REMOVING OIL AND SAVING OILED MARSH GRASS USING A SHORELINE CLEANER

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ABSTRACT: *A new shoreline cleaner, which was specially developed during the cleanup of the* Valdez *spill in Alaska, was tested to determine its effectiveness in removing oil from Louisiana Gulf Coast marsh grass thus minimizing the oil impact. Intact plugs of* Spartina alterniflora *containing living plants, roots, and soil microbial communities were collected from salt marshes and transferred to a greenhouse. Plant photosynthesis, respiration, and stomatal conductance were monitored following various oiling and cleaning scenarios. The treatments included: oiled, oiled and cleaned after one day, oiled and cleaned after two days, cleaner only, and control. Plant recovery depended upon the degree of oiling and the type of oil used. Fouling with bunker C oil caused almost total plant mortality unless the plants were cleaned with the shoreline cleaner. South Louisiana crude oil was less toxic but cleaning accelerated recovery as was evident by photosynthetic activity and other plant functions such as regeneration of new shoots. Collectively, these studies demonstrate the potential for saving an oiled* Spartina alterniflora *marsh by use of this shoreline cleaner in a real oil spill.*

Marshes are an important part of riverine, estuarine, and coastal ecosystems. A significant amount of petroleum is refined, stored, or transported through these areas and some marshes are therefore subject to occasional fouling with oil. Marshes provide fish and wildlife habitat and can improve water quality.[13] Thus, there is generally great public attention and pressure to minimize negative impacts of oil in marshes.

Oil can have adverse effects on marsh vegetation ranging from short-term depressions of photosynthesis to near total mortality.[1,2,15] This may in turn affect adjacent aquatic habitats because marsh vegetation provides detritus to food webs there.[13] Fortunately, the adverse effects of oil on marsh vegetation can be temporary.[4]

Oil can also have adverse effects on soil microbial communities. Burns and Teal[3] found that oil persisted in salt marsh sediments even seven years after one spill. The microbial community is important because it regulates the flow of energy from plants to food webs and also controls nutrient regeneration in marsh soils, which affects plant growth.[10] Thus, oil may have long-term effects on marsh functions even after plant growth resumes.

Chemical and mechanical methods are available to minimize negative effects of oil in wetlands. Oil can be physically removed from the wetland or concentrated with absorbent materials or burned. But wetlands are particularly vulnerable to mechanical damage during removal operations.[14] Determining a cleaning strategy therefore involves trade-offs balancing physical damage to the marsh and oil toxicity. Short-term damage to wetland ecosystems can be acceptable when it prevents long-term ecological impacts.[9] Sometimes, the best approach may be no action because physical damage and toxicity problems associated with the available cleaning techniques may be greater than allowing the oil to remain in the wetland.[12,14]

A need therefore exists for techniques that reduce adverse impacts of oil on wetlands without causing additional toxicity problems or affecting adjacent ecosystems. Teas and colleagues[19] recently showed that Corexit 9580 prevented mortality of oiled red mangroves (*Rhizophora mangle*) by removing oil that otherwise suffocated roots. Corexit 9580 shoreline cleaner, developed during the cleanup in Alaska of the *Valdez* spill,[7] is a low toxicity, low dispersion cleaner whose cleaning effectiveness and low toxicity have been confirmed in tests at Environment Canada[6] as shown in Table 1. This paper examines the effectiveness of Corexit 9580 for cleaning oiled *Spartina alterniflora Loisel.*, which is common in salt marshes. Although other plant species may be exposed to oil spills in riverine and estuarine habitats, *S. alterniflora* often dominates salt marshes.

Materials and methods

Vegetation samples. Intact plugs of living salt marsh were collected from Louisiana salt marshes and acclimated to greenhouse conditions. The plugs were 15 cm in diameter and 20 to 30 cm deep; they contained rooted plants roughly 40 cm tall. Plugs were collected by inserting a 30 cm long, sharpened PVC pipe into the marsh, removing the pipe and enclosed soil, and placing a PVC cap over the bottom of the plug. Plugs were returned to the greenhouse where they were stored in 38 L plastic tubs filled with diluted artificial seawater. Seawater was at 8 ppt salinity, similar to the bayou water where the plugs were collected. Plugs were watered with tap water as needed to maintain 5 to 10 ppt

Table 1. Toxicity and cleaning effectiveness of various cleaners[1]

Product[2]	Toxicity to rainbow trout (LC-50 ppm)	Oil removal in lab tests (%)
Corexit 9580	>5600	42
Value 100	4250	2
BP 1100X AB	2900	23
Tornado	1350	3
Corexit 7664	850	27
Pyrr	650	11
Firezyme	521	3
Siallon emulsifier	375	8
Breaker-4	340	13
Balchip 215	157	3
IDX 20	140	7
Oil Spill Eater	135	11
Bioversal	120	11
Nokomis 3	110	13

1. After Fingas et al[6]
2. Ranked by toxicity

salinity in the water contained within individual plugs. Vegetation was also washed weekly with a mist of tap water to remove salt that accumulated on the leaves.

Oiling and cleaning vegetation. The South Louisiana crude or bunker C oil was applied to vegetation. The South Louisiana crude was weathered by storing the oil in uncovered pans inside a greenhouse. Oil was applied to individual plants using a cotton glove dipped in oil. Oil remained on the vegetation from 1 to 3 days. Corexit 9580 was used to remove oil from vegetation. Cleaner was applied to vegetation, approximately 4 oz of chemical per test core, with a manual squeeze-type hand sprayer, and allowed to soak for 15 minutes. Plants were then gently washed with a garden hose. Oil-contaminated seawater was replaced with clean seawater after cleaning.

Whole plug gas exchange measurements. Photosynthesis rates for each plug were estimated from the difference between the rates of CO_2 change in light and dark chambers.[11] Light and dark chambers were placed over entire plugs in standing water to make an airtight chamber 1 m tall and 25 cm in diameter. Light chambers were constructed of clear Plexiglas and equipped with a rubber stopper for withdrawing gas samples. Dark chambers were similar, but covered with aluminum foil to block sunlight. The internal air volume was determined from the chamber diameter, chamber height, and water depth.

Before sampling, chamber air was mixed by repeatedly withdrawing and reinjecting air with a 60 mL syringe. Air samples (15 mL) were collected 15 minutes after the chambers were placed over the plugs. Trial runs indicated that detectable changes in the CO_2 content of the air sample were noticed in 6 minutes. The amount of time elapsed between the initial and final sample was recorded to the nearest minute. Air samples were stored in 10 mL Vacutainers (Becton Dickinson and Co., Rutherford New Jersey) and returned to the laboratory. Subsamples (1 mL) were analyzed for CO_2 content with a gas chromatograph (Shimadzu, model GC-14A) and thermal conductivity detector.

Plug respiration, which included vegetation and soil communities, was estimated from the increase in CO_2 concentration after a plug was placed in the dark chamber. Measurements made during darkness reflect only respiration, which produces CO_2. Measurements made during light reflect both CO_2 from respiration and CO_2 depletion by photosynthesis.

Leaf area gas exchange measurements. Measurements of photosynthetic photon flux (PPF), leaf temperature, and leaf conductance to water vapor were made on replicated sample leaves on several days following treatment initiation in each experiment. Leaf conductance was measured using a steady-state porometer (LI-1600, Li-Cor, Inc., Lincoln, New England). After recording leaf conductance, the same leaf was used for rapid net photosynthesis measurement using a portable gas exchange system (DC, Model A120, Field Analytical System, Analytical Development Co., England). The leaf was enclosed in the chamber, and PPF and differential CO_2 levels were recorded. Rates of net photosynthesis were calculated from the flow rate of air through the chamber and from the CO_2 partial pressure differences between incoming and outgoing air. These data are very labor intensive to collect, but they are very precise and particularly suited for evaluating short-term plant responses to environmental perturbation.

Experiment One. Eight plugs were randomly assigned to each of five treatments. South Louisiana crude oil was weathered to a 13 percent weight loss and applied to the lower 12 inches of vegetation, which simulated oiling during calm water but high tide conditions. The five treatments were: (1) oiled and never cleaned, (2) oiled and cleaned after one day, (3) oiled and cleaned after two days, (4) never oiled but cleaned after one day, and (5) never oiled and never cleaned.

- *Whole chamber gas exchange*—Gas samples were collected from the headspace of each plug at least once before day 0, when the oil was applied. Stem count data were also collected prior to oiling. Gas samples were collected day 0, day 1, day 2, day 14, day 29, and day 44. Whole plug photosynthesis and whole plug respiration data were analyzed as repeated measures. This statistical method allows correlated observations, such as those collected from the same plug on different days, to be analyzed properly for differences among plugs.[16,18] Data were analyzed with SAS software. All aboveground biomass was harvested on days 111 to 113. Those data were analyzed using a completely randomized design. This experimental design is best suited for detecting small differences that persist over the course of an experiment.

  Results—Oil appeared to have a toxic effect on the plants, but new leaves quickly appeared. These new leaves originated primarily from the tops of oiled stems. The cleaner removed almost all traces of oil from the vegetation. The cleaner also appeared to have a delayed, temporarily adverse effect on the plants. Leaves became yellow and stunted; some died, others eventually recovered.

  Aboveground biomass varied 2-fold among the plugs by the end of the experiment and no significant differences were detected among the treatments (Chi-square = 3.7034, 4 df, $P >$ Chi-square = 0.4476). There was great variability in whole-plug photosynthesis and whole-plug respiration rates among plugs (Figure 1). However, statistical analyses indicated no significant differences among the treatments in whole-plug photosynthesis or whole-plug respiration; differences were attributed to substantial variations in the amount of foliages among the study plugs.
- *Leaf area gas exchange*—Gas exchange measurement on all treatments were conducted on six leaves per treatment on days 2, 7, 14, 23, 28, 38, 56, and 78 with the exception of the oiled and never cleaned plug. With this treatment, original leaves were dead by day 38; thus beginning with day 56, data were collected from new shoots that had emerged following death of the original shoots.

  Results—Time-course response of stomatal conductance shows significant recovery in plugs that were cleaned as compared to control plants (Figure 2). Much of the recovery was noted within the first two weeks following initiation of the treatments. Time-course response of net photosynthesis shows a similar pattern (Figure 3); however, net photosynthetic rates, compared to control plants, did not fully recover throughout the course of this experiment.

  When data were pooled across treatments over the course of the experiment, oiled plants had significantly reduced stomatal conductance (Figure 4) and no photosynthetic activity. The leaves on which these measurements were taken died by day 38. The new shoots that subsequently emerged had stomatal conductance and net photosynthetic rates similar to the control plants, showing no detectable stress effects.

  The application of cleaner after one and two days had similar effects on stomatal conductance but cleaning after one day resulted in significant improvement of net photosynthesis recovery relative to plants cleaned after two days. Application of cleaner alone did not affect mean leaf conductance (Figure 4), but caused significant reductions in net photosynthesis (Figure 5). It is important to note that while cleaner application caused reductions in net photosynthesis, plants treated with cleaner only or oiled and cleaned after one day did not show massive leaf death as seen in the oiled plants. By the end of the experiment, natural leaf regeneration replaced all dead leaves in all treatments, which partially explains why there were no differences in biomass among the treatments.

Experiment Two. Eight plugs were oiled with the same South Louisiana crude used in Experiment 1, but with additional weathering that resulted in a total weight loss of 33 percent. There were two treat-

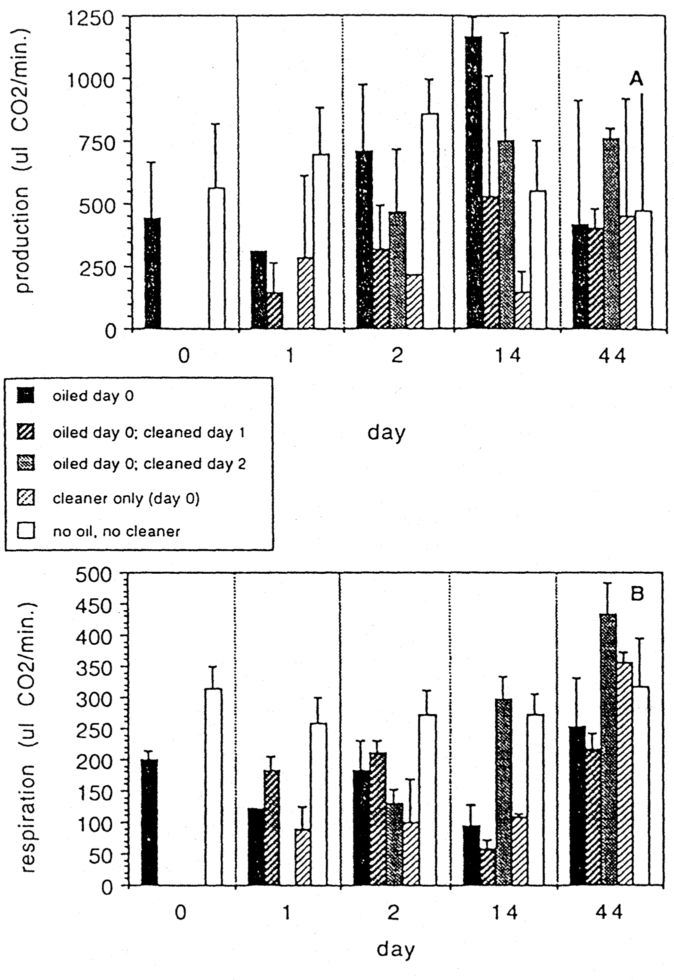

Figure 1. Whole plant gas exchange (A) and soil respiration (B) in plugs over the course of Experiment 1

ments: oiled and cleaned, and oiled and uncleaned. This experiment also differed in that these plants were oiled from top to bottom, which simulated oiling during foul weather conditions. Four of the plugs were cleaned the next day.

- *Whole chamber gas exchange*—Gas samples were collected from all eight plugs on day 0 immediately following oiling, and on days 1, 8, 34, and 47. Whole-plug photosynthesis and whole-plug respiration data were also analyzed using a repeated measures analysis-of-variance design.

  Results.—Complete oiling without cleaning caused massive, rapid death to all existing leaves. Leaf death in the oiled and cleaned plants did not appear as extensive as in the oiled and uncleaned plants. But as in Experiment 1, all plants appeared to recover by the production of new leaves produced by existing stems.

  Aboveground biomass at the end of the experiment averaged 41 g/plug in uncleaned plugs and 34 g/plug in cleaned plugs, but the difference was not significant ($P = 0.8852$). Dead aboveground biomass averaged 37.2 g/plug in oiled plugs and 30.8 g/plug in oiled and cleaned plugs; the difference was not significant ($P = 0.0545$).

  Whole-plug photosynthesis averaged 237 μL CO_2/min in cleaned plugs and 353 μL CO_2/min in uncleaned plugs, but variability among plugs was so great that the difference was not significant ($F = 2.2659$, 1 and 30 df, $P = 0.1439$). Respiration averaged 147 μL CO_2/min in cleaned plugs and 247 μL CO_2/min in uncleaned plugs, but again variability among plugs was so great that the difference was not significant ($F = 2.0503$, 1 and 26 df, $P = 0.1632$).

- *Leaf area gas exchange*—Stomatal and photosynthetic response

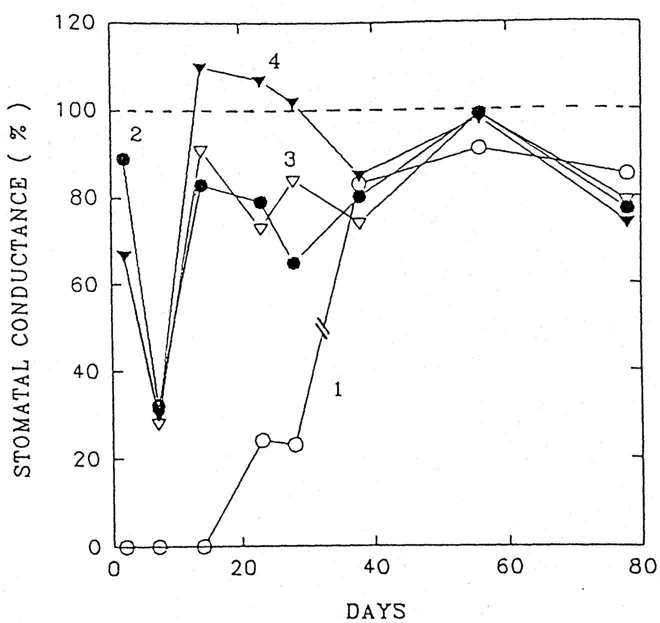

Figure 2. Time-course responses of stomata to various treatments in Experiment 1—Values represent the mean for six measurements shown as percentage of control. Numbers represent treatments: 1. oiled, 2. oiled and cleaned after 1 day, 3. oiled and cleaned after 2 days, and 4. cleaner only. The break in curve shown for treatment 1 represents leaf death; subsequent measurements were conducted on newly emerged leaves.

measurements on plants were conducted on 6 sample leaves per treatment on days 1, 9, 19, 34, and 53 following treatment initiation.

Results—Photosynthetic rates of cleaned leaves were significantly greater than in uncleaned leaves (Figure 6). Beginning on day 34, all original leaves were dead, and the gas exchange mea-

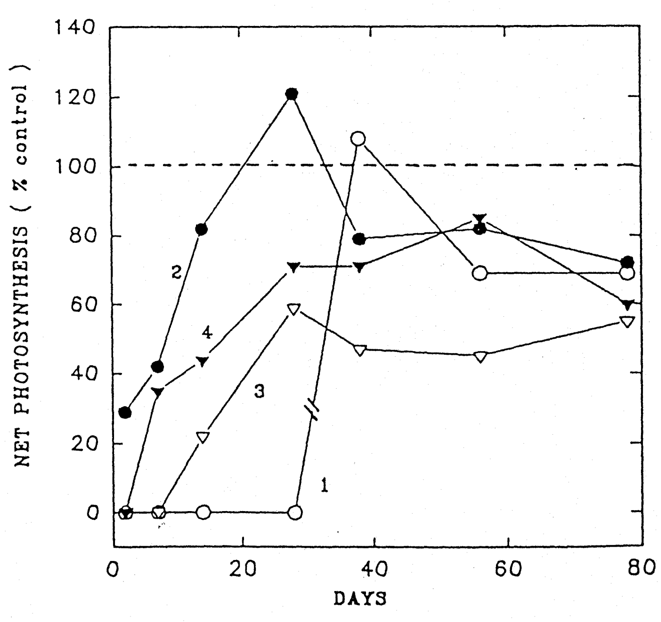

Figure 3. Time-course responses of net photosynthesis to various treatments in Experiment 1—Values represent the mean for six measurements shown as percentage of control. The break in curve shown for treatment 1 represents leaf death; subsequent measurements were conducted on newly emerged leaves. Numbers represent treatments: 1. oiled, 2. oiled and cleaned after 1 day, 3. oiled and cleaned after 2 days, and 4. cleaner only.

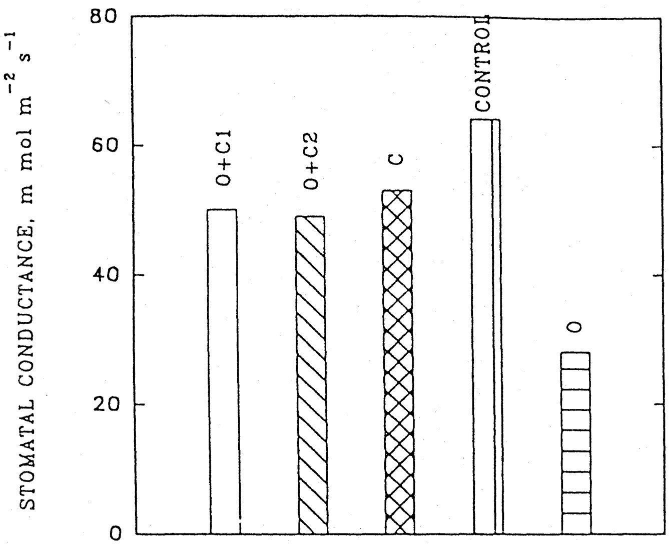

Figure 4. Response of stomatal conductance to various treatments in Experiment 1—Values represent the mean for measurements over the experimental period. O = oiled, O + C1 = oiled and cleaned after 1 day, O + C2 = oiled and cleaned after 2 days, C = cleaner only

surements were conducted on leaves that had emerged after the treatments were applied (Figure 7). The newly emerged leaves had similar gas exchange measurements in the two treatments (Figures 8 and 9).

Experiment Three. Eight plugs were randomly assigned to one of three treatments: completely oiled with bunker C, completely oiled and cleaned, and unoiled and uncleaned (control). Two plugs were assigned to the control treatment; three plugs were assigned to the other treatments.

- *Whole chamber gas exchange*—Gas samples were collected on day 5. Aboveground biomass was harvested on day 49.

  Results—Even though the bunker C formed an extremely thick coating on the plants, the cleaner removed virtually all the oil. Plants in the uncleaned plugs did not recover. Unlike oiling with South Louisiana crude, stems as well as leaves died. Original stems produced no new leaves and only five wilted new stems emerged. On the other hand, the cleaned plants recovered and there was no apparent difference between them and the control plants after eight weeks.

  Five days after treatment initiation, whole-plug photosynthesis was greatest in the control plants, intermediate in the oiled and cleaned plants, and least in the oiled and uncleaned plants (Figure 10). By day 49, live aboveground biomass averaged only 12.2 g in uncleaned plugs, but 43.5 g and 59.1 g in cleaned and control plugs, respectively (Figure 11).

- *Leaf area gas exchange*—Plant photosynthetic activity and stomatal conductances were measured on days 5, 13, 21, and 35 following treatment initiation.

  Results—The overall responses of stomatal conductance and net photosynthesis are presented in Figures 12 and 13. The cleaner improved plant functions as indicated by the greater stomatal conductance and net photosynthesis as compared to plants that were oiled and not cleaned.

Discussion

Effects of oil on *Spartina alterniflora*. Oiled and uncleaned plants had reduced stomatal conductance and no photosynthetic activity. This indicated the potential breakdown of photosynthetic apparatus in leaves directly subjected to oil application. Such breakdown of leaf structure and/or chlorophyll system may occur because of blocked leaf transpiration leading to dramatic temperature increases and/or direct adverse effects of oil penetrating into the leaf tissue. This led to leaf death within 40 days of oil applications. However, new leaves that emerged from oiled plants had photosynthetic rates similar to unoiled plants.

Spartina alterniflora recovered from oiling with South Louisiana crude by the rapid production of new leaves. New leaves were growing from oiled plants within seven days of oil applications. Thus, whole-plug photosynthesis rates quickly recovered from oiling with South Louisiana crude. The emergence of new leaves was the most likely reason that oiled plugs had similar photosynthetic rates as unoiled plugs. It is important to note, however, that the whole-plug technique is not as accurate as the leaf chamber technique for two reasons. Natural variability exists in the number of stems, height of stems, and number of leaves per stem among the plugs. This introduces variability into estimates of whole-plug photosynthetic and respiration rates that can mask treatment effects when the latter are relatively small. Also, the water used to seal the chambers can absorb and release CO_2, which also introduces variability into the estimates of whole-plug photosynthesis and respiration. This variability combined with the extended period of time needed to test for long-term effects masked the short-term adverse effects of oil on photosynthesis. Photosynthetic rates as determined by leaf area and whole-plug techniques were similar in unoiled *Spartina alterniflora* and *Spartina alterniflora* oiled with South Louisiana crude by the end of the measurement period. This finding is in agreement with previous work by DeLaune and colleagues[4] and Smith and colleagues,[17] who also found that *S. alterniflora* stands recovered from oiling with South Louisiana crude. This was not the case when plants were oiled with bunker C, which caused almost complete plant mortality. Whole-plug photosynthetic rates in oiled plants were less than 50 percent the rates in unoiled plants. Oiled stems did not produce new leaves as they did when they were fouled with South Louisiana crude. Several new stems emerged in one oiled plug, but there were too few to counter the death of all other stems and leaves during the course of our experiment.

Whole-plug respiration did not appear to be affected by either South Louisiana crude or bunker C. This agreed with previous studies that found that oil had little effect on soil microbial communities.[5,8] As noted, however, the whole chamber method used was capable of detecting large or modest effects.

Effects of cleaning oiled *Spartina alterniflora*. These experiments indicated that cleaning with Corexit 9580 was beneficial because clean-

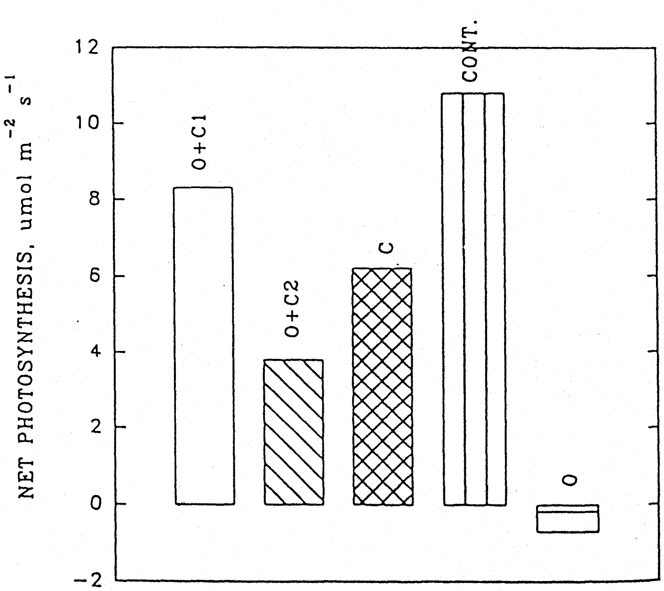

Figure 5. Response of net photosynthesis to various treatments in Experiment 1—A negative value indicates no photosynthesis but tissue was still respiring. Values represent the mean for measurements over the experimental period. O = oiled, O + C1 = oiled and cleaned after 1 day, O + C2 = oiled and cleaned after 2 days, C = cleaner only, CONT. = control

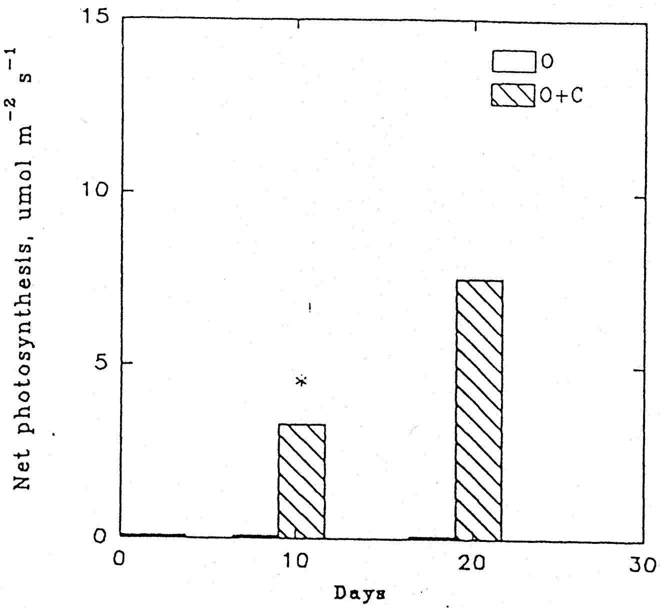

Figure 6. Time-course response of net photosynthesis to various treatments in Experiment 2—Measurements were conducted on the existing leaves during the 30-day period following treatment initiation. O + C = oiled and cleaned; O = oiled but not cleaned (bars not visible because values were close to zero)

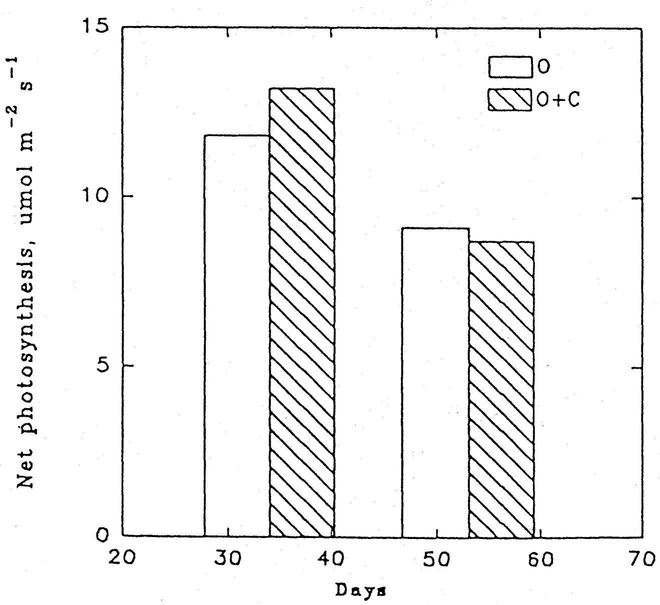

Figure 7. Time-course response of net photosynthesis to various treatments in Experiment 2—Measurements were conducted on new leaves that emerged during the 30-day period following treatment initiation. O = oiled and not cleaned, O + C = oiled and cleaned

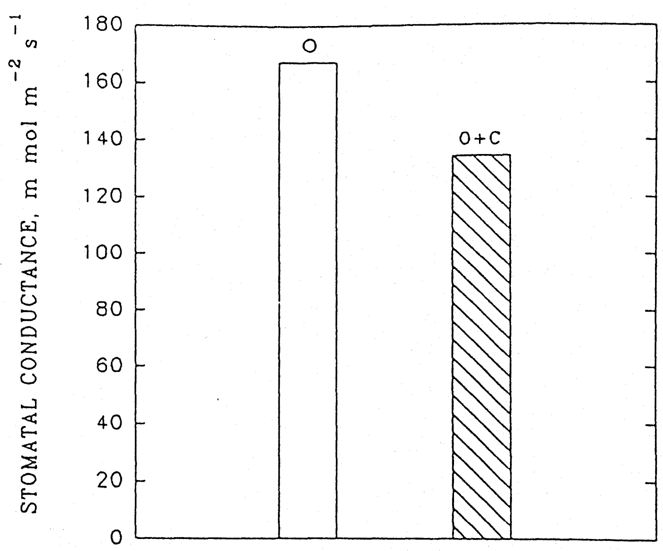

Figure 8. Mean values of stomatal conductance in new tissue developed after treatment initiation in Experiment 2—Values represent the mean for measurements over the experimental period. O = oiled and not cleaned, O + C = oiled and cleaned after 1 day

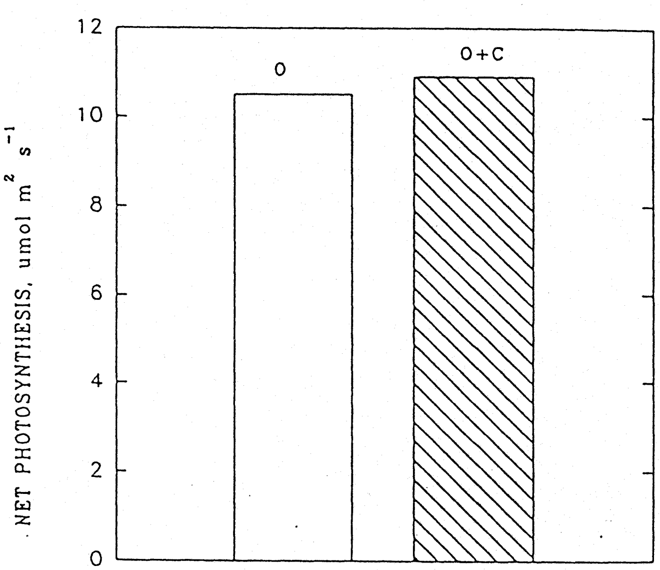

Figure 9. Net photosynthesis in new tissue developed after treatment initiation in Experiment 2—Values represent the mean for measurements over the experimental period. O = oiled and not cleaned, O + C = oiled and cleaned after 1 day

ing limited the adverse effects of oil on photosynthetic activity and also prevented subsequent leaf death. Cleaning was necessary for vegetation to survive fouling with bunker C. Cleaning was not necessary for plants to survive oiling with South Louisiana crude but was beneficial because it improved photosynthetic recovery of oiled leaves. Preventing death to oiled leaves in response to immediate application of cleaner is significant because the living tissue continued to photosynthesize and function albeit at a slower rate than unoiled vegetation. This probably enhanced production of new leaves that replaced oiled leaves. No beneficial effect of the cleaner on the soil microbial community was detected because there was no detectable adverse effect of oil on microbial respiration.

Response implications. In responding to oil spills in *S. alterniflora* marshes, the responding agency should consider how much tissue is covered and the type of oil. *Spartina alterniflora* marshes oiled with South Louisiana crude should recover without cleaning, but recovery should be enhanced by cleaning with Corexit 9580. On the other hand, *S. alterniflora* marshes oiled with bunker C require cleaning to survive, because of the severity and persistence of the adverse effects of the oil. Corexit 9580 removes the oil and allows *S. alterniflora* to recover. Regardless of the type of oil to be cleaned, it appears that the cleaner should be applied as soon as possible following an oil spill to prevent massive plant tissue death and to speed up the recovery of normal plant functions.

Marshes fouled with South Louisiana crude and bunker C benefit

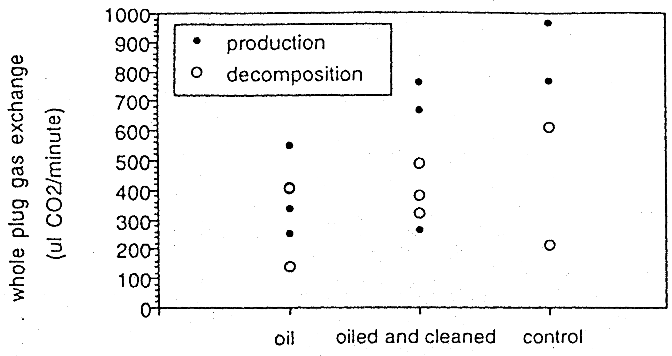

Figure 10. Production and respiration in Experiment 3 with bunker C

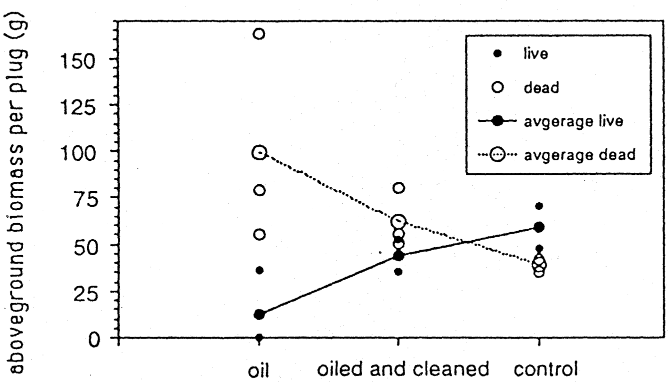

Figure 11. Aboveground biomass at the end of Experiment 3

from cleaning with Corexit 9580, but variations in the level of toxicity of the different oils suggest different response strategies when resources are limited. When *S. alterniflora* marshes are fouled with South Louisiana crude, limited resources might best be spent on other aspects of cleaning because the marsh will recover as shown by the present studies as well as the previous studies.[4,17] However, when *S. alterniflora* marshes are fouled with bunker C, limited resources might best be spent on cleaning the marsh because it may not recover otherwise.

Specific data are needed to determine the toxicity of South Louisiana crude and Corexit 9580 to other important marsh plant species before response strategies can be formulated for other marsh types and other oil types. For instance, the Louisiana Offshore Oil Port pipeline carries a mixture of crude oils across more than 25 miles of marshes, the majority dominated by other species, such as *Panicum hemitomon* and *Spartina patens*.

Summary

Coastal, estuarine, and riverine marshes are occasionally fouled by oil from navigation channels, pipelines, and storage facilities. Fouling can stress or kill vegetation, but some available cleaning strategies may be more harmful than taking no action. This project investigated the effectiveness of Corexit 9850 for cleaning *Spartina alterniflora*.

Intact plugs containing living plants, roots, and soil microbial communities were collected from Louisiana salt marshes and transferred to a greenhouse. Whole-plug photosynthesis, whole-plug respiration, stomatal conductance, and leaf area gas exchange were monitored following different fouling and cleaning scenarios. Experiment 1 evaluated cleaning plants that were partially oiled with Louisiana South crude. Experiment 2 evaluated cleaning plants that were completely oiled with Louisiana South crude. Experiment 3 evaluated cleaning plants that were completely oiled with bunker C.

Plant recovery depended on the degree of fouling and type of oil.

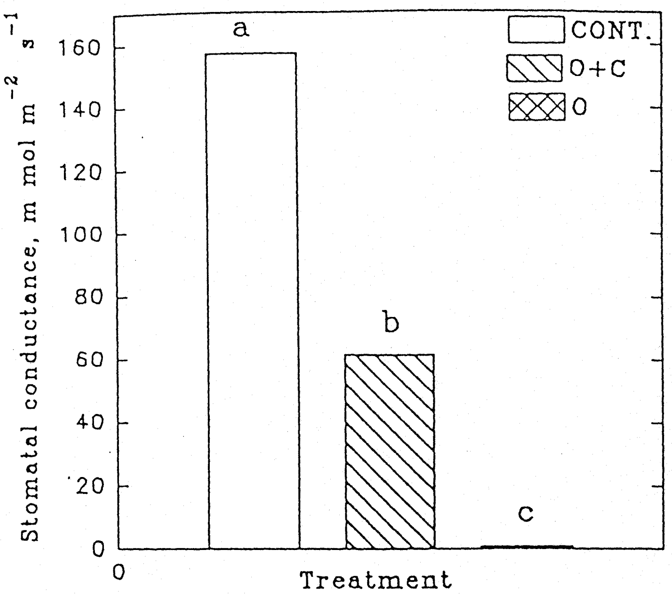

Figure 12. Response of stomatal conductance in *Spartina altuniflora* to various treatments using bunker C oil in Experiment 3—Values represent the mean for measurements over the experimental period; a = control (neither oiled or cleaned), b = oiled and cleaned after 1 day, c = oiled and not cleaned. Values across treatments labeled by different letters are significantly different at the 0.05 level.

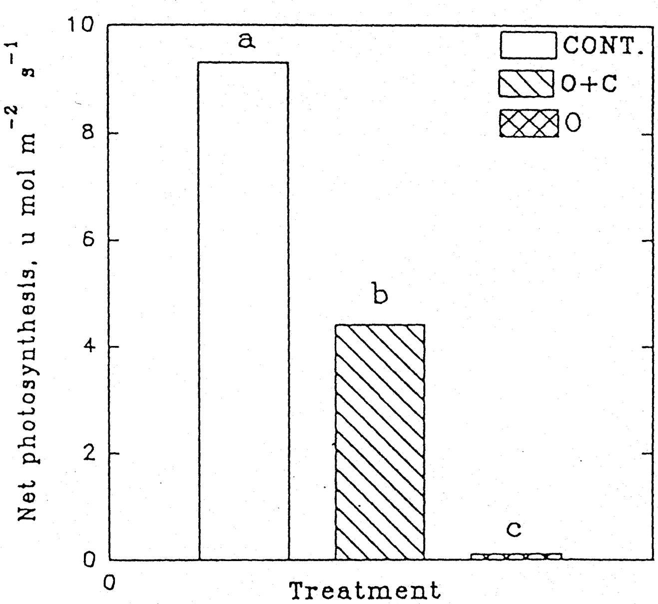

Figure 13. Response of photosynthesis in *Spartina alterniflora* to various treatments using bunker C oil in Experiment 3—Values represent the mean for the measurements over the experimental period; a = control (neither oiled or cleaned), b = oiled and cleaned after 1 day, c = oiled and not cleaned. Values across treatments labeled by different letters are significantly different at the 0.05 level.

Fouling with bunker C caused almost total plant mortality unless the plants were cleaned. South Louisiana crude was less toxic, but cleaning enhanced recovery because cleaned plants had more photosynthetic activity to help speed recovery.

Collectively, these studies indicated that oiled *S. alterniflora* marshes benefited from cleaning with Corexit 9580; other important species also may benefit from such cleaning.

Acknowledgment

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