also control the amount of oil that remains within the soil. Therefore, particular concern should be paid to dealing with oil contamination in marshes of high soil organic matter, such as the *S. patens* marsh type used in this study. The plant response observed was also likely due to differences in plant sensitivities to oil. For example, even though oil content in the *Spartina patens and Sagittaria lancifolia* soils were high and very similar at the 8 l m^{-2} level, *S. patens* showed negative effects while *S. lancifolia* was unaffected. The stimulation of *S. lancifolia* growth by petroleum hydrocarbons at all oil levels, even up to 24 l m^{-2}, suggests the potential use of *S. lancifolia* in oil contaminated fresh

marsh sites, either for protecting sediment erosion or accelerating oil degradation by generating a more oxidized soil.

The marsh plants showed different responses to oiling their aboveground portions. *Sagittaria lancifolia* was relatively resistant to oil coverage, with no detectable effect from oiling the aerial portions of the plant. The two *Spartina* species exhibited a greater sensitivity to oiling their aerial portions, with decreased live and total biomass production and stem density. However, oiling the lower aerial portion of plants did not cause long-term damage in any of the species studied, with regrowth after oil coverage similar to that of the control. This means that mechanical oil cleanup might be avoided if the oil spill mainly coated aboveground components of the marsh plants and the oil did not appreciably sorb into the soil. Mechanical cleanup is often more damaging to the marsh than the oil itself.

In contrast, oil incorporation into the substrate caused both severe short- and long-term damages to the plant species, with a reduction in photosynthesis, biomass, stem density and regrowth. The type of soil substrate largely controlled the intensity of impact. The effects of oil application to the high organic matter, coarse-texture soil were more severe for all three marsh plant species apparently due to a greater amount of fresh oil that incorporated rapidly into the soil. The findings of the present study suggest that long-term impacts will be likely when large amounts of oil incorporate rapidly into the soil. Oil spills in the marshes which favor oil incorporation into the sediment, such as highly organic or sandy substrates, and long-term presence of the oil on the soil surface should be given higher priority relative to cleanup. Cleanup techniques such as low-pressure water flushing might be necessary.

The extent of recovery of marsh plants to oil coverage of the leaves depended largely upon the plant species. *Sagittaria lancifolia* exhibited a relatively greater recovery to oiled leaves, with more complete recovery of photosynthetic and transpiration rate compared to the *Spartina* species. Effect of oil coverage was more

severe in the sunlight than in shade. Unlike *Sagittaria lancifolia*, *Spartina alterniflora* and *S. patens* were very sensitive to oil coverage of their leaves. Photosynthetic and transpiration rates were completely inhibited and never recovered after oil was applied to leaves of the two *Spartina* species regardless of the light condition.

Plant leaf surface structure plays an important role in the sensitivity of leaves to oil coverage. The smooth, cuticle-covered surface on both sides of the *Sagittaria lancifolia* leaf retarded oil from penetrating the leaf. Thus, photosynthesis could partially recover after the oil on the leaf surface, which blocked to stomata, evaporated. In contrast, many deep furrows occur on the adaxial leaf surface of the *Spartina* species. Oil accumulated on and penetrated into the furrows and completely inhibited photosynthesis. Therefore, the great sensitivity of the *Spartina* species to leaf oiling was mostly due to the furrows on the adaxial surface of leaves.

The season when oil spills occur is another important factor controlling the intensity of oil impacts on plants. Both *Sagittaria lancifolia* and *Spartina alterniflora* were highly resistant to crude oil applied to the soil substrate in the fall (season of declining growth), but showed little resistance in the summer (season of active growth). Regrowth biomass of *S. lancifolia* and *S. alterniflora* one year after application of 8 L/m^2 of oil was not significantly different from that of the control. My findings suggest that an oil spill during the active growing season (spring to summer) has more potential for damage, especially if the circumstances favor oil incorporation into sediment. If oil spills occur during the fall to winter (season of declining growth), cleanup activities need not be as intensive; the oiled marsh may best be left to recover naturally rather than attempting to clean it. However, if free, unweathered oil concentrates on the marsh surface or on water covering the marsh, cleanup may be necessary to prevent new shoots of Spartina, growing from belowground rhizomes, from coming in contact with the oil. *Sagittaria*, which is relatively resistant to leaf oiling, would be able to grow through the oil layer.

Revegetation of the bare marsh soil caused by an oil spill is important to restoring the marshes. This study showed that it was possible to revegetate an oil contaminated marsh by transplanting. Transplant regrowth was not adversely affected by oil as high as 250 mg g^{-1} of dry soil. My findings also supported the general rule that weathered oil is less toxic to vegetation than fresh oil since fresh oil killed the *Spartina* species but the same concentration of the more weathered oil did not. *S. alterniflora* showed greater oil tolerance than *S. patens*, with decreased biomass for *S. patens* and increased biomass for *S. alterniflora* with higher oil levels. These results indicate that if an oil spill occurs in a marsh with a mixture of these species, *S. alterniflora* may out compete *S. patens* and become dominant. Fertilizer greatly enhanced the biomass of the transplants and the oil degradation rate, suggesting that fertilization is important in accelerating recovery of the marsh vegetation and restorating oil contaminated marshes. Since the *Spartina* transplants could grow in a soil with relatively high oil content, it would be unnecessary to remove the highly oiled surface soil before transplanting. Revegetation of an oiled marsh through transplanting and fertilizing could be the cost-efficient way to rapidly restore oiled marshes with recovery of the vegetation and acceleration of oil degradation.

Finally, the findings of the present study suggest that future research is need to (1) evaluate the effectiveness of using the dominant coast marsh vegetation to restore oil-polluted marshes and prevent from shoreline erosion after an oil spill, (2) evaluate the effectiveness of phytoremediation, the use of pollutant-resistant vegetation, such as *Sagittaria* species to oil, for the in situ treatment of oil-contaminated soils and sediments, as an low-impacted oil spill cleanup method, (3) select the high oil-resistant marsh plants to restoring and phyto-remediating oil contaminated marshes.

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Major Field: Oceanography and Coastal Sciences

Title of Dissertation: Factors Controlling the Impacts of South Louisiana Crude Oil on the Vegetation and Revegetation of Coastal Wetlands

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