EVALUATION OF HABITAT RESPONSE TO *IN SITU* BURNING AS METHOD OF OIL REMOVAL: PHASE II - *SPARTINA ALTERNIFLORA* SALT MARSH FIELD STUDY

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Abstract

A field study was initiated to evaluate habitat responses to burning as a method of oil removal. Two *in situ* burns were conducted in a *Spartina alterniflora* salt marsh located south of Houma, Louisiana in Terrebonne Basin. Treatments included (1) control, (2) oiling, and (3) oiling plus burning. South Louisiana Crude was applied to the oiled and oiled plus burned treatments at a rate of 2 L m^{-2}. The first burn was conducted in August, 1995 and the second in April, 1996. Shoot regeneration, biomass production, plant height, and carbon fixation were monitored over the post-burn evaluation periods.

Plant responses to oiling and oiling plus burning were monitored for 50 weeks after the first burn. At the conclusion of the study, carbon fixation in the oiled and oiled plus burned treatments was not significantly different from the control treatment fixation values. Plant height and shoot regeneration in the burned plots increased steadily and were comparable to control plot values at 50 weeks. At the conclusion of the study, no significant differences were observed in total above ground biomass production among the three treatments.

Habitat responses to oiling and oiling plus burning were monitored for 15 weeks after the second burn. Carbon fixation measured in the oiled and oiled plus burned plots was about 14% and 50% of the control values at the conclusion of the study. Plant heights measured in the burned plots were equal to the control plot values at 15 weeks, but oil treatment plant heights were only 64% of the control values. Shoot regeneration in the oiled and oiled plus burned treatments increased but were still below the control plot values at the conclusion of the study.

1.0 Introduction

1.1 Background

Coastal wetlands bordering the northern Gulf of Mexico account for 58% of all the coastal wetlands in the United States (Alexander *et al.*1986). These coastal areas support a rich, diverse wildlife that is heavily dependent upon production of estuarine flora and fauna. In many areas of this region, considerable quantities of petroleum are transported, received and stored on a regular basis. Intense activity associated with oil and gas production has the potential for reducing productivity of the area through oil spills. The potentially adverse effects of oil spills on marsh vegetation could have widespread repercussions on the food web for the entire estuarine ecosystem. Studies by Webb *et al.* (1981) and Hershner and Moore (1977) suggest that the effects of oil on vegetation depend on many factors including oil type and concentration, the extent of coverage, and the timing of the oil spill. These effects may also be species specific (Hershner and Moore, 1977; Webb *et al.* 1981, 1985).

1.2 Effects of Oil Spills on Vegetation

Data in the literature show great variations in responses of macrophytes to oil hydrocarbons (Crapp, 1971; American Petroleum Institute, 1985; Mendelssohn *et al.* 1995). This is a reflection of several factors including the differences in: the species susceptibility to oil compounds, the types of oil used, the experimental conditions, and the stage of growth for a given species during its life cycle. The toxicity of crude oil is primarily due to the lower boiling point of volatile aromatic hydrocarbons. Oil type and the degree of weathering largely determine the toxicity levels (Crapp, 1971).

Exposure to oil may adversely affect marsh vegetation (Cowell, 1969; Holt*et al.* 1978; de la Cruz *et al.*1981; Pezeshki and DeLaune, 1993). An oil spill resulted in severe reduction of growth in a salt marsh in Texas (Holt *et al.* 1978). Exposure to crude oil at 1.5 L m^{-2} resulted in death of *Juncus roemerianus,* with recovery reported within three years (de la Cruz *et al.* 1981). Petroleum hydrocarbons at 2 L m^{-2} adversely affected gas exchange functions of *Juncus roemerianus* and *S. alterniflora* under laboratory conditions (Pezeshki and DeLaune, 1993). Net photosynthesis decreased in both species shortly after treatment initiation and remained within 71 to 94% of control in *J. roemerianus* and within 53 to 80% of control plants in *S. alterniflora*. However, there were no lethal effects observed. Under field conditions, plants will likely recover once residual oil is removed by rainfall or tidal action. Growth responses, including growth of new shoots and overall plant health, show adverse effects of oil application in several species. However, these effects are likely to be short-term (Sjotun and Lein, 1993). In

previous studies (DeLaune *et al*.1979, 1984), experimental application of oil to a Louisiana salt marsh caused no reduction in biomass production as measured by above ground biomass at the end of the second growing season following oil addition. Application of 0.25 L m^{-2} of crude oil to *S. alterniflora* salt marsh caused little damage either to the existing stocks or to the regeneration of new plants. DeLaune *et al.*(1979) observed no significant changes in the regeneration of new shoots and above ground biomass of *S. alterniflora* four and 16 months after adding one, two, three, four, and eight L m^{-2} of oil to the marsh under field conditions. Various studies have indicated that biomass in *S. alterniflora* is not sensitive to crude oil application at as much as 32 L m^{-2} (DeLaune *et al.* 1979, 1984; Smith *et al.* 1981, 1984).

1.3 Effects of Oil Spills on Microbial Biomass

In general, microbial biomass increases initially after an oil spill in marsh sediments. Studies have documented changes in wetland microbial populations in response to oil impacts (e.g., Kator and Herwig, 1977; Hood*et al.* 1975). These responses were generally increases in total microbial populations and increases in the ratio of hydrocarbon degraders to total heterotrophs. However, little is understood about how these changes in microbial numbers affect the turnover of oil components and the length of time for remediation of wetland systems.

The rate and extent of microbial degradation of petroleum hydrocarbons is largely determined by environmental conditions. These conditions include temperature (Bartholomew and Pfaender, 1983), salinity (Bourquin and Pryzybyszewski, 1977), Eh (Hambrick *et al.* 1980, Pardue *et al.* 1988), pH (DeLaune *et al.* 1981), and the oxygen and nutrient status of the environment (Cooney, 1984). Biodegradation of petroleum hydrocarbons is primarily an aerobic process, requiring the presence of molecular oxygen (high Eh). Several novel microbial processes have been identified that degrade oil components under anaerobic conditions (e.g., degradation of BTEX compounds (Hutchins *et al.* 1991) and naphthalene under denitrifying conditions (Milhelcic and Luthy, 1988a, b). It is likely that other anaerobic processes have yet to be determined, however, it is recognized that aerobic processes act on a broader spectrum of compounds and are more rapid and complete (e.g., mineralization to CO_2 and H_2O).

1.4 Vegetative Response to Burning

Fires in wetland habitats occur naturally (Wilbur and Christensen, 1983; Davison and Bratton, 1988) and as a marsh management tool fire is used extensively in North America (Kirby *et al.* 1988). Periodic or annual burning is also used as a marsh management tool in Louisiana. In areas of intense management such as state refuges, as much as 80% of the marsh is burned annually as compared to the estimated 25 to 30% of

marshes burned in other areas (Feijtel *et al.* 1985). After a fire, there is an increase in species richness in marsh habitats (Davison and Bratton, 1988). Burning changes the relative importance of species in *S. cynosuroides* and in *Juncus* marshes (Hackney and de la Cruz, 1983). In other marsh systems, no change in species composition was found due to fire (Vogl, 1973; Van Arman and Goodrick, 1979). Shifts in the relative importance of species were reported due to fire in Florida marshes (Schmalzer *et al.* 1991).

The post-fire recovery of productivity is dependent on many factors including the species present when burning occurs during the growing season. Significant increases in regenerating culms, plant gas exchange, and above ground production were found in annually burned *S. pectinata* as compared to biennially burned *S. pectinata* in a natural tallgrass in Kansas (Johnson and Knapp, 1993). Similar findings are reported in the coastal marsh species *S. bakeri* in Florida (Schmalzer *et al.* 1991). Live biomass in burned *S. bakeri* marsh did not recover to preburn levels in one year (Schmalzer *et al.* 1991). Total biomass of *Cladium jamaicense* was only 38% of the unburned stand 18 months after burning (Steward and Ornes, 1975). On the other hand, productivity in a *S. cynosuroides* marsh was enhanced by burning (Hackney and de la Cruz, 1983). In other marsh habitats, studies report little change in productivity or reduced production due to burning (Smith and Kadlec, 1985a, b; Turner, 1987). The recovery appears to be rapid in marshes that die back annually. For example, burned *Panicum hemitomon* marshes produced greater live biomass within six months of burning compared to unburned stands (Vogl, 1973; van Arman and Goodrick, 1979).

Grass burning stimulates new growth of above ground biomass, but the effects on root biomass in most cases are unknown. The effects on root biomass, however, may be significant to marsh ecology because roots make up 90 to 95% of most organic peat soils. Plant health, growth, and productivity are important since intertidal marshes with limited mineral sediment deposition are maintained primarily by plant growth and organic detritus accumulation (DeLaune *et al.* 1983). Marsh burning reduces the organic source, which may indirectly affect marsh aggradation in areas experiencing aggradation deficits. Burning during dry periods can cause damage to plant root systems, which in turn may accelerate marsh deterioration in unstable coastal areas and lead to pond formation (Hoffpauer, 1968). While in stable coastal marsh regions burning may be an acceptable practice, in certain other areas where large aggradation deficits exist, marsh burning may reduce the source of organics below the critical level needed for maintaining a viable marsh.

1.5 Burning as a Method of Oil Removal

The technique involves igniting the oil (Freiberger and Byers, 1971); the procedure results in rapid burning of the volatile oil components. Various igniters (including Knotax) and primers (such as gasoline and kerosene) are used to facilitate the ignition. To increase effectiveness, combustion promoters such as wicking agents, thermal insulators, and volatility modifiers may also be used (Energetex Engineering, 1979). A successful

burning operation, however, may leave a thin, viscous film ranging between 0.5 to 1.2 mm in thickness on the marsh surface (American Petroleum Institute, 1985). The technique is controversial at best. It is considered inefficient in certain habitats (Ford, 1970; Der and Ghormley, 1975; Logan *et al.*1975), while in other habitats burning is regarded as one of the most effective cleanup techniques available (Vandermeulen and Ross, 1977). For instance, in an oil affected *Spartina* marsh in Texas, burning partially removed oil from vegetation. Some heavily oiled vegetation still showed an oily residue on unburned portions of stems. Within six months, however, burned unoiled and lightly oiled vegetation had recovered rapidly while heavily oiled unburned marshes showed only moderate recovery (Holt *et al.* 1978).

1.6 Rationale and Significance

Various agencies have examined both the susceptibility of individual wetland habitats to oil spills, and the proper cleanup methods for insuring habitat recovery. The potential impacts of burning include disturbance and death of biota from the direct effects of burning as well as the potentially toxic effects of residual compounds. These compounds include unburned oil and burned oil products that may penetrate the lower sediment where degradation is slow and the potential for long-term harm to biota is great. These residual materials may have lethal or sublethal effects on various organisms. Marsh burning also results in a temporary loss of cover, the loss of detrital materials important to the food web, and the loss of other functions such as feeding and resting areas for birds and other wildlife species. In marsh habitats, burning destroys vegetation and other organisms through direct heat effects. Uncontrolled burning may also have adverse effects on adjacent marshes not directly affected by oil. Some species are particularly sensitive to burning and may recovery slowly or not at all from a surface burn. Despite the potential risks, the effects of surface burns on oiled marsh biota have not been studied in detail.

While the existing work does address some oil effects on vegetation it does not address the combined oiling and burning effects. In addition, very little is known about the behavior and fate of hydrocarbon compounds (or residual compounds left by burning) in various marsh systems. We recently completed two oil related research projects (Pezeshki and DeLaune, 1993; DeLaune *et al.* 1994). These studies, however, addressed macrophyte responses to oiling and did not address oiling and burning. Using a multidisciplinary approach, we are attempting to address not only the effects of such stressors on biota but also the movement and degradation of hydrocarbon compounds (with or without burning) in various habitats. We are examining several questions including: What are the specific effects of oil spills on biota in each marsh type (salt and fresh)? What are the consequences of burning for marsh biota in various marsh habitats? What percentages of carbohydrate components are removed by burning? What happens to the specific residual carbohydrate compounds that are byproducts of burning? What are the degradation rates of these compounds?

While our project will by no means answer all of these questions, we have attempted to provide some insights into various aspects of oil spill burning. Our research provides immediate benefits to Louisiana's coastal/interior wetlands by allowing us to evaluate burning as a method of oil removal in specific habitats. Our research also addresses several areas of concern listed in the RFP for OSRADP such as the environmental consequences and effectiveness of *in situ* burning in a salt marsh (second year) and fresh marsh (third year). Finally, our research will contribute to an understanding of U.S. Gulf Coast marsh habitats' sensitivity to oil spills followed by *in situ* burning and how the spill timing affects the life cycles of marsh species. The recovery of various habitats and the post-treatment lethal/sublethal effects of burned and unburned oil residual components are being investigated. Based on this information, the feasibility of burning as a method of oil removal in various marsh habitats is being evaluated and its effectiveness quantified.

1.7 Hypotheses

1. Burning of oiled marsh can be used as a remediation technique in selected marsh habitats of the U.S. Gulf Coast.
2. Burning impact is short-term (1-2 years). Marsh recovery following oil spills is enhanced by the oil removal effects of burning.

1.8 Objectives

Our study allows quantification of oil spill and burning impacts on several species representing salt marsh, brackish and fresh water habitats (greenhouse studies-Year 1), a field *in situ* study in a salt marsh habitat (Year 2), and a field *in situ* study in fresh marsh habit (Year 3). The specific objectives to evaluate:

1. The effects of oiling and burning on flora in selected marsh habitats.
2. The mechanisms of such impacts on marsh macrophytes.
3. The impact of oiling and burning on flora recovery and dynamics during the post-treatment period.
4. The percentage removal of oil compounds by fire and the degradation of various residual compounds that are byproducts of burning (Phase I).

2.0 Methodology

2.1 Methods

The study is being conducted in three phases. Phase I of the study was conducted in the greenhouse and laboratory (Year 1). Phases II and III are being conducted in the field and are designed to complement and reconcile the laboratory experiments performed in Year 1. The work plan and time schedule for Phase II are shown in Table 2.1.

2.1.1 Laboratory and Greenhouse Studies (Phase I-Year 1) Completed (1994/1995)

South Louisiana "sweet" Crude (SLC), which is enriched with light aromatic hydrocarbons, paraffins and olefins, was used in the greenhouse studies. The SLC is moderately toxic to various organisms and is degraded by indigenous microflora. Sediment cores 30 cm deep and 15 cm in diameter containing *Spartina alterniflora/S. patens* (representing salt marsh/brackish habitat) and *Panicum hemitomon/Sagittaria lancifolia* (representing fresh water habitat) were collected from Louisiana marshes. The cores were transferred to a greenhouse for the study. Replicated cores were randomly assigned to main treatments of (1) oiling, (2) oiling plus burning, (3) no oiling or burning, plants clipped at marsh surface, and (4) a control (no oiling or burning).

Cores containing plants were placed in large containers, 75 cm deep. The containers were partially filled with water from the field site where plants were collected. Oil was added to these containers at 2 L m^{-2}. The water level was raised slowly to mimic high tide conditions until it reached 25 cm above the soil surface of each pot. After eight hours, the water was released to mimic low tide by removing rubber stoppers and allowing the water level to fall slowly to the soil surface. In this manner, the plants were coated with oil in a way which mimicked the rise and fall of tides. The pots designated for burning were then subjected to burning by ignition. The plants were monitored continuously for the entire period of post-treatment evaluations. Sediment/plant cores were kept waterlogged and/or a two to three inch floodwater layer was maintained. We compensated for evaporation by adding freshwater to the pots daily. Each month, the soil was flushed and water from the respective field sites was used to fill the pots.

The study was conducted in a greenhouse over the evaluation period allowing observation and measurements of plant responses to oiling, oiling plus burning, and control treatments. The experimental design for the greenhouse was a randomized block design with a factorial treatment arrangement with four replications.

2.1.2 Field Studies (Phase II - Year 2)

Based on the greenhouse experiments, a study area was established in a salt marsh located in Terrebonne Basin in the Pointe Au Chien Wildlife Management Area (Phase II - 1995/1996). An additional *in situ* burn study will be conducted in a fresh marsh during Phase III (1996/1997).

Table 2.1 Work plan and time schedule of salt marsh field burn study conducted in Year 2

	1995						1996					
FIELD STUDIES (salt marsh)	**J**	**A**	**S**	**O**	**N**	**D**	**J**	**F**	**M**	**A**	**M**	**J**
- site selection	X											
- obtain permit	X											
- plot layout	X											
- oiling/burning		X								X		
- plant growth	X	X	X	X	X	X		X	X			X
- regeneration	X	X	X	X	X	X		X	X			X
- biomass	X	X	X	X	X	X		X	X			X
- carbon/nitrogen flux	X	X	X	X	X	X		X	X			X
- data analyses, report writing									X	X	X	X

Each plot was 2.4 m x 2.4 m with enclosures made of plywood and aluminum sheets installed to a depth of 15 cm into the sediment to minimize oil leaks. Oil was applied slowly at 2 L m^{-2} to the surface of the marsh in each plot. Seventy-two hours after the completion of treatment, the designated plots were burned following the procedure described in detail by Breuel (1981) and the American Petroleum Institute (1985 Manual). Any floating oil remaining on the unburned plots was collected for proper disposal. The retainers were removed to allow normal water exchange between each plot and the surrounding area.

The experimental design was a randomized block field design with a factorial treatment arrangement. Four replications were performed. Two burn times (summer and spring), two oiling levels (oiled and unoiled), and two burning levels (burned and unburned) were used. Oil (South Louisiana Crude) was applied at a rate of 2 L m^{-2} and designated plots were burned. Data analysis was conducted using the Statistical Analysis System (SAS). The package is available to researchers through the Louisiana State University Network Computer Systems.

Proposed measurements include those outlined below. Field measurements were taken about one week, two weeks, one month, two months, four months, and eight months after each burn cycle (see Work Plan/Time Schedule).

2.1.2.1 Site Selection and Burn Permit

The Pointe Au Chien Wildlife Management Area South of Houma, Louisiana was selected as the site for conducting the salt marsh and fresh marsh *in situ* burn studies. The management area was ideal for the studies because it had limited public access and roads leading to the marsh sites. The roads were used to transport plot construction material during the post monitoring phases of the studies. Use of the roads kept marsh damage to a minimum.

A Louisiana coastal use permit for the study received final approval from the Coastal Management Division of the Department of Natural Resources on June 19, 1995. The approved coastal use permit (C.U.P. No. P950232) is in the possession of the principal investigator (C.W. Lindau). Additional approvals from the Louisiana Department of Health and Hospitals and the Department of the Army, Corps of Engineers (New Orleans District) were received and are in the possession of the principal investigator.

On October 17, 1995 a second coastal use permit was requested to conduct the proposed burn study in the fresh marsh during the third year of our project (96/97). The permit was approved on December 27, 1995 and is in the possession of the principal investigator. Additional approvals from the Department of Wildlife and Fisheries, the Department of Health and Hospitals, and the Department of the Army have also been received.

2.1.3 Proposed Measurements for Phase I, II and III

Various measurements outlined in this proposal are being conducted during Phase I, II and III as applicable. These methodologies and the necessary instruments are presently available at the Wetland Biogeochemistry Institute and Nuclear Science Center.

2.1.3.1 Plant Growth and Regeneration

Culm density (vegetative and reproductive) and the density of culms in flower is being recorded for each plot/treatment throughout the study.

To assess changes in carbon flux and net photosynthesis from plant/soil systems, light and dark chambers are placed over each sub-plot in the field for determination of CO_2 and other biogeochemical gas fluxes (Smith *et al.* 1981). Light chambers constructed from 3 mm clear Plexiglass with 0.366 m^2 in the cross-sectioned area and an internal volume of 281 liters are used for measurements. Chambers similar in dimension insulated with Styrofoam (2 cm thick) and covered with a reflective space blanket are used for dark CO_2 flux measurements (respiration). Method and calculations are performed according to Smith *et al.* (1981).

Measurements are conducted using portable infra-red gas analyzers (ADC, model A120 and PP systems, Model CIRAS-1). These techniques have been previously used for evaluation of plant responses both in the laboratory and in the field (Pezeshki and DeLaune, 1988; Pezeshki *et al.* 1989). Implementation of these methods provides useful information on seasonal patterns of plant gas exchange for each study habitat.

2.1.3.1.1 Biomass

Changes in above ground and below ground biomass are being determined in Phase I, II and III experiments. In the greenhouse, replicated pots were harvested for assessing the above ground and below ground biomass components. In the field, the above ground biomass is being measured by cutting the vegetation at sediment level using a 1.00 m^2 quadrat in randomly selected sub-plots. The subplots are carefully marked in each area to avoid resampling. The above ground materials are cut at sediment level and sealed in plastic bags. In the laboratory, biomass is separated into live and dead fractions and dried to a constant weight. In addition, the live materials are separated into stem and leaf components. In determining below ground production (in the field), replicated surface cores are taken simultaneously with the above ground sampling in each plot. The below ground samples are processed as described by Schubauer and Hopkinson (1984) and Hopkinson and Dunn (1984). Sections (10 cm) from 15 cm diameter cores are placed in a solution of sodium metaphosphate, shaken, and then washed through a sieve to separate the organic size fraction. The organic material is then separated into live and dead fractions. After determining the live and dead components, production is calculated according to Schubauer and Hopkinson (1984).

2.1.3.1.2 Plant Community Structure

The plant composition, structure and density is determined in study plots in the field. In addition, plots are being photographed for a visual record of change. The procedure is described in detail by Kadlec and Wentz, 1974; and Smith and Kadlec, 1985 a, b.

2.1.3.2 Degradation and Compositional Changes in Hydrocarbon Components

Following burning, two degradation mechanisms are being quantified: (1) loss of oil components during burning, and (2) potential for microbial degradation by wetland microbiota following burning. To assess loss mechanisms from burning, soil cores (10 cm x 15 cm deep) are taken from each plot immediately following treatment to determine the crude oil fraction remaining in the sediment. Cores are extruded and vertically sectioned into 4 cm increments according to DeLaune *et al.* (1983). The core section is dried at 28C and ground to pass through a 25 mesh sieve. Soil is extracted using supercritical fluid extraction with a suitable modifier. The extract is fractionated on activated alumina. Hydrocarbon fractions are analyzed with GC-MS using a modification of EPA Method 8720. A mass balance approach is used in all studies.

To assess loss from microbial degradation, identical cores are removed from each plot at one, four and eight weeks following treatment. Cores are extracted and analyzed as described above. Loss of oil during this post-burn period is caused primarily by microbial degradation plus abiotic processes such as volatilization. Loss rates are correlated with measurements of microbial biomass, as described above.

2.1.3.2.1 Oil Extraction and GC-MS Techniques

Sediment samples (about 4 g) with and without oil (South Louisiana Crude and Arabian Crude), that are both burned and unburned are extracted for hydrocarbons using a modified extraction procedure similar to Koques *et al.* (1994). Sediment/oil samples are transferred to Teflon centrifuge tubes for hydrocarbon extraction. The extracting solution is a 1:1 mixture of hexane and acetone. Twenty ml of the extracting solution is added to the Teflon tubes and the tubes are then shaken for 12 hours. After shaking, the Teflon tubes are centrifuged at 10,000 rpm for 13 minutes at laboratory temperature. The hexane/acetone solvent is decanted at the top and anhydrous sodium sulfate is added to remove trace amounts of water. The solvent mixture is evaporated down to 10 ml using ultra high, pure nitrogen gas. The solvent/hydrocarbon mixture is then diluted 100 times to permit GC-MS analysis. From the diluted sample, 1 ml is added to GC-MS reduced volume vials and 0.04 ml of internal standard is added to each vial before GC-MS instrumental analysis.

Hydrocarbon analyses are performed on a gas chromatograph (Hewlett Packard 5890 Series II plus) equipped with an automatic sampler and a HP-5 high resolution capillary column (30 m, 0.25 mm film thickness, 0.25 mm i.d.). The capillary column is directly interfaced to a quadruple mass spectrometer (Hewlett Packard 5972 Mass Selective Detector). The carrier gas is ultra high pure helium. Flow rate is 1.0 ml/min, injector temperature is 300 ^{o}C, column temperature is programmed from 50 ^{o}C to 310 ^{o}C at 8 ^{o}C/min rate with an initial 3.0 minute delay and a 15.0 minute hold at the end of the run. The interface to the mass selective detector is maintained at 28 ^{o}C. Sample and

standard injections are made using a Hewlett Packard 7673 automatic liquid sampler into a splitless injection port.

A Hewlett Packard Vectra 486/66 XM computer system and Hewlett Packard 61034C software for the MS chem station (DOS series) are used to collect and analyze data. Hydrocarbon peaks are identified using the G1033A NIST PBM Library software.

3.0 Results and Discussion: Phase II - Salt Marsh

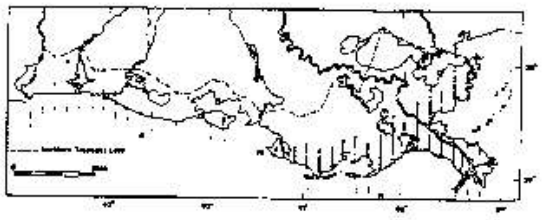
3.1 Plant Species

Spartina alterniflora Lois. (Figure 3.1) is a perennial grass that grows from extensive rhizomes and forms dense stands over broad areas of the marsh. The *Spartina alterniflora* plants grow in brackish and salt marshes; culms are thick and two to four feet tall with wide tapering leaves. *Spartina alterniflora* is a major detritus contributor to aquatic food chains and is also called oystergrass or smooth cordgrass.

3.2 Field Design

Plywood plot enclosures (2.4 m x 2.4 m) were constructed and installed in the salt marsh prior to each burn cycle. Metal sheeting was used to line the burn treatment plot enclosures. Treatments consisted of: (1) control (no oiling or burning), (2) oiling, and (3) oiling plus burning. Oil treatments received 2 L m^{-1} of South Louisiana Crude (Figure 3.2). Treatments were replicated four times for statistical analysis. A 10 to 15 cm floodwater layer above the salt marsh sediment surface was required before the burn plots were ignited. A propane torch was used to ignite the South Louisiana Crude.

Two burns were initiated in the *Spartina alterniflora* marsh. The late summer/fall burn was conducted on August 17, 1995 and the spring burn was initiated on April 17, 1996.



Spartina alterniflora Lois.

Gramineae

Oyster grass
Smooth cordgrass
Seacane

Spartina alterniflora is a perennial grass growing from extensive rhizomes. Culms are erect, 2 to 4 feet tall, thick, and spongy; leaves are wide and tapering. The inflorescence is a long panicle with tight, erect spikes. The plant grows in intermediate to saline marshes, often forming dense stands over broad areas. It is a major contributor of detritus to aquatic food chains; rhizomes are eaten by muskrats and nutria.

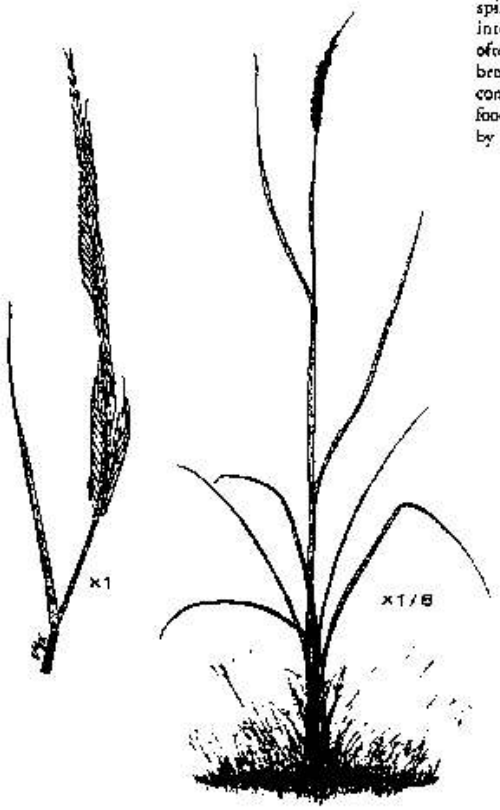


Figure 3.1 *Description of Spartina alterniflora grass.*

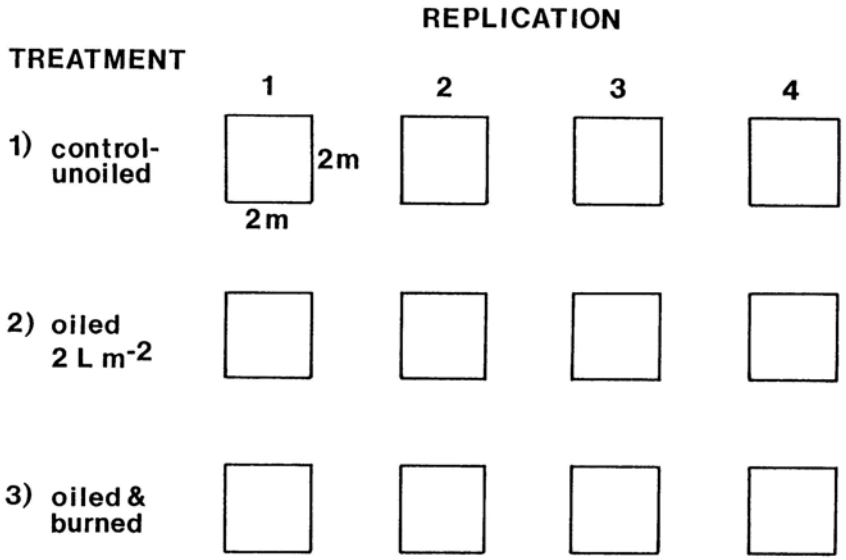


Figure 3.2 *Field plot experimental design for salt marsh burn (Phase II).*

3.3 Results From First *In Situ* Burn

3.3.1 Shoot Regeneration

Shoot regeneration capacity of the control, oiled, and oiled plus burned plots for the summer/fall burn are graphed in Figure 3.3 and Figure 3.4 for the *Spartina alterniflora* marsh site. Shoot regeneration was evaluated at two, four, 12, 16, 41, 46 and 50 weeks after oiling and burning. Measurements were not taken during the winter and early spring months (16 to 41 weeks). Figure 3.3 displays the number of new or live stems measured on each sampling date for each treatment. Each number represents the mean of four replications per treatment. For the control treatment, the number of live stems per plot ranged from 10.6 (week four) to a high of 32.2 live stems per chamber at 50 weeks. Application of South Louisiana Crude reduced shoot regeneration. In the oiled plots, the number of live shoots ranged from 4.5 (week four) to 23.3 recorded 50 weeks after the burn. The greatest effect on stem count was observed in the oiled plus burned treatment. Two weeks after the burn, 3.6 new shoots per chamber were measured and stem count steadily increased to 34.3 at 50 weeks.

Figure 3.4 displays the percentage of live stems of the oiled and oiled plus burned treatments compared to the control mean (100%) over the seven sampling dates. Oiling reduced the stem count to about 35% of the control 12 weeks after oiling, but at 50 weeks stem count was up to 72% of the control mean value. Two weeks after burning, the percentage of new shoots/stems was about 30% of the control replications, and stem count steadily increased to approximately 120% at 41 and 46 weeks. At 50 weeks, the

percentage was about 105% compared to the control mean stem count. At the end of the 50 week period, shoot regeneration in the burn plots was equal to the control, but regeneration in the oiled plots was about 30% below the control mean.

3.3.2 Plant Height

Plant height was monitored over a 50 week period (two, four, 12, 16, 41, 46 and 50 weeks) after the first burn (8/17/95) for the three treatments (Figures 3.5 and 3.6). Plant height (cm) of the *Spartina alterniflora* plants in the control plots remained fairly constant over the seven sampling intervals and ranged from 78 to 93 cm. Plant height in the oiled treatment plots did not differ much from control values. For the oiled plots, plant height decreased for the initial 16 week period after oil application (89 to 68 cm), but during the spring of 1996 oiled plant height increased from 90 to 95 cm (Figure 3.5). Burned plots showed the greatest change in plant height over the 50 week study period. Two weeks after the burn, average plant height was about 24 cm. *Spartina alterniflora* height steadily increased in the burned plots, and at 50 weeks a mean height of 105 cm was measured.

Figure 3.6 compares the plant height measurements of the oiled and oiled plus burned treatments to the control mean value. Height measurements taken in the oiled plots averaged about 97% of the control mean height over the seven sampling intervals. In the burned plots, the plant heights' percentage of the control increased from 24% (at two weeks) to 105% at 50 weeks.

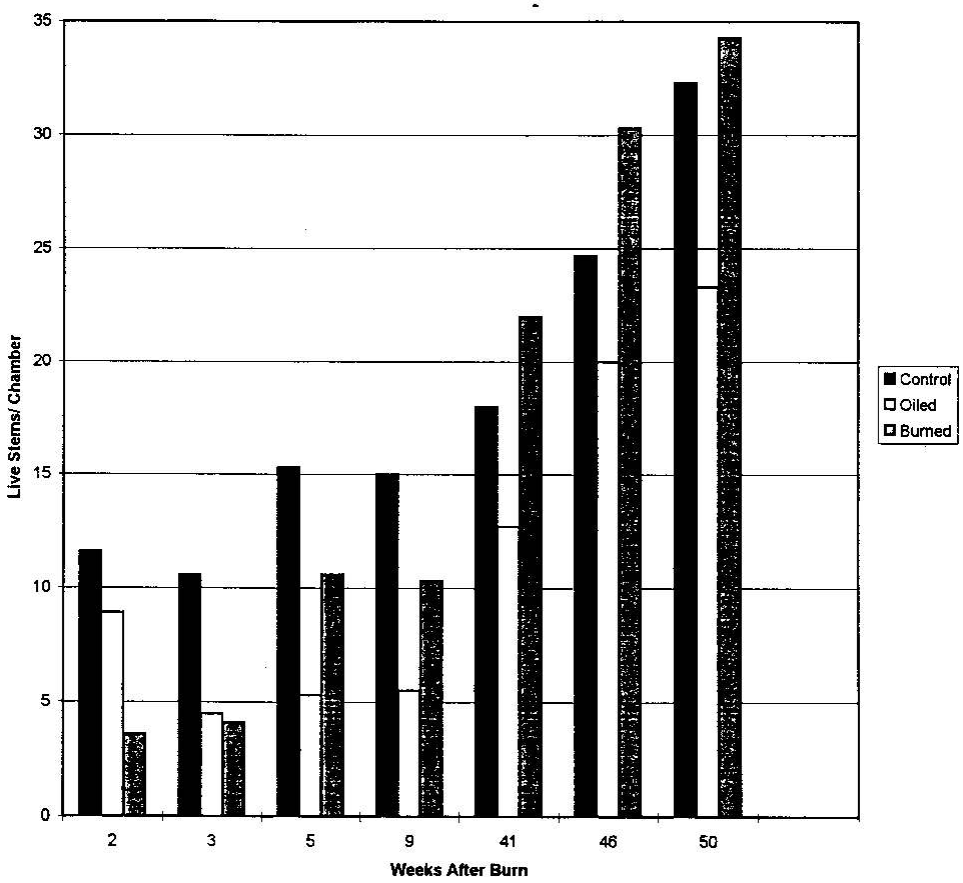


Figure 3.3 *Number of live stems of Spartina alterniflora, by treatment, after the first burn. Each observation is a mean of four values.*

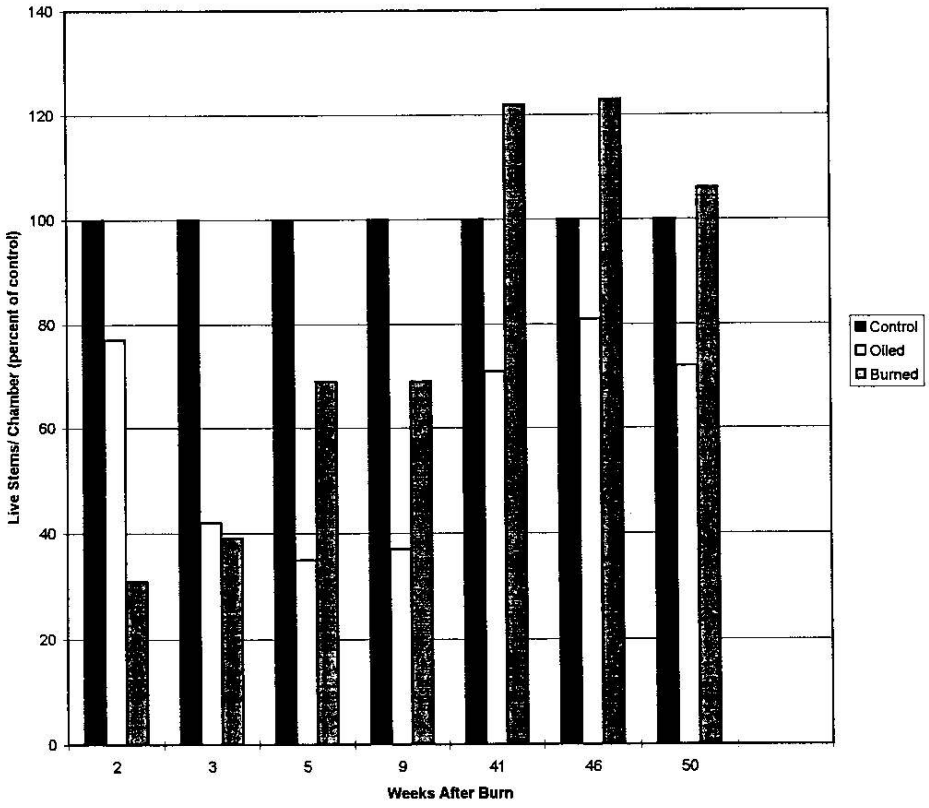


Figure 3.4 *Percentage of the number of live stems of the oil and burn treatments compared to control values (100%).*

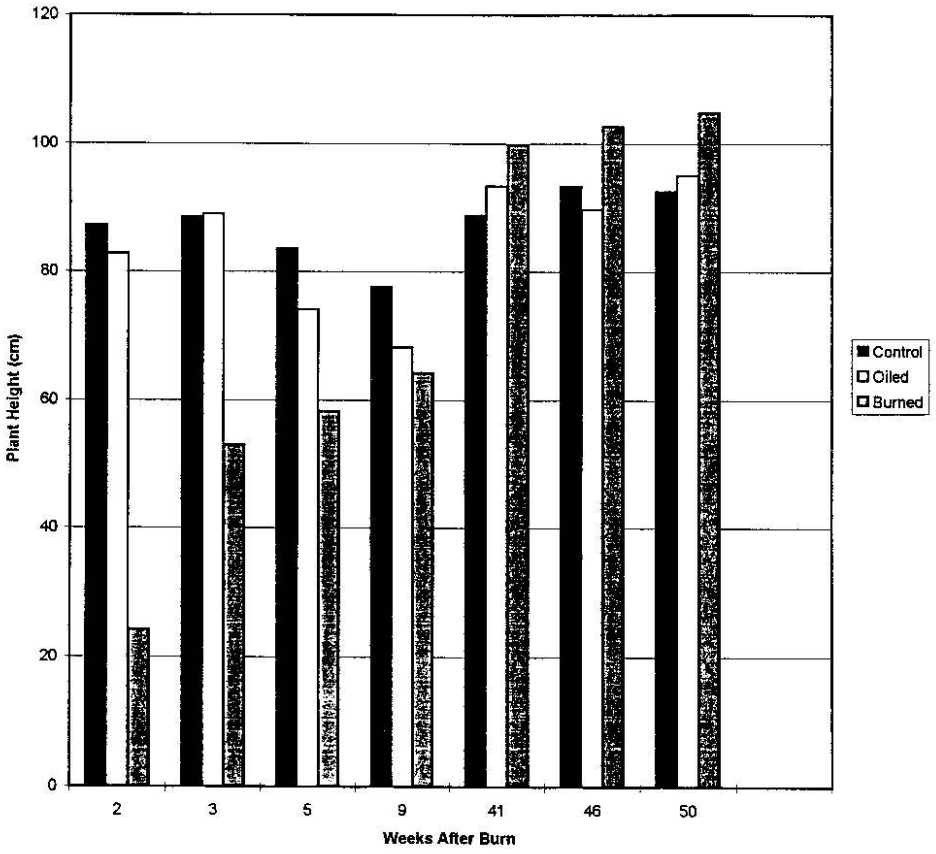


Figure 3.5 *Maximum height of Spartina alterniflora after the first burn. Each observation is a mean of four values.*

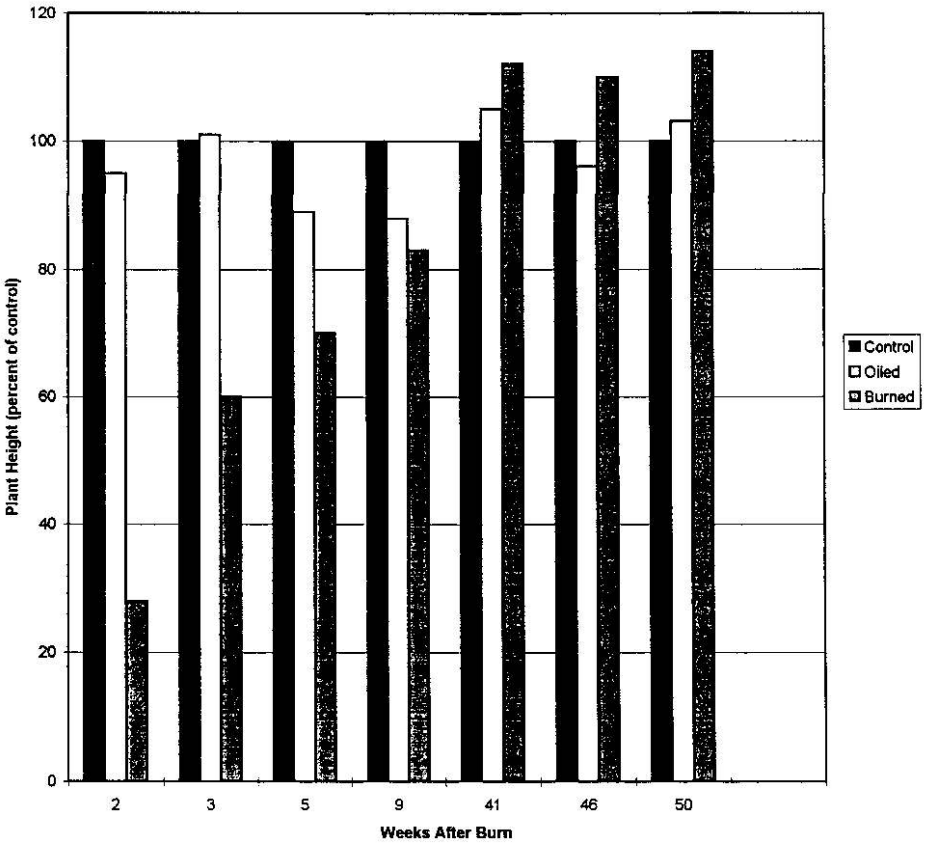


Figure 3.6 *Percentage of plant height of the oil and burn treatments compared to control values (100%).*

3.3.3 Carbon Fixation

Gross carbon fixation for the control, oiled, and oiled plus burned treatments was measured in the salt marsh experimental plots two, three, five, nine, 15, 41, 46, and 50 weeks after the 8/17/95 burn. Carbon fixation mean rates are graphed in Figures 3.7 and 3.8 and are expressed in g CO_2-C fixed $m^{-2}h^{-1}$ and as percentages of the control means (Figure 3.8). Carbon fixation in the control plots remained fairly constant over the eight sampling periods and ranged from a low of 0.74 g CO_2-C $m^{-2}h^{-1}$ (15 weeks) to a high of 1.40 g CO_2-C $m^{-2}h^{-1}$ recorded during the summer of 1996 (46 weeks after the burn). Carbon fixation in the oiled plots was drastically reduced for the first 15 weeks after oiling (Figure 3.7). Over this period, gross carbon fixation rates ranged from 0.24 to 0.56 g CO_2-C $m^{-2}h^{-1}$ and the low values were attributed to the oil coating on the *Spartina alterniflora* leaves and stems. In the spring and summer of 1996 (41, 46 and 50 weeks), carbon fixation in the oiled plots increased and averaged 0.96 g CO_2-C $m^{-2}h^{-1}$. The largest range in fixation rates was observed for the oiled plus burned treatment. Two to three

weeks after burning, fixation rates averaged 0.12 g CO_2-C $m^{-2}h^{-1}$ and steadily increased in 1995 to 0.51 g CO_2-C $m^{-2}h^{-1}$. At 41, 46, and 50 weeks, carbon fixation by plants in the burned plots increased, and the means averaged 1.19 g CO_2-C $m^{-2}h^{-1}$ (Figure 3.7).

In Figure 3.8, the percentage of carbon fixation in oiled and oiled plus burned plots is compared to the control mean rates (100%). For the oiled treatment, carbon fixation rates averaged 27% to 87% (41 weeks) of the control means. In the oiled plus burned plots, the carbon fixation percentage ranged from a low of 10% at three weeks to a high of 112% recorded at 50 weeks (Figure 3.8). At 50 weeks after the burn, carbon fixation in the oiled plots was only 68% of the control mean values, but for the burn plots, carbon fixation was 112% of the control mean.

Carbon fixation data collected at the 50 week period was subjected to statistical analysis to determine if control, oiled and oiled plus burned treatment means were significantly different (alpha = 0.05). A general linear model and Tukey treatment means procedures were used (SAS, 1988). Statistical analysis revealed no significant differences in carbon fixation among the control (1.11), oiled (0.76) and oiled plus burned (1.24 g CO_2-C $m^{-2}h^{-1}$) treatment mean values.

3.3.4 Biomass Production

3.3.4.1 First Cutting (12/7/95)

Above ground biomass produced in the control, oiled and burned plots was measured on 12/7/95 and 8/7/96. Biomass production for the two cutting times is shown in Figures 3.9 and 3.10. Biomass is divided into live, dead, and total fractions. Biomass plotted in Figure 3.9 was cut from chamber subplots (0.087 m^2), but on 8/7/96 biomass was cut from 1 m^2 plots at the termination of the study (Figure 3.10). Each value plotted represents a mean of four replications per treatment.

Sixteen weeks after the first burn, biomass cut and collected (12/7/95) from the control plots averaged 25.5 g for the live fraction and 25.8 g per subplot for the dead component. Live biomass for the oiled treatment was much less (8.3 g per subplot), but the dead fraction was higher (30.6 g) compared to the control live and dead biomass fractions, respectively. As expected, the burned treatment *Spartina alterniflora* biomass components were greatly reduced compared to the control fractions. Live plant material totalled 9.3 g per subplot, and the dead plant component averaged 5.4 g for the burn treatment. Total biomass produced (live plus dead fractions) from the control, oiled and oiled plus burned treatments averaged 51.3, 38.9 and 14.7 g per subplot, respectively, 16 weeks after initiation of the first burn cycle (Figure 3.9). Total biomass produced from the oiled plots averaged about 76% of the control total mean, and the burned plots only averaged approximately 29% at 16 weeks.

3.3.4.2 Second Cutting (8/7/96)

Fifty-one weeks after burning, biomass was cut from the *Spartina alterniflora* 1 m^2 control, oiled and burned plots. The results are plotted in Figure 3.10. Control plot above ground biomass components averaged 662 g m^{-2} and 14 g m^{-2} for the live and dead fractions, respectively. The live component cut from the oiled plots averaged 559 g m^{-2}. This amount was about 100 g m^{-2} less than the control live biomass measured (Figure 3.10). The dead biomass fraction of oiled plots averaged 364 g m^{-2} over the four replications. This represents a 26-fold increase in dead biomass compared to the control plots and was mainly attributed to the presence of oil and residual oil components. The greatest live biomass was measured in the burned treatment plots. Approximately 722 g m^{-2} were clipped from each burn plot (Figure 3.10). The amount of dead biomass (burn plots) averaged 115 g m^{-2}. Total biomass cut from the control, oiled, and oiled plus burned plots averaged 676, 923, and 867 g m^{-2} respectively, over four replications per treatment. Total biomass measured in the oiled plots represented 136% of what was cut from the control plots. Total burn plot above ground biomass increased approximately 28% above control values.

A statistical analysis was run on the above ground biomass fractions cut and collected from the 1 m^{-2} plots. Statistical procedures (SAS, 1988) were used to determine the significance between biomass treatment means.

No significant differences were calculated between live biomass for the control (662 g), oiled (559 g), and oiled plus burned (752 g) treatment means. Significant differences were observed for the dead biomass fractions. The control dead biomass mean (14.1 g) was significantly lower (= 0.05) than the oiled dead biomass mean of 364 g. The burned treatment dead biomass mean (114.6 g) was not significantly different than the oiled and control means. Total biomass (live plus dead) was not significantly different among the three treatments (control-676 g, oiled-923 g and burned-867 g).

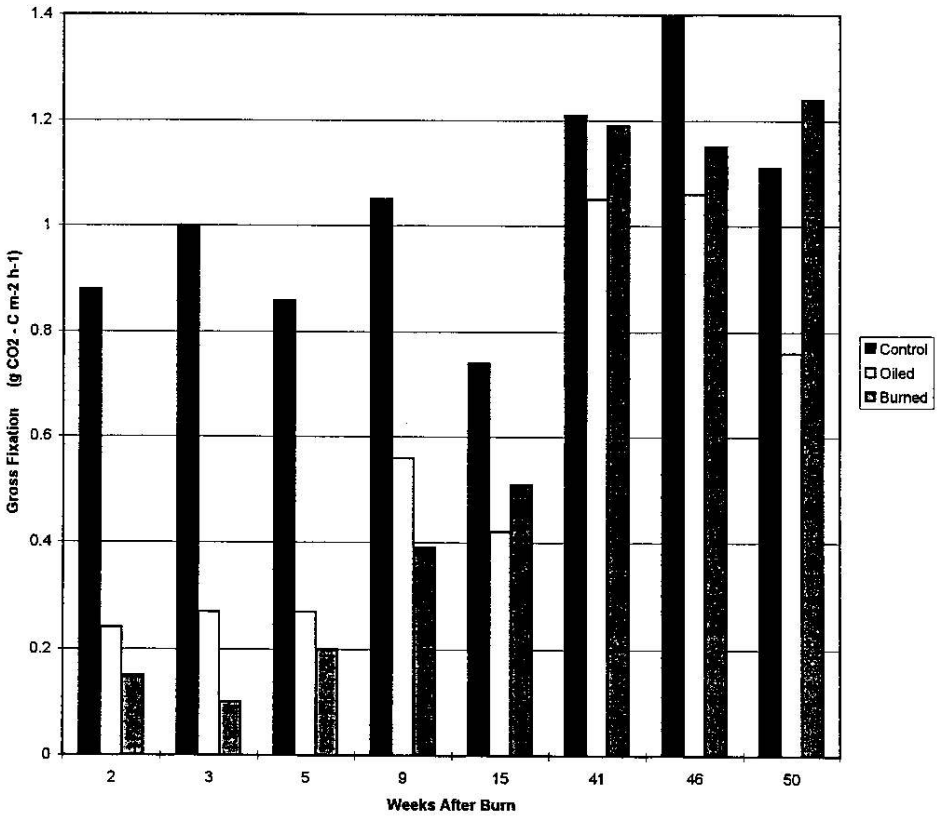


Figure 3.7 *Carbon fixation of Spartina alterniflora treatments after the first burn. Each observation is a mean of four values.*

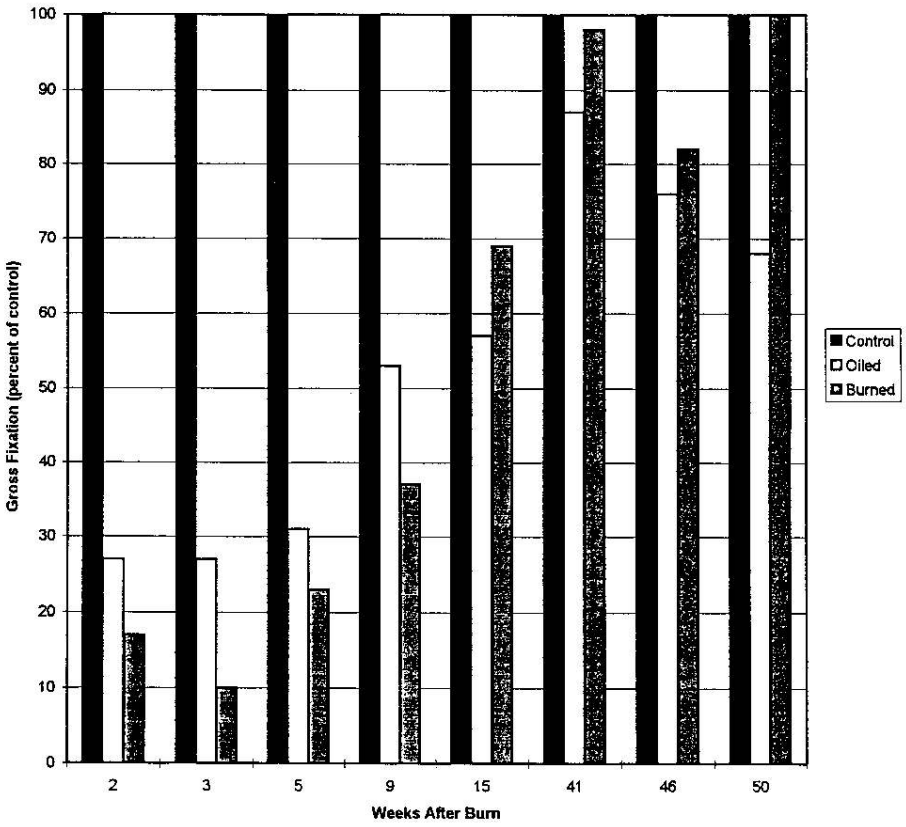


Figure 3.8 *Percentage of carbon fixation of the oil and burn treatments compared to control values (100%).*

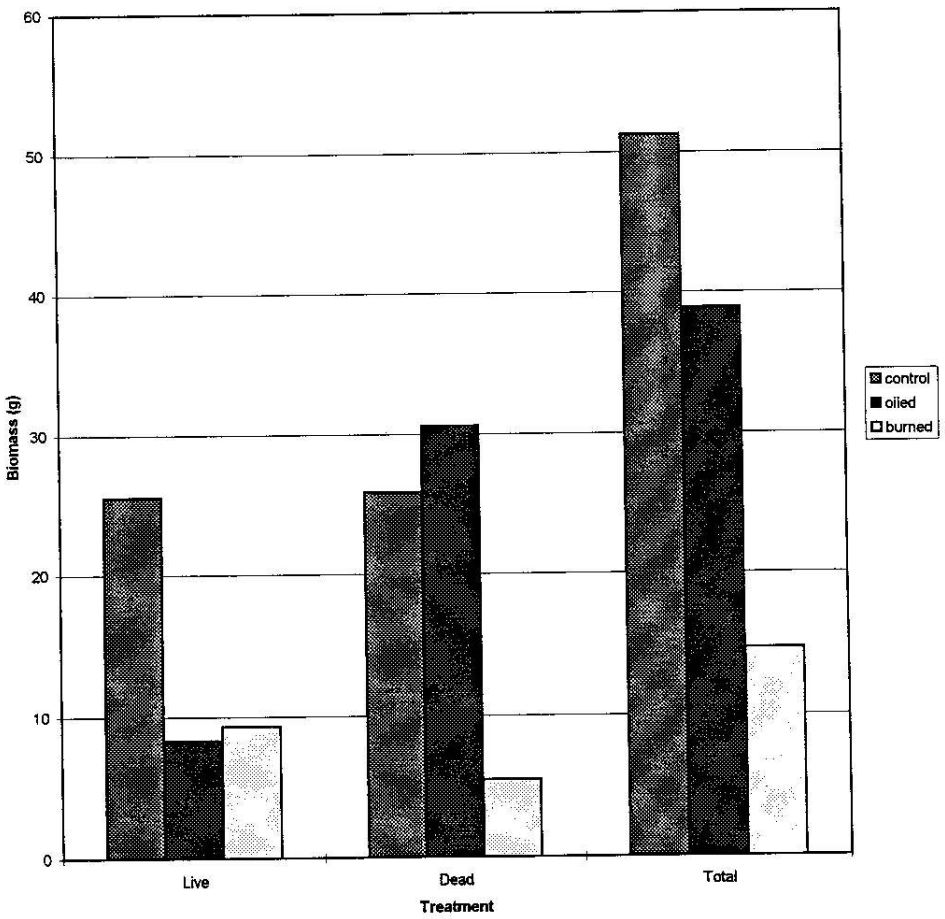


Figure 3.9 *Biomass production of Spartina alterniflora treatments at the first cutting (12/7/95). Each observation is a mean of four values.*

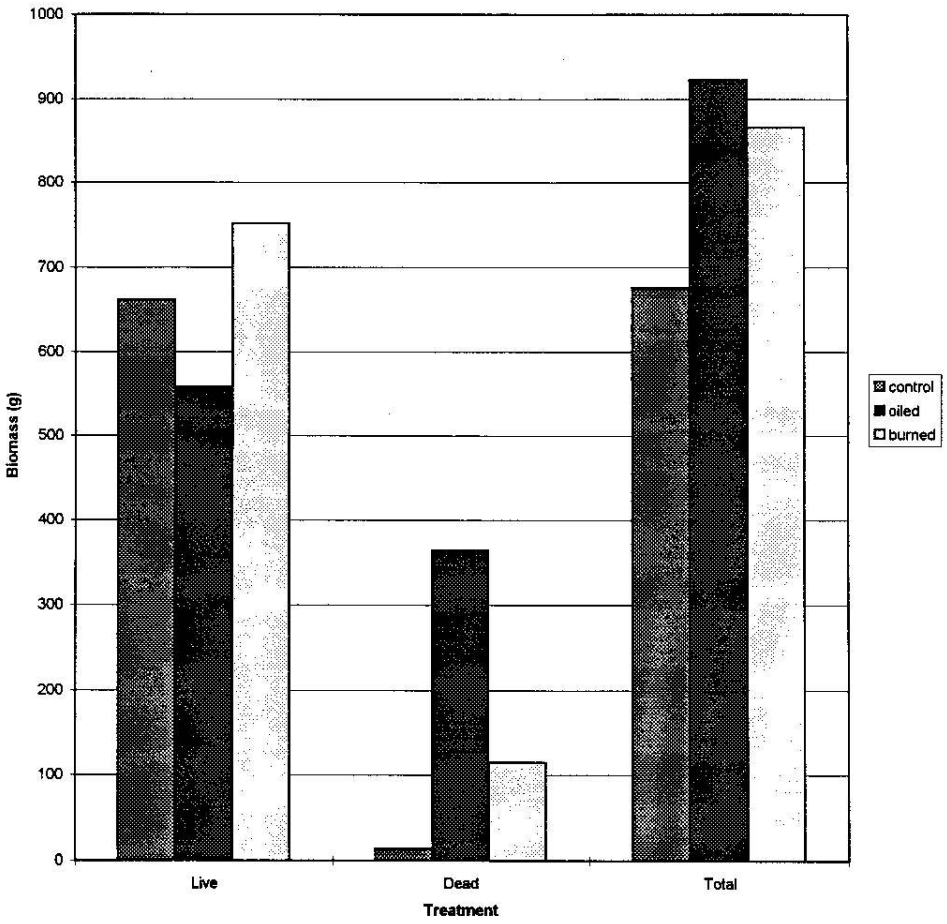


Figure 3.10 *Biomass production of Spartina alterniflora treatments at the second cutting (8/7/96). Each observation is a mean of four values.*

3.4 Results From Second *In Situ* Burn

3.4.1 Shoot Regeneration

Shoot regeneration of the control, oiled and oiled plus burned treatments for the spring 1996 burn cycle are plotted in Figures 3.11 (live shoots per chamber) and 3.12 (percentage of the control). Shoot regeneration was evaluated at five, seven, 10, 12 and 15 weeks after the burn (4/17/96). *Spartina alterniflora* shoot regeneration in the control plots steadily increased from 14.8 live stems per chamber (week five) to 32.3 recorded at 15 weeks. Regeneration in the oiled plots was severely reduced after oiling. Live stems averaged 0.8 per plot at seven weeks and increased to 5.5 stems at 15 weeks (Figure 3.11). Live stems recorded in the burn chambers were also much lower than the control values but were higher than the oiled treatment values. For the burned plots, live stems

per chamber increased from 4.0 (week five) to 14.2 recorded 15 weeks after the burn. At 15 weeks, the number of live stems per chamber for the oiled and oiled plus burned treatments was approximately 17% and 44% of the control plot mean values, respectively (Figure 3.12).

3.4.2 Plant Height

Plant height (cm) was monitored for 15 weeks after the second burn. Heights were measured and recorded for each treatment replication. Measurements were made at five, seven, 10, 12, and 15 weeks and are graphed in Figures 3.13 (cm) and 3.14 (percent of control). Over the study period, *Spartina alterniflora* plant height measured in control plots ranged from a low of 75.8 cm (week five) to a high of 97.5 cm recorded at week 12 (Figure 3.13). Oiled treatment plant heights were much less than control heights and ranged in height from 37.5 cm (week five) to 59.5 cm at week 15. The greatest increase in plant height occurred in the burned plots. At five weeks, the average plant height was 26.3 cm and at the final measuring, the average plant height was 91.5 cm (Figure 3.13).

Figure 3.14 plots the oiled and oiled plus burned treatments as a percentage of the control plant heights. Over the five sampling dates, the mean plant heights measured in the oiled plots ranged from 49% to 64% of the control mean height values. For the burned plots, plant height percentages steadily increased from 35% (week five) to 99% at week 15 (Figure 3.14). The oil only treatment appeared to have the greatest affect on *Spartina alterniflora* plant height.

3.4.3 Carbon Fixation

Gross CO_2-C fixation for the control, oiled and burned treatments was measured at five, seven, 10, 12, and 15 weeks after the second burn. Gross fixation in g CO_2-C $m^{-2}h^{-1}$ is plotted in Figure 3.15 and the percentage of gross carbon fixation is graphed in Figure 3.16.

Control plot carbon fixation means ranged from a low of 1.04 g CO_2-C $m^{-2}h^{-1}$ (week 15) to a high of 1.96 g CO_2-C $m^{-2}h^{-1}$ (week 10). Averaged over five sampling dates, the control treatment carbon fixation rate was 1.47 g CO_2-C $m^{-2}h^{-1}$. Carbon fixation in the oiled plots was drastically reduced. Rates ranged from 0.02 to 0.34 g CO_2-C $m^{-2}h^{-1}$ with an average fixation value of 0.19 g CO_2-C $m^{-2}h^{-1}$. Fixation rates in the oiled plus burned plots were also much lower than the control mean values but higher than oil carbon fixation means (Figure 3.15). Burned plot fixation means varied from 0.12 g CO_2-C $m^{-2}h^{-1}$(week 5) to 0.66 g CO_2-C $m^{-2}h^{-1}$ measured 12 weeks after oiling and burning. Average burn plot fixation was 0.42 g CO_2-C $m^{-2}h^{-1}$ over the five sampling dates.

The percentage of carbon fixation in the oiled plots compared to the control plot fixation rates ranged from 1% (five weeks) to 18% measured at 12 weeks (Figure 3.16). For the oiled plus burned treatment, percentages ranged from 8% (week five) to 50% (week 15). After 15 weeks, the burned plot carbon fixation rate was one-half of the control plot values and the oil treatment fixation rate was approximately one-seventh (Figure 3.16).

Carbon fixation data collected at 15 weeks was subjected to statistical analysis. The General Linear Model Procedure (SAS, 1988) showed significant (= 0.05) differences among treatment carbon fixation means. The control treatment fixation mean (1.04 g CO_2-C $m^{-2}h^{-1}$) was significantly higher than the oiled mean (0.15 g CO_2-C $m^{-2}h^{-1}$) and the burned mean value (0.52 g CO_2-C $m^{-2}h^{-1}$). No significant difference was computed between the burned and oiled carbon fixation treatment means.

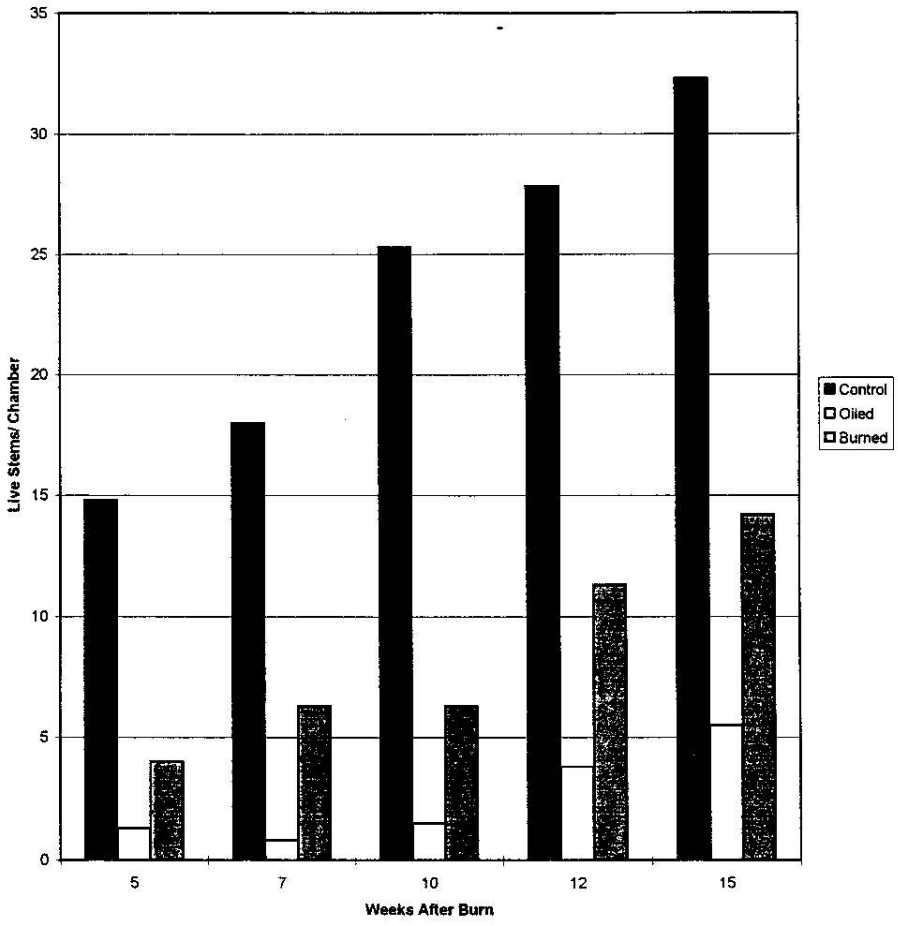


Figure 3.11 *Number of live stems of Spartina alterniflora, by treatment, after the second burn. Each observation is a mean of four values.*

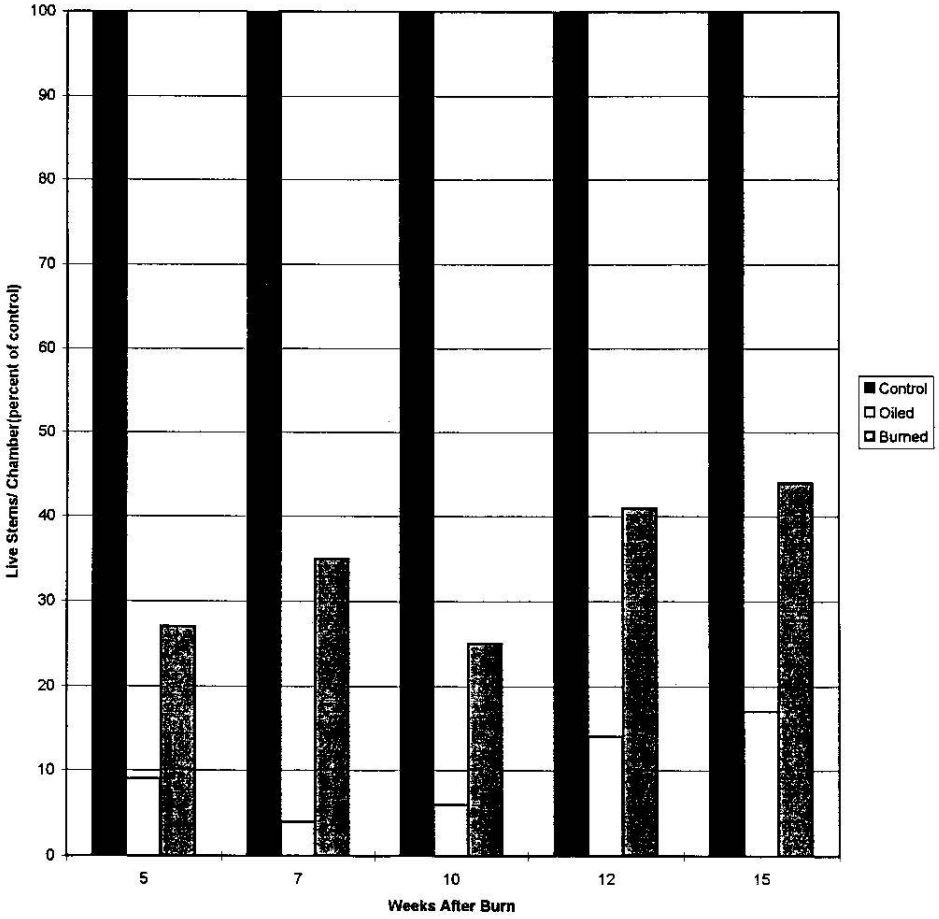


Figure 3.12 *Percentage of the number of live stems of the oil and burn treatments compared to control values (100%).*

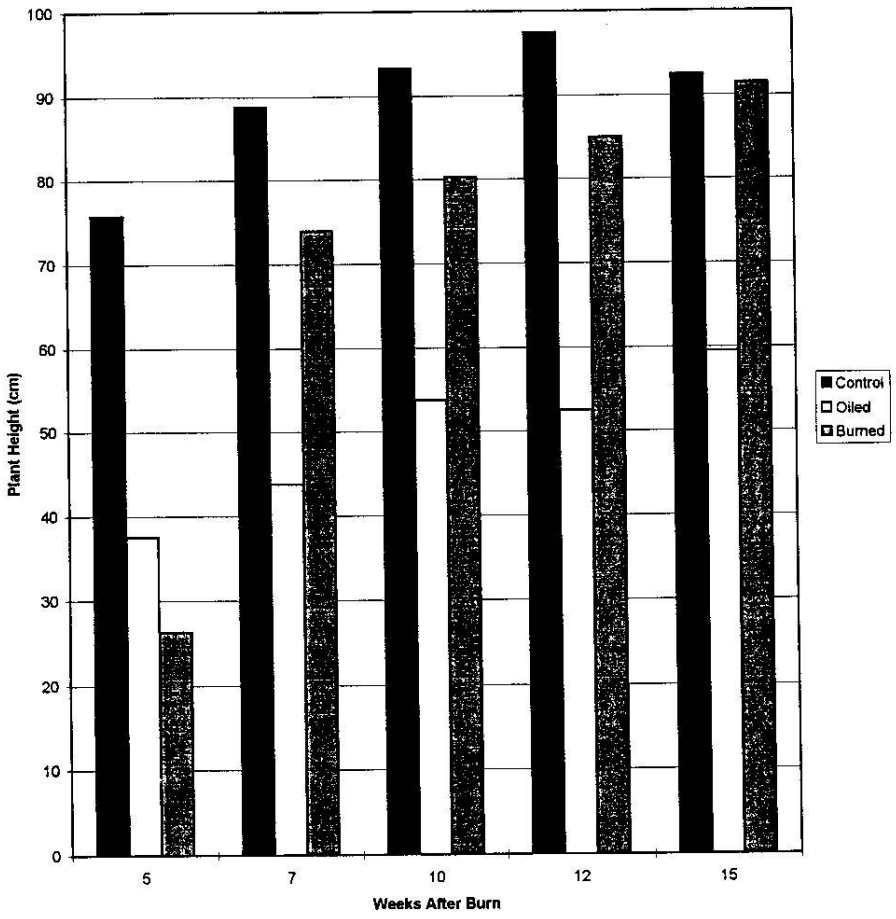


Figure 3.13 *Maximum height of Spartina alterniflora after the second burn. Each observation is a mean of four values.*

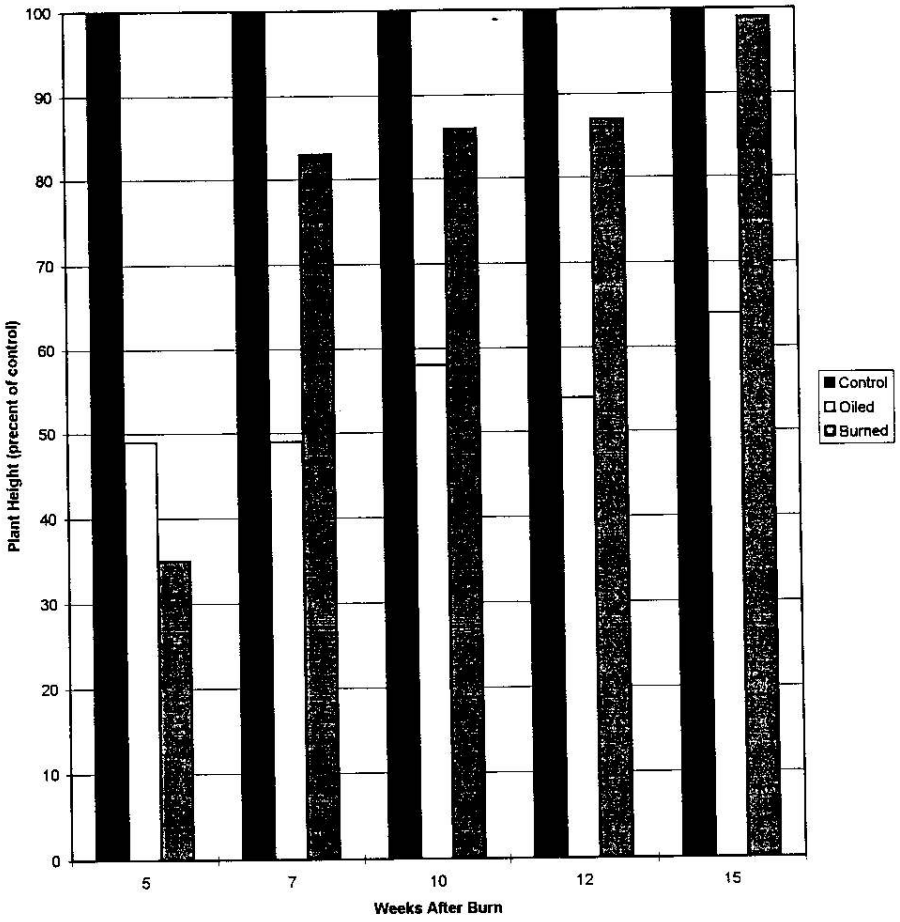


Figure 3.14 *Percentage of plant height of the oil and burn treatments compared to control values (100%).*

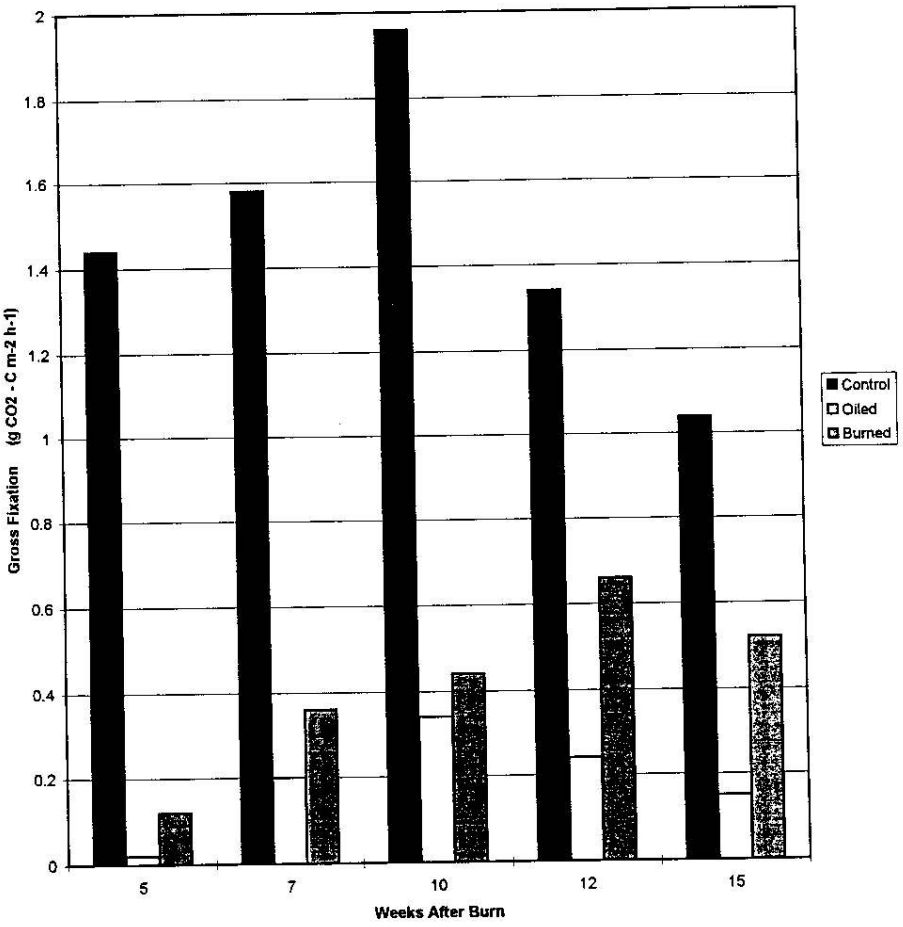


Figure 3.15 *Carbon fixation of Spartina alterniflora treatments after the second burn. Each observation is a mean of four values.*

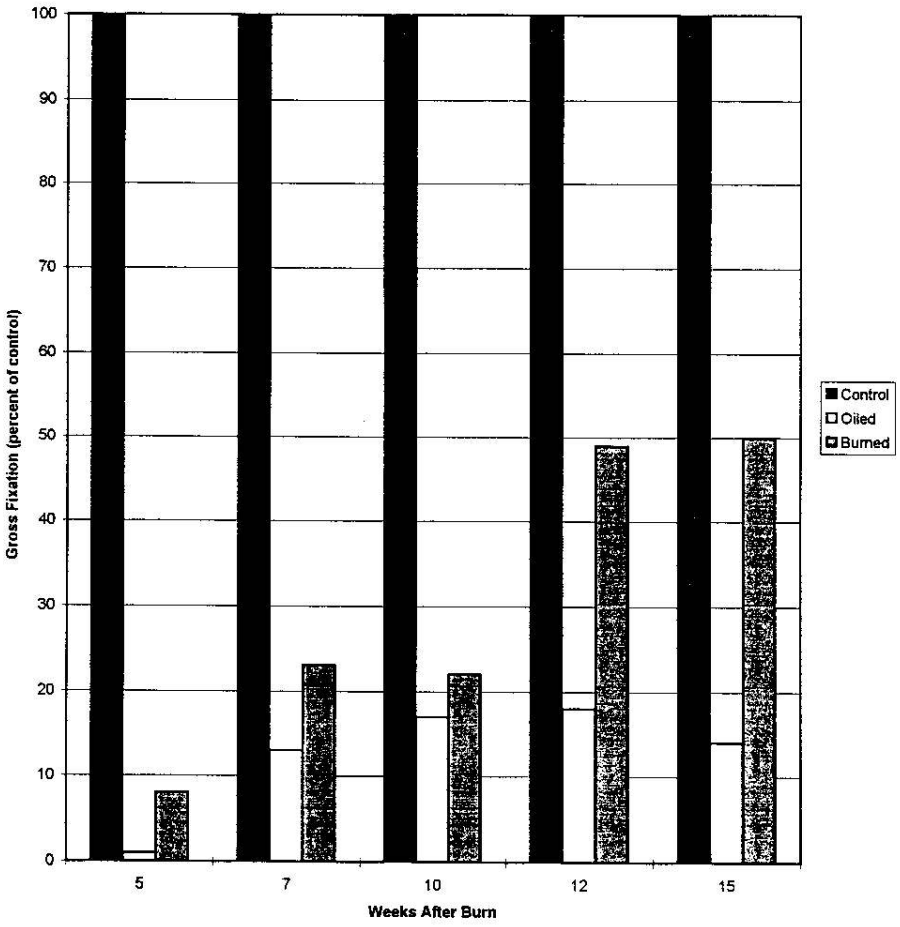


Figure 3.16 *Percentage of carbon fixation of the oil and burn treatments compared to control values (100).*

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