OCS Study
MMS 2000-042

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Potential for Accelerated Bioremediation and Restoration of Oil-Impacted Marshes through the Selection of Superior Oil-Tolerant Vegetation

MMS U.S. Department of the Interior
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May 2000

Prepared under MMS Contract
14-35-0001-30660-19927
by
Coastal Marine Institute
Louisiana State University
Baton Rouge, Louisiana 70803

Published by

U.S. Department of the Interior
Minerals Management Service
Gulf of Mexico OCS Region

Cooperative Agreement
Coastal Marine Institute
Louisiana State University

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CITATION

Suggested citation:

Hester, M. W., Q. Lin, I. A. Mendelssohn, and D. J. DesRoches. 2000. Potential for accelerated bioremediation and restoration of oil-impacted marshes through the selection of superior oil-tolerant vegetation. OCS Study MMS 2000-042 U.S. Department of the Interior, Minerals Management Service, Gulf of Mexico OCS Region, New Orleans, La. 48 pp.

ABSTRACT

The cleanup of oil spills in coastal marshes remains a problematic issue because wetlands can be extremely sensitive to the disturbances associated with cleanup activities. As a result, there has been interest in developing alternative, less intrusive, oil spill cleanup/bioremediation techniques. The research reported herein assessed the feasibility of identifying superior oil-tolerant genotypes of Spartina patens and Spartina alterniflora (dominant brackish and salt marsh grasses, respectively) that may be utilized in the restoration and bioremediation of oil-impacted marshes, as well as in the creation of marshes in areas with a high amount of oil activity and, hence, a high incidence of an oil spill occurring.

Genotypes of S. patens and S. alterniflora were collected from Gulf Coast marshes and established in a uniform potting mix of approximately 10% organic matter content by weight. The soil was then oiled with South Louisiana crude oil at rates known to be stressful from previous research (5 L oil m^{-2} for S. patens and 8 L oil m^{-2} for S. alterniflora). Measurements of plant growth and physiological responses were conducted during the growing season, after which an initial harvest of aboveground biomass was conducted. Vegetative regrowth through the oiled soil was monitored for an additional three months, after which a final harvest was conducted and residual oil in the soil determined.

Highly significant genotype differences in a number of plant responses were detected in both species when oiled. Differences between genotypes generally became more pronounced with time, with the exception of leaf expansion rate, which proved to be a less sensitive short-term indicator of oil stress than photosynthesis (net CO_2 assimilation rate). Three genotypes of S. patens and three genotypes of S. alterniflora were identified that when oiled maintained high rates of photosynthesis and also generally performed better than the other genotypes in terms of a number of plant growth response variables, including regrowth through the oiled soil. However, residual oil concentration showed no significant univariate effect of genotype in either species.

A multivariate analysis (factor analysis) was conducted on a suite of plant growth response variables and residual oil. Factor analysis confirmed that three superior, oil-tolerant genotypes could be identified in each species that performed significantly better than other genotypes. When oiled, these oil-tolerant genotypes characteristically displayed less tissue death, maintained higher plant productivity, and demonstrated a greater ability to successfully produce new shoots through the oiled soil. Analysis of oil-degradation factor scores in S. patens further revealed that two of the three oil-tolerant genotypes demonstrated significantly greater oil-degradation potential than other genotypes. This study has shown that there is a tremendous amount of natural genetic variation in oil tolerance in Gulf Coast populations of S. patens and S. alterniflora that may potentially be exploited in the restoration and bioremediation of oiled brackish and salt marshes. Although significant genotype differences were not uniformly detected in oil degradation potential, it is likely that in the long term (and under field conditions) greater rates of oil degradation would be associated with those genotypes that are less stressed and have greater rates of plant productivity.

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INTRODUCTION

Coastal wetlands are among the most productive ecosystems in the world. Louisiana's wetlands are the most expansive in the U.S., comprising 41% of the nation's total (Mitsch and Gosselink 1986; Turner and Gosselink 1975). Coastal marshes are known for their role in improving water quality and enhancing productivity by providing protection and valuable nursery grounds for commercially important fisheries (Mitsch and Gosselink 1986). The living marsh grasses are vital elements in these highly productive coastal ecosystems. If the plants become stressed and die, either from natural or anthropogenic causes, a delicate balance is upset, and without the production and binding properties of the living shoots and roots, the marsh surface erodes.

The coastal marshes of Louisiana have been experiencing rapid rates of marsh loss and erosion (Dunbar et al. 1992). Although some marsh loss can occur from direct erosion and scouring of vegetated marsh (Nyman et al. 1994), the high rates of marsh loss in Louisiana have generally been attributed to plant stress (both natural and anthropogenic) followed by plant dieback and subsequent erosion of the marsh substrate. Potential causes of plant stress include waterlogging stress, due to insufficient elevation of the marsh surface, and salinity stress resulting from salt-water intrusion into the more interior brackish and fresh marshes (Mendelssohn et al. 1983; Sasser et al. 1986). Oil pollution and oil spills are additional sources of anthropogenic stress to wetland vegetation that can similarly stress the vegetation and lead to plant dieback and marsh erosion (Mendelssohn et al. 1993a; Getter et al. 1984).

The Louisiana coastal zone and outer continental shelf region are among the most intensively developed oil and gas areas in the world (Turner and Cahoon 1987). As a region, the Gulf of Mexico, and especially the central Gulf of Mexico, produce the vast majority of all U.S. offshore oil. In 1985, U.S. offshore oil wells in the Gulf of Mexico accounted for 92% of the total U.S. offshore oil production, with 94.3% of this oil coming from the central Gulf of Mexico region (EPA 1985). However, the tremendous volume of oil-related activities in the central Gulf of Mexico and in Louisiana's coastal zone also leads to a proportionately higher risk of an oil spill or oil leakage impacting Louisiana's fragile and productive coastal marshes.

Once a marsh is impacted by an oil spill, a decision must be made concerning the best method of cleanup and restoration. This issue has caused much controversy. Often it appears that the best course of action may be to let the marsh recover on its own to avoid secondary impacts to the marsh during the cleanup operation (McCauley and Harrel 1981; Long and Vandermeulen 1983; Getter et al. 1984; Baker et al. 1993; Mendelssohn et al. 1993a). Foot traffic and equipment traffic on the marsh surface during cleanup operations are considered secondary impacts that in themselves can have significant adverse effects on the recovery of the marsh by trampling vegetation, accelerating erosion, and burying oil into anaerobic soils where it may persist for years (Getter et al. 1984). Since it is known that wetland environments are sensitive to the impacts of cleanup (McCauley and Harrel 1981; Long and Vandermeulen 1983; Getter et al. 1984; Kiesling et al. 1988; Baker et al. 1993; Mendelssohn et al. 1993a), there is a need to develop alternative, less intrusive, oil spill cleanup/bioremediation techniques that are compatible with the delicate wetland environment and may be utilized singularly

(without other cleanup methods) in marshes oiled by minor spills, or utilized in conjunction with existing cleanup methodologies in heavily oiled marshes following initial cleanup efforts and impacts. The selection and utilization of superior oil-tolerant populations of dominant Gulf Coast marsh grasses may provide such a technique for the restoration and bioremediation of oil-impacted marshes.

The natural recovery of an oil-impacted marsh would be expected to be greatest when the oiled vegetation is somewhat tolerant to the initial impact of the oil and when new shoots originating from belowground rhizomes are able to emerge through the oiled soil and survive. If the vegetation is oil sensitive and the oil impact is severe enough to both kill the aboveground tissue and inhibit vegetative regrowth, then soil instability and erosion of the marsh surface may occur. Once this type of marsh dieback and erosion occurs, vegetation rarely again colonizes the open water (Mendelssohn et al. 1990). Rather, small dieback ponds often coalesce and form larger, shallow open water bodies. If superior oil-tolerant vegetation is available, it may be possible to re-establish vegetation in an oil-impacted marsh before substantial erosion of the marsh surface occurs and at an earlier date than what may be tolerated by non-oil-tolerant vegetation. The early establishment of superior oil-tolerant vegetation at a spill site may also aid in accelerating the restoration and bioremediation of the oil-impacted marsh. Furthermore, it may be possible to utilize superior oil-tolerant vegetation in certain marsh restoration and creation projects, such that oil-tolerant vegetation could be established in areas subjected to a high amount of oil-related activity and, hence, subjected to a greater probability of an oil spill occurring.

Spartina patens (Aiton) Muhl. is the single most important coastal grass species found in Louisiana's extensive coastal marshes, encompassing intermediate, brackish and saline marsh habitats. Spartina patens has been reported to be nearly twice as common as the second most important coastal marsh species, Spartina alterniflora Loisel (Chabreck 1972). Spartina patens is especially dominant in the expansive brackish marshes of Louisiana, whereas Spartina alterniflora dominates the higher salinity coastal salt marshes (Chabreck 1972). Both of these species are widespread marsh dominants throughout the Gulf and Atlantic coasts.

Although a number of studies have shown that different wetland plant species may respond quite differently to oiling (interspecific variation), we are aware of no studies to date that have investigated differences within single species (intraspecific variation) for differential tolerance to oiling. Furthermore, although there has been considerable effort and a fair amount of success in breeding agricultural crops for stressful environments (Blum and references therein 1988), there has been little emphasis to date on selecting superior stress-tolerant wetland vegetation. The research that has been conducted on wetland vegetation has focused on the natural environmental stresses of salinity (Pezeshki and DeLaune 1993; Hester et al. 1996; Hester et al. 1998) and flooding (Lessmann et al. 1997). To the best of our knowledge there has been no published research on selecting superior oil-tolerant lines of wetland plant species. The research reported herein specifically addresses the question of whether superior oil-tolerant populations of Spartina patens and Spartina alterniflora (dominant Gulf Coast brackish and salt marsh grasses) can be identified that display 1) superior growth response and plant production under oil stress, 2) superior vegetative regrowth through oiled soil, and 3) superior oil degradation potential.

MATERIALS AND METHODS

METHODS

Stock populations of Spartina patens and Spartina alterniflora were collected from various coastal marsh communities in Louisiana, Texas, and Florida and propagated under fresh conditions in a greenhouse for seven vegetative generations prior to use in this study. These populations have been shown by our previous research to be genetically diverse based on highly significant population differences in morphological characteristics and salinity tolerance (Hester et al. 1996; Hester et al. 1998). From these stock populations, a subset of 10 populations of each species was selected for the experimental screening of oil tolerance. Plants from each population were established in a uniform potting mix (approx. 10% organic matter by weight) and potted in 12-liter plastic pots equipped with a drain and water-level sight gauge. Ten pots were planted per population. For each population, five pots were randomly designated as control pots and five randomly designated as treatment (oiled) pots. The S. patens oiling experiment was conducted in the Fall of 1995. The S. alterniflora oiling experiment was conducted in the Spring of 1997.

Once the plants became established in the experimental vessels, water levels were raised slightly above the soil surface and South Louisiana crude oil was applied at a rate of 5 L m^{-2} for Spartina patens and 8 L m^{-2} for Spartina alterniflora (equivalent to a 5 mm thick and an 8 mm thick layer of oil, respectively, on the marsh surface). The oil was applied directly to surface flood water of the treatment pots. The drain valve was then opened and the applied oil was allowed to slowly drain through and oil the soil column over a period of about two hours. The water-level gauge of each pot was monitored during this process and drainage was stopped just prior to the drainage of any oil, thereby ensuring even oiling of the substrate without loss of oil. For each pot, the drain water was collected in a reservoir bucket and subsequently slowly returned to the top of the treatment pot soil. The control pots received no oil, but were similarly drained and had the solution returned to the surface of the pots. Throughout the study, water levels were maintained at the level of the soil surface.

Measurements of leaf expansion rate and plant photosynthesis (net CO_2 assimilation rate) were conducted one month and three months after oiling. Plant photosynthesis (net CO_2 exchange rate) was measured on young, but fully expanded leaves (generally the second expanded leaf from the growing tip) during midday conditions on a randomly selected mature stem. Measurements were conducted with a portable infrared gas analyzer (Analytical Development Company, Herts, England; model LCA-2). The selected leaf was clamped into an ADC Parkinson leaf chamber and the difference in CO_2 concentration between inlet and outlet air was measured. Sampling air was taken outdoors at 5 m above the ground surface in order to obtain a relatively stable CO_2 concentration, which was led through an ADC air supply unit with silica columns to obtain a dry inlet airstream. The flow rate was held constant at 6.25 ml s^{-1} and measurements were conducted under light saturated photosynthetic conditions provided by a Kodak projector lamp at a quantum flux density of 1500 - 2000 µmol m^{-2} s^{-1}. Gas exchange was determined on a per unit leaf area. Molar air flow, transpiration rate, and CO_2 uptake were calculated according to von Caemmerer and Farquhar (1981). Water-use efficiency was calculated as the ratio of µmol CO_2 fixed per mmol H_2O transpired

(Griffiths 1993). Leaf expansion rate was measured on terminal leaves that were neither newly emergent nor fully expanded, but in the range of one-third to two-thirds expanded. Leaf length was measured from the tip of the terminal leaf to the ligule of the youngest expanded leaf over a time interval of three days. This method has been used successfully with these and other grasses and generally produces results which parallel changes in net CO_2 assimilation and biomass (Hester et al. 1998; Hester et al. 1996; Hester and Mendelssohn 1990).

An initial harvest of plant aboveground biomass was conducted three months after oil application and a final harvest conducted six months after oil application. During the initial harvest the number of live and dead stems per pot were counted and aboveground biomass clipped at the soil surface, separated into live and dead components, oven-dried at 65° C for three days and weighed. To assess regrowth potential, the clipped pots were monitored for emergence of new shoots through the oiled surface for three additional months. Newly emergent aboveground biomass was then harvested during the final harvest in the same manner as outlined above for the initial harvest. To assess differences in oil degradation potential between the different genotypes, residual crude oil remaining in the soil was analyzed on a soil core (3.6 cm diameter x 10 cm depth) from each pot using a modification of the gravimetric method (Alexander and Webb 1985) and DCM (dichloromethane) as the extractant.

STATISTICAL ANALYSES

The experimental design of both the Spartina patens and the Spartina alterniflora oiling experiments was a 10 x 2 factorial of ten genotypes and two oiling treatments (control and oiled) with five complete replicates, yielding 100 experimental units total per experiment. The S. patens factorial was set up within a randomized block design (RBD) with one replicate in each of five blocks positioned along a potential temperature gradient in the greenhouse. Upon statistical analysis, the effect of block in the S. patens experiment was determined to not be significant, so the subsequent S. alterniflora factorial experiment was set up in a completely randomized design (CRD). All data were analyzed with SYSTAT 7.0.1 statistical software (SYSTAT 1997) using analysis of variance (ANOVA). All data were inspected for meeting the assumptions of parametric statistical analysis. Data were also assessed for outliers. Suspect data points were deemed outliers and deleted from the data set only when the studentized residuals were greater than 3.0 or less than -3.0 (SYSTAT 1997). For all analyses a significance level of 0.05 was used unless otherwise stated.

Additional one-way ANOVAs (genotype effects only) were run on a set of variables expressed as a percentage of the control response. For these analyses the average control response of each genotype was calculated for each variable. Then the treatment response (of each treatment replicate within a genotype) was expressed as a percentage of the mean genotype control response. This allowed for genotypes to be assessed not only in terms of absolute performances (actual treatment responses), but also in terms of relative performances (treatment responses relative to control responses).

Factor analyses were performed on each species to assist in identifying those genotypes with the most desirable responses to oiling. All factor analyses were run on the correlation matrix using the principal component solution and varimax rotation of

SYSTAT 7.0.1 statistical software (SYSTAT 1997). Initial factor analyses included all of the oiled response variables, which were then subsequently reduced until stable and interpretable solutions were obtained. Factor scores were then outputted for analysis of genotype differences using a one-way ANOVA.

RESULTS

Spartina patens

Significant genotype and treatment (oil vs. no oil) differences were detected in plant photosynthetic response (net CO_2 assimilation) in Spartina patens within the first month after oiling (Figure 1). These differences became even more pronounced by the third month, in which there was also a significant genotype x treatment interaction, indicating that genotype responses to oiling relative to their controls were different (Figure 1). For example, it can be seen that genotypes C, G, and J showed no significant reduction in photosynthesis relative to their controls by the third month (94%, 104%, and 93% of their controls, respectively), whereas the other genotypes had photosynthetic rates in the range of 31% to 55% of their respective controls (Table 1). The analysis of genotype response expressed as a percentage of the control supports this and yielded a highly significant ($P<0.01$) genotype effect on net CO_2 assimilation, both one month and three months after oiling (Table 1). Overall, genotypes C, G, and J had the highest treatment photosynthetic responses whether expressed as actual responses or as percentages of the control (Figure 1; Table 1). Water-use efficiency in S. patens was not a very sensitive indicator and failed to yield significant genotype, treatment, or interaction effects in either month one or month three (Figure 2).

Highly significant genotype differences in leaf expansion rate were evident throughout the study (Figure 3). Interestingly, oiling did not significantly affect leaf expansion rate until the third month (Figure 3). Also, no significant interaction of genotype x treatment was detected in leaf expansion rate. When analyzed as a percentage of the control response, there was a highly significant genotype effect, but again this was not detected until three months after oiling (Table 1). After three months of oiling many of the genotypes (including genotypes C and G) had leaf expansion rates that were equivalent to, or greater than, their controls (Figure 3; Table 1).

Live, dead, and total aboveground biomass all displayed a highly significant effect of genotype (Figures 4, 5, and 6). The treatment effect of oiling was significant for live aboveground biomass and highly significant for dead aboveground biomass (Figures 4 and 5), but was not significant for total aboveground biomass (Figure 6). The number of live and dead stems generally paralleled these biomass responses (Figures 4 and 5). These results are interpreted to mean that the relative proportion of live and dead aboveground biomass was more affected by oiling than was total aboveground biomass. An analysis of the proportion of dead aboveground biomass (dead aboveground/total aboveground) supports this interpretation and yielded highly significant genotype and treatment effects (Figure 6). There were no significant genotype x treatment interactions for any of these biomass responses. When analyzed as a percentage of the control response, dead biomass showed a significant effect of genotype, as did the proportion of dead aboveground biomass, which approached statistical significance ($P=0.065$; Table 1). Genotypes C, G, and J again performed well when oiled and produced some of the greatest amounts of live biomass, but not necessarily the lowest amounts of dead biomass (Figures 4 and 5). Genotype C consistently produced very little dead biomass whether oiled or not, whereas genotypes G and J produced intermediate amounts of dead biomass when oiled (Figures 5 and 6).

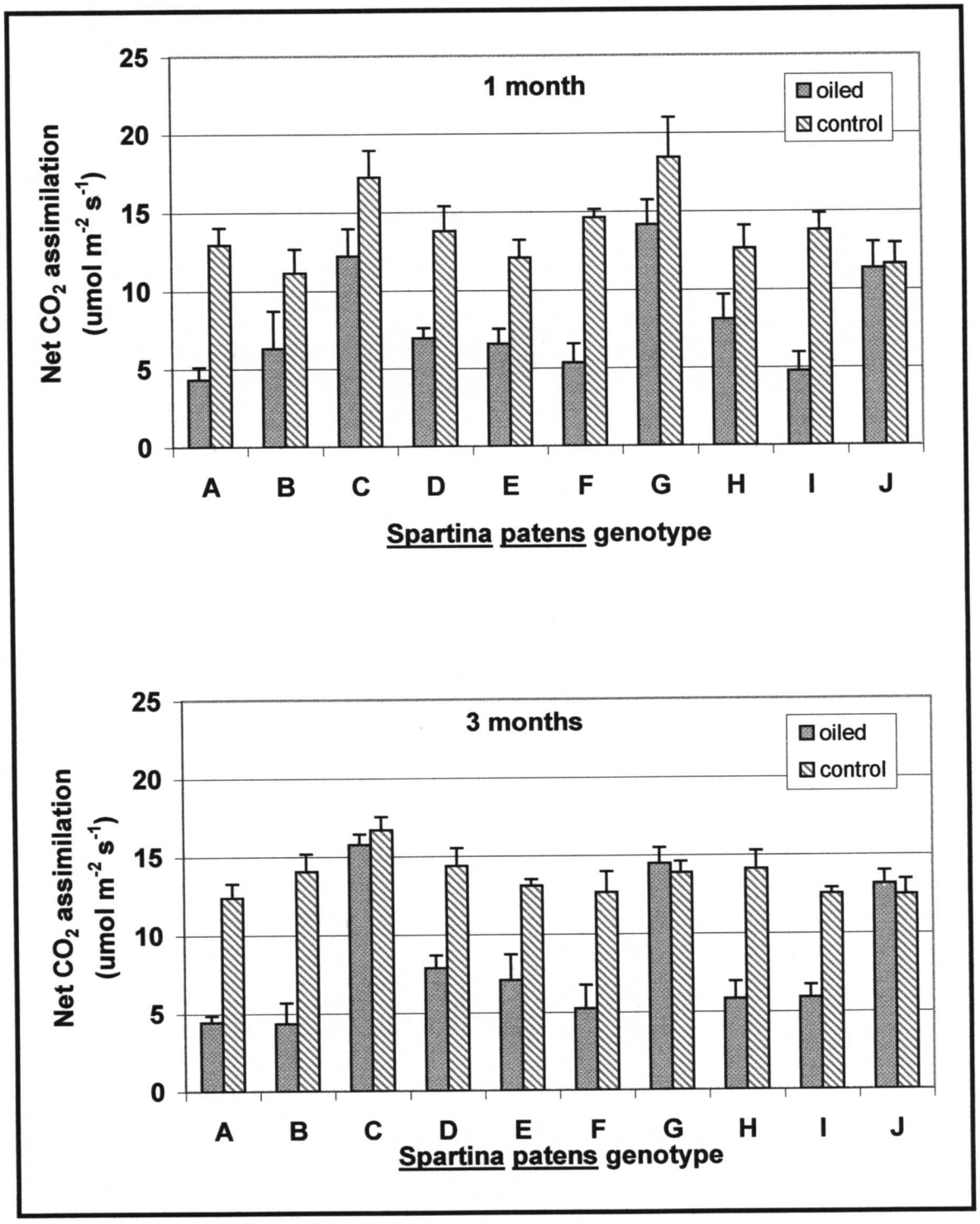

Figure 1. Mean (± standard error) net CO_2 assimilation rates (μmol m^{-2} s^{-1}) of Spartina patens genotypes one month (top panel) and three months (bottom panel) after oiling at a rate of 5 L oil m^{-2} (n=5).

Table 1. Responses of Spartina patens genotypes to oiling (5 L m^{-2}) expressed as percentages of the controls. Shown are mean responses and standard errors in parentheses (n=5). The probability of a genotype effect for each response is indicated as follows: NS = P > 0.05, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Response	**Genotype**									
	A	B	C	D	E	F	G	H	I	J
Photosynthesis ** After 1 month	33.40 (6.34)	68.00 (17.0)	71.20 (10.04)	50.60 (4.62)	54.40 (7.95)	36.20 (8.55)	76.8 (8.36)	65.80 (13.12)	34.8 (8.8)	97.25 (14.55)
Photosynthesis *** After 3 months	35.60 (3.67)	31.00 (9.52)	94.20 (4.09)	54.80 (5.76)	54.00 (12.33)	41.20 (11.65)	103.75 (7.31)	41.20 (7.83)	47.00 (6.44)	92.80 (4.10)
Leaf Expansion NS 1 month	99.80 (1.91)	99.20 (2.50)	101.0 (1.27)	101.20 (2.46)	104.60 (3.16)	99.40 (1.78)	101.20 (3.34)	103.40 (1.08)	100.00 (1.05)	97.00 (2.35)
Leaf Expansion * 3 months	101.20 (26.03)	158.40 (37.71)	152.60 (35.92)	237.60 (32.67)	133.4 (11.16)	104.20 (28.71)	159.60 (27.14)	71.00 (29.63)	279.75 (34.28)	84.40 (12.36)
Live Stem *** Count	48.35 (3.62)	33.33 (8.95)	86.46 (2.08)	47.67 (9.11)	58.0 (6.99)	26.99 (5.5)	73.67 (5.53)	67.33 (11.23)	40.41 (2.66)	71.69 (1.97)
Live Biomass NS	74.60 (9.90)	95.75 (16.13)	101.60 (8.81)	92.60 (13.48)	76.00 (10.99)	61.80 (15.15)	91.40 (4.11)	105.40 (8.08)	84.00 (7.18)	95.60 (7.27)
Dead Stem *** Count	81.39 (18.75)	255.7 (105.6)	522.7 (276.2)	2437.5 (62.5)	433.2 (135.4)	455.6 (81.33)	100 (100)	287.5 (109.3)	333.3 (166.7)	125 (125)
Dead Biomass *	72.60 (14.41)	154.60 (30.06)	165.2 (25.04)	141.8 (7.55)	184.33 (18.81)	130.40 (15.04)	99.00 (9.61)	113.80 (11.22)	125.80 (13.64)	132.40 (25.71)
Total Biomass NS	74.14 (10.21)	97.06 (12.19)	115.02 (6.68)	102.98 (11.02)	103.76 (11.66)	77.44 (14.78)	92.64 (4.51)	107.70 (7.66)	91.75 (5.97)	102.43 (8.55)
Proportion Dead NS Biomass/Total	101.54 (12.47)	132.49 (25.05)	145.17 (20.50)	142.07 (14.23)	139.84 (26.44)	185.15 (26.22)	104.52 (7.76)	105.48 (7.35)	146.65 (15.60)	127.64 (20.05)
Stem Count *** Regrowth	25.80 (3.92)	27.60 (8.93)	91.40 (7.39)	67.00 (9.32)	67.60 (13.00)	30.60 (11.14)	71.20 (6.18)	67.80 (9.30)	30.60 (4.73)	87.00 (5.18)
Biomass *** Regrowth	17.40 (2.66)	27.20 (12.91)	82.80 (9.56)	53.00 (10.12)	61.00 (13.57)	24.60 (12.53)	80.80 (7.01)	62.40 (7.85)	22.00 (3.95)	72.00 (8.45)

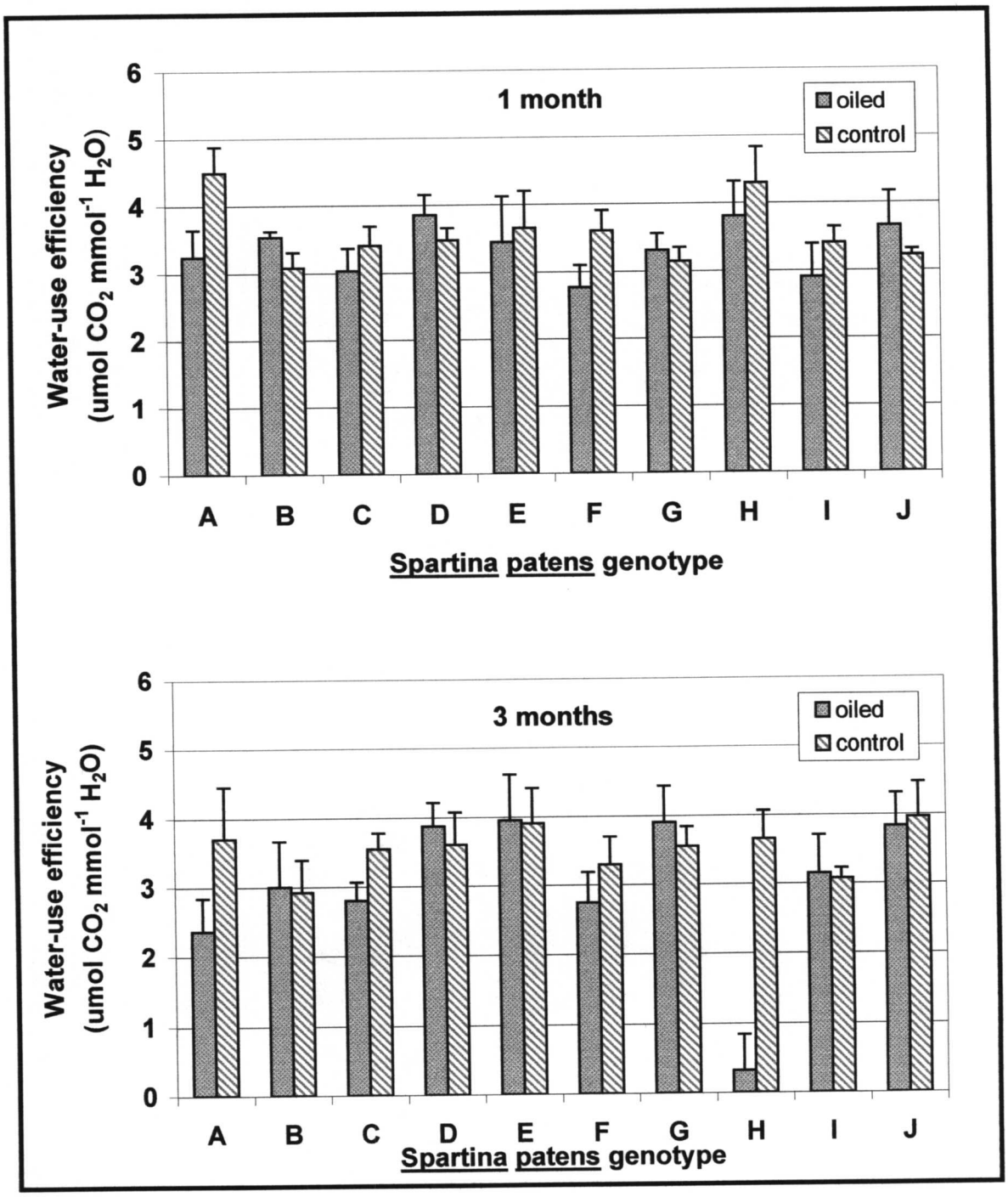

Figure 2. Mean (± standard error) water-use efficiencies (μmol CO_2 $mmol^{-1}$ H_2O) of Spartina patens genotypes one month (top panel) and three months (bottom panel) after oiling at a rate of 5 L oil m^{-2} (n=5).

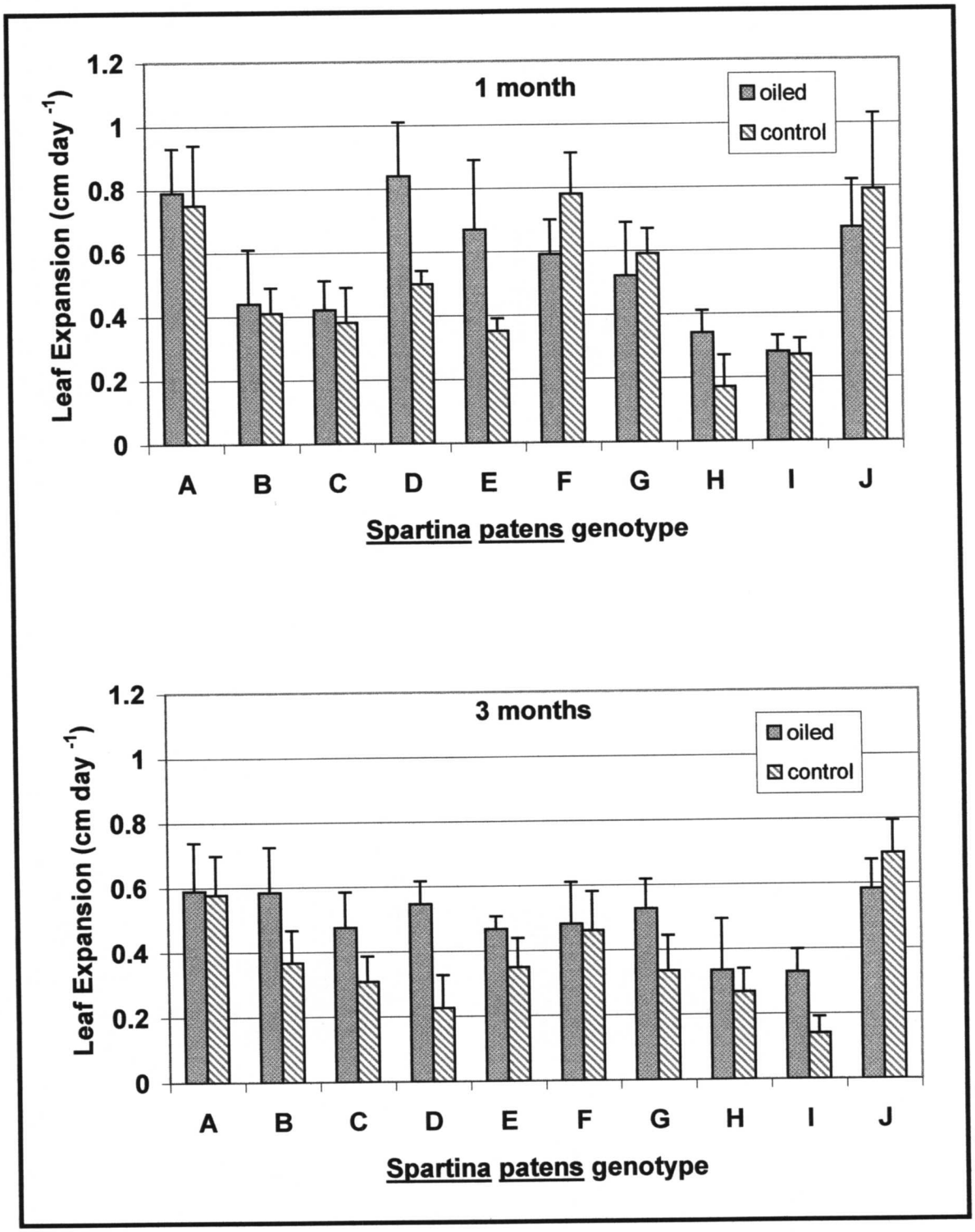

Figure 3. Mean (± standard error) leaf expansion rates (cm day^{-1}) of Spartina patens genotypes one month (top panel) and three months (bottom panel) after oiling at a rate of 5 L oil m^{-2} (n=5).

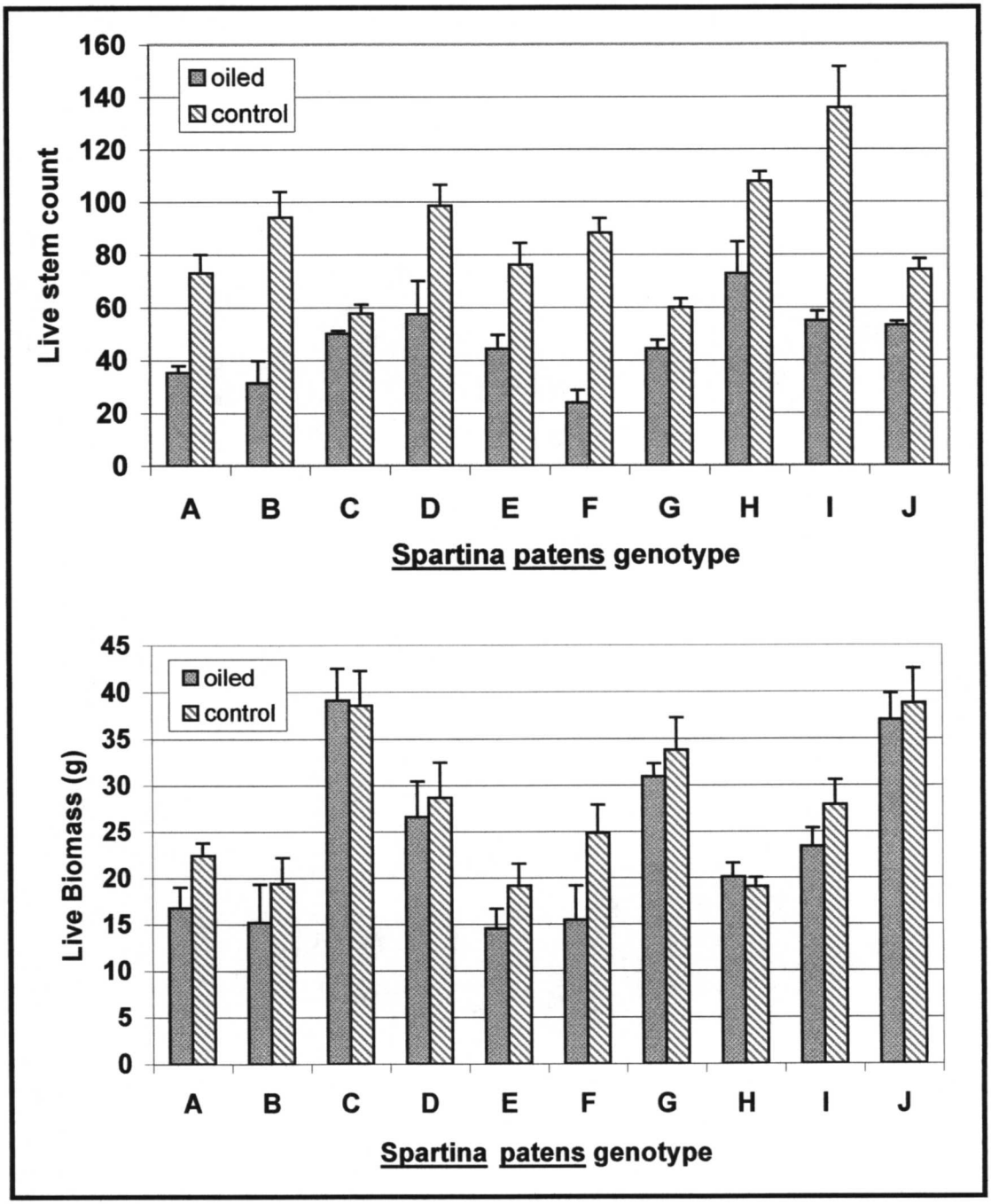

Figure 4. Mean (± standard error) number of live stems (# per pot; top panel) and live aboveground biomass (g per pot; bottom panel) of Spartina patens genotypes three months after oiling at a rate of 5 L oil m^{-2} (n=5).

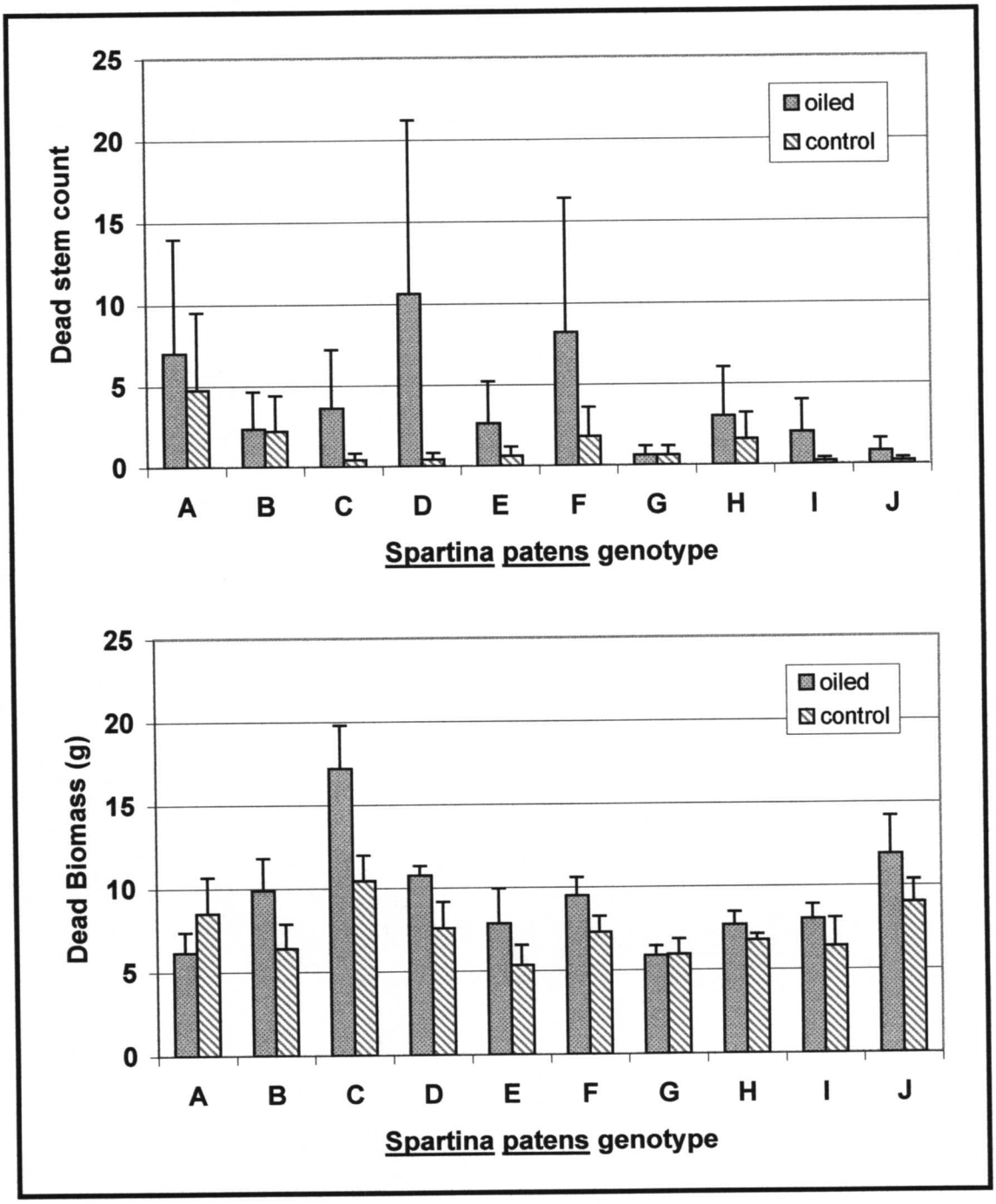

Figure 5. Mean (± standard error) number of dead stems (# per pot; top panel) and dead aboveground biomass (g per pot; bottom panel) of Spartina patens genotypes three months after oiling at a rate of 5 L oil m^{-2} (n=5).

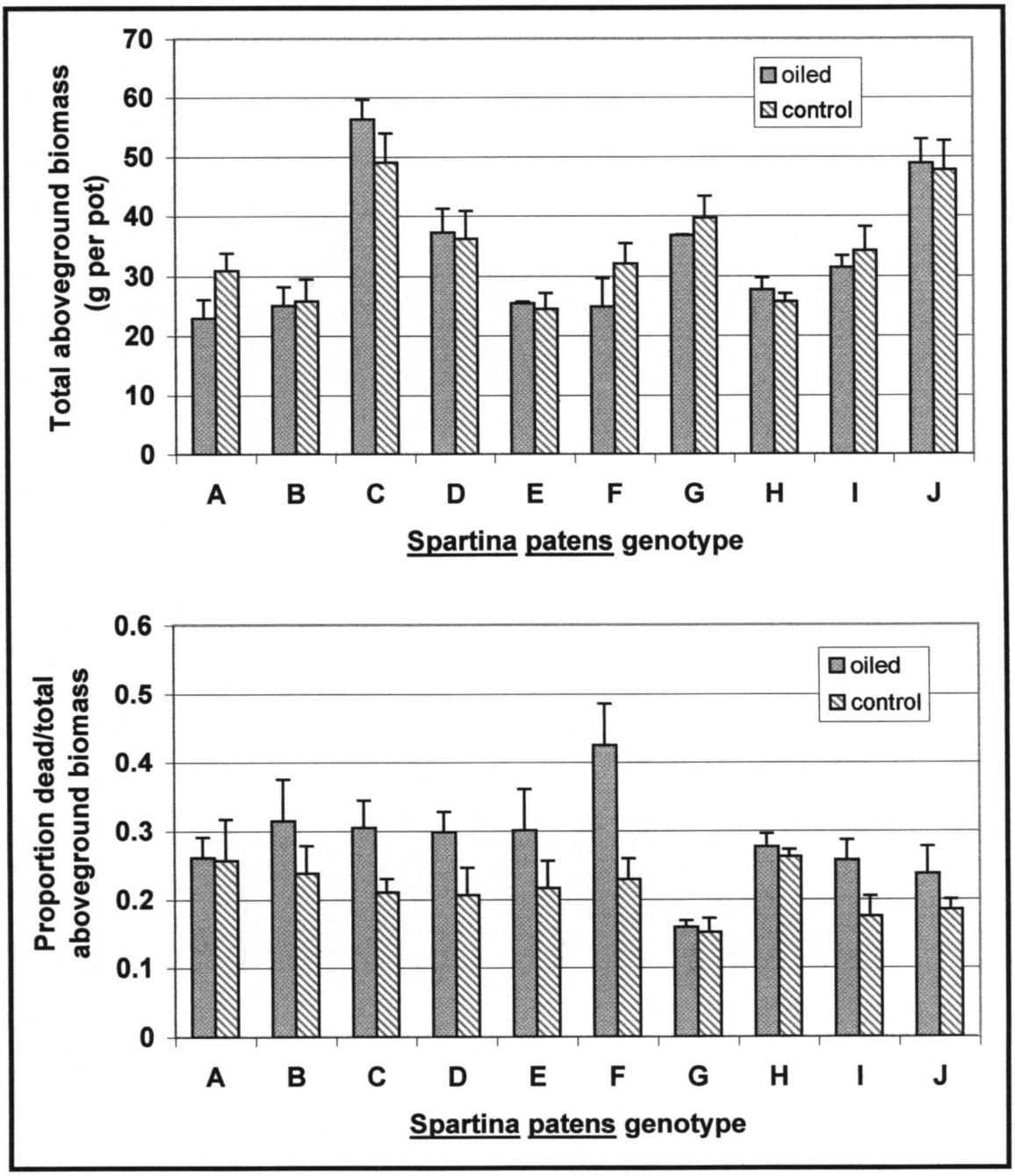

Figure 6. Mean (± standard error) total aboveground biomass (g per pot; top panel) and proportion of dead aboveground biomass (dead/total aboveground biomass) of Spartina patens genotypes three months after oiling at a rate of 5 L oil m^{-2} (n=5).

The amount of regrowth through the oiled soil in the three months following the initial aboveground harvest displayed highly significant effects of genotype, treatment, and genotype x treatment. This was true for both the number of new stems produced and the amount of aboveground biomass produced (Figure 7). Although all genotypes of S. patens assessed were able to successfully tiller and produce live shoots through the oiled soil, genotypes C, G, and J were particularly successful in absolute terms and relative to their controls, thereby contributing to the significant genotype x treatment interactions (Figure 7). When analyzed as a percentage of the control, a highly significant genotype effect was detected for both of these regrowth responses (Table 1). Genotypes C, G, and J had very high regrowth responses that ranged from 71% - 91% of their controls, whereas other genotypes had responses that ranged from 17% - 67% of their controls (Table 1).

Results from the analysis of residual oil in the soil six months after oiling were highly variable within populations and overall displayed a lack of significant population differences in oil degradation (P=0.15; Figure 8). However, the amount of residual oil remaining in the soil of genotypes C and J were generally in the lower half of all genotype responses and ranged from about 11 - 12 mg oil g^{-1} dry weight of soil (Figure 8). In comparison, genotype G had a greater amount of residual oil remaining in the soil, which averaged about 15 mg g^{-1} (Figure 8).

Factor analysis of the S. patens genotype responses in the oiled treatment agreed with and helped to clarify the univariate results. The clearest interpretation was obtained when utilizing plant growth response variables in addition to the amount of residual oil remaining in the soil at the end of the study. The following six variables were utilized: photosynthesis (net CO_2 assimilation rate) at one month and three months, total aboveground biomass at the three month harvest, the proportion of dead aboveground biomass at the three month harvest, the amount of aboveground biomass regrowth that occurred through the oiled soil, and residual oil in the soil. This model resulted in a two factor solution that accounted for 72% of the variation in these variables (Figure 9). The first factor accounted for 54.7% of the variation and is best described as a plant growth/regrowth factor. This factor had high positive loadings of photosynthesis at one month (0.835) and three months (0.883), total aboveground biomass (0.884), and aboveground biomass regrowth (0.920), with a negative loading of proportion of dead aboveground biomass (-0.498). Therefore, genotypes with high factor 1 scores would be classified as having high rates of photosynthesis and growth, with very little tissue death when oiled, and also high rates of new aboveground biomass production through oiled soil. The ANOVA that was run on these factor scores detected highly significant differences between genotypes, with genotypes C, G, and J having the highest factor 1 scores (Figure 10). The second factor accounted for an additional 17.3% of the variation and is described solely by a high negative loading of residual oil. Therefore, genotypes that have a high factor 2 score would be classified as genotypes whose presence was associated with less residual oil remaining at the end of the study (Figure 9). The ANOVA of the factor 2 scores failed to detect an overall significant genotype effect (P=0.224). However, genotypes C and J tended to be associated with the greatest amount of oil degradation and a linear contrast did show that genotypes C and J had significantly greater factor 2 scores (and hence less residual oil associated with them) than the other genotypes (P=0.014; Figures 8 and 10).

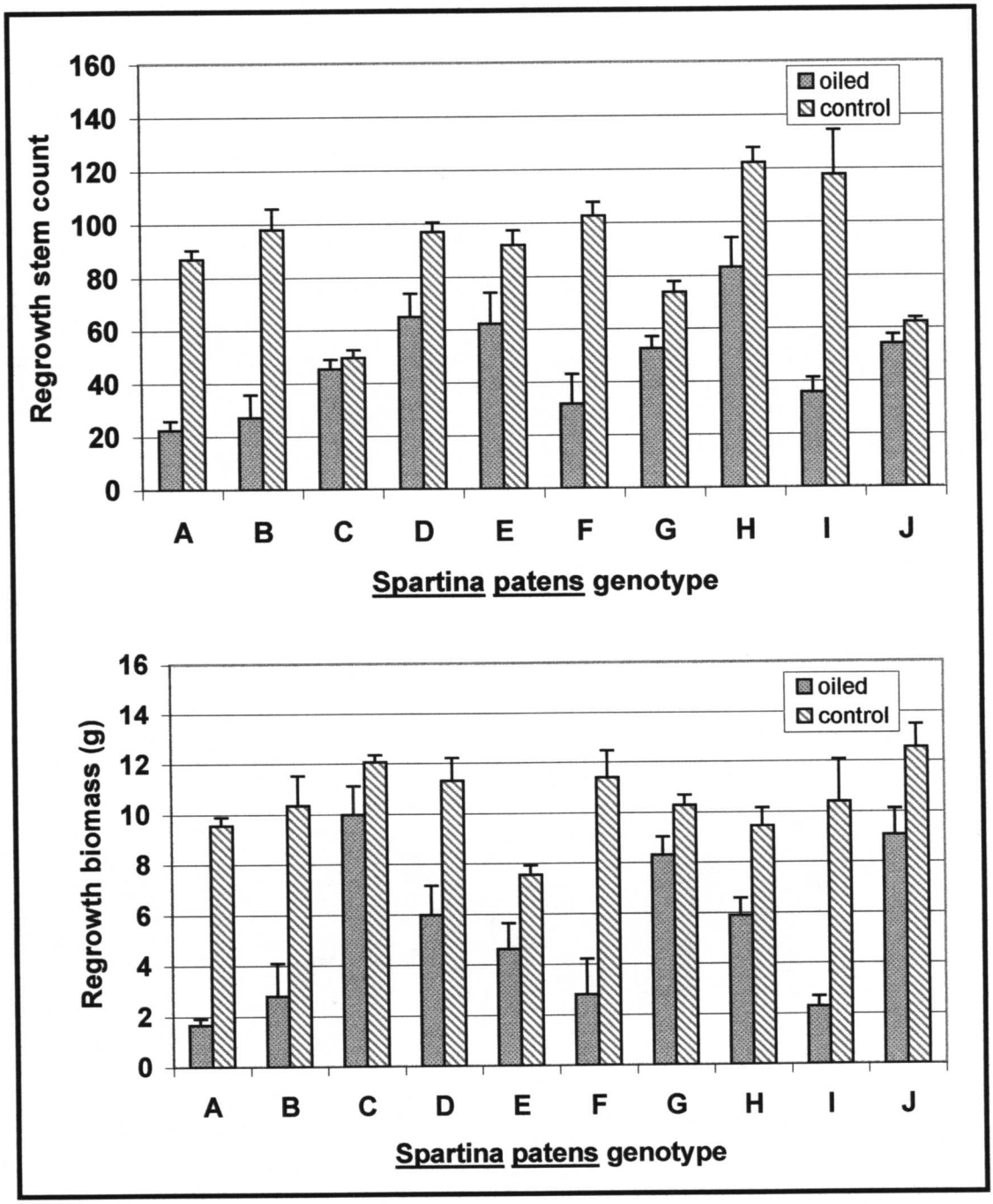

Figure 7. Mean (± standard error) number of regrowth stems (# per pot; top panel) and aboveground biomass regrowth (g per pot; bottom panel) of Spartina patens genotypes three months after the initial harvest (n=5).

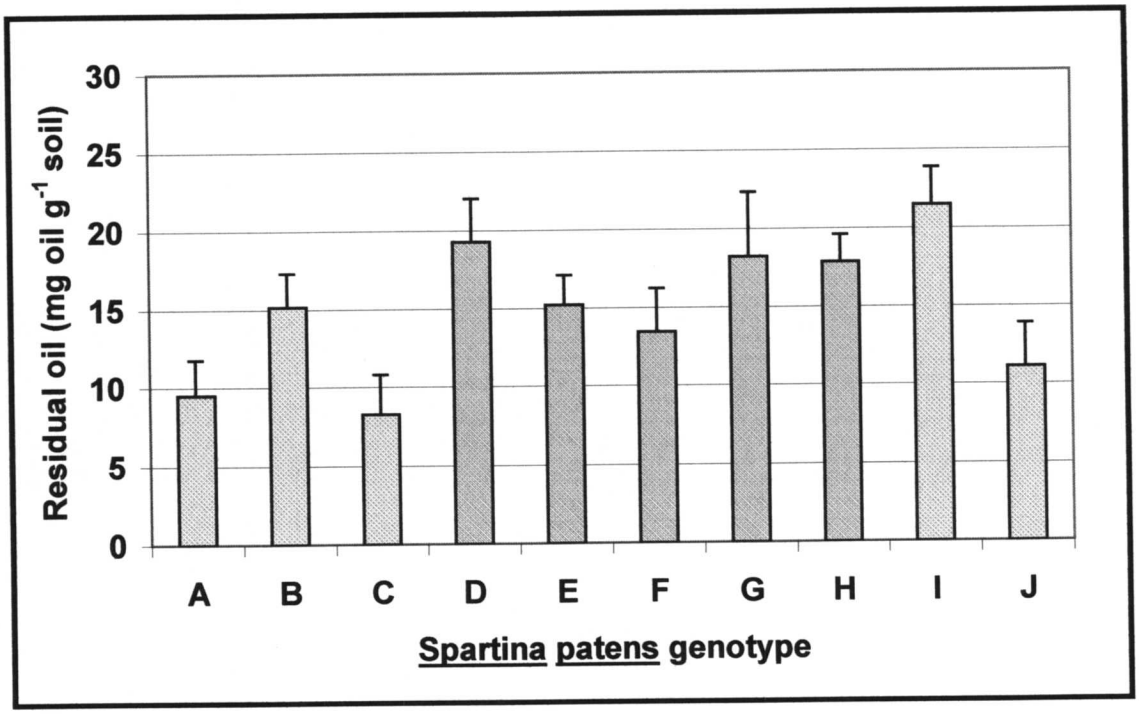

Figure 8. Mean (± standard error) residual oil (mg oil g^{-1} soil) remaining in the soil of Spartina patens genotypes six months after oiling at a rate of 5 L oil m^{-2} (n=5).

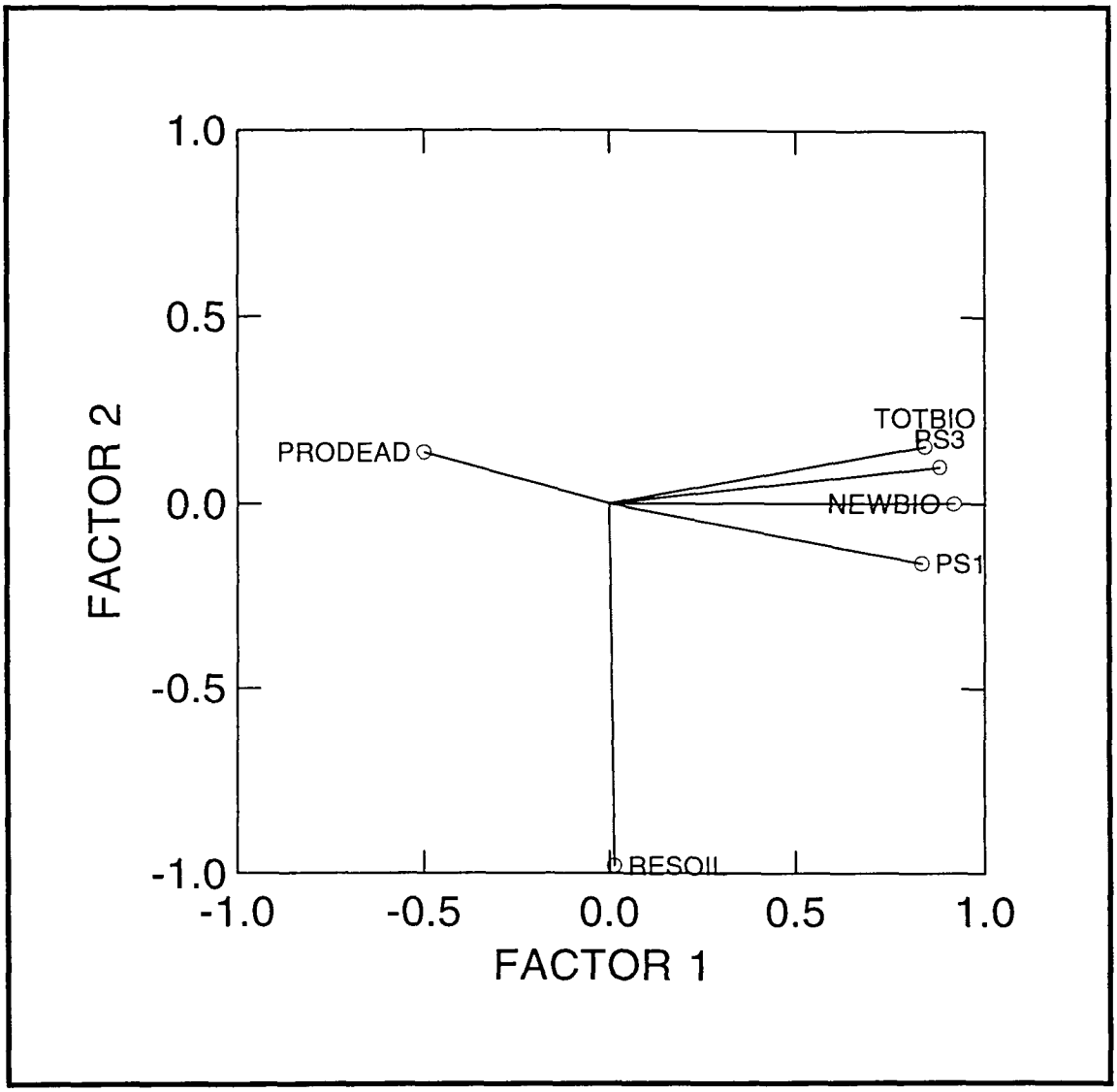

Figure 9. Factor loadings plot resulting from a factor analysis of residual oil and five plant response variables of oiled Spartina patens genotypes . Abbreviations are as follows: PS1=photosynthesis (net CO_2 assimilation rate) one month after oiling, PS3=photosynthesis three months after oiling, TOTBIO=total aboveground biomass three months after oiling, PRODEAD=proportion of dead aboveground biomass three months after oiling, NEWBIO=amount of aboveground biomass regrowth between three and six months after oiling, and RESOIL=residual oil in the sediment six months after oiling (n=5).

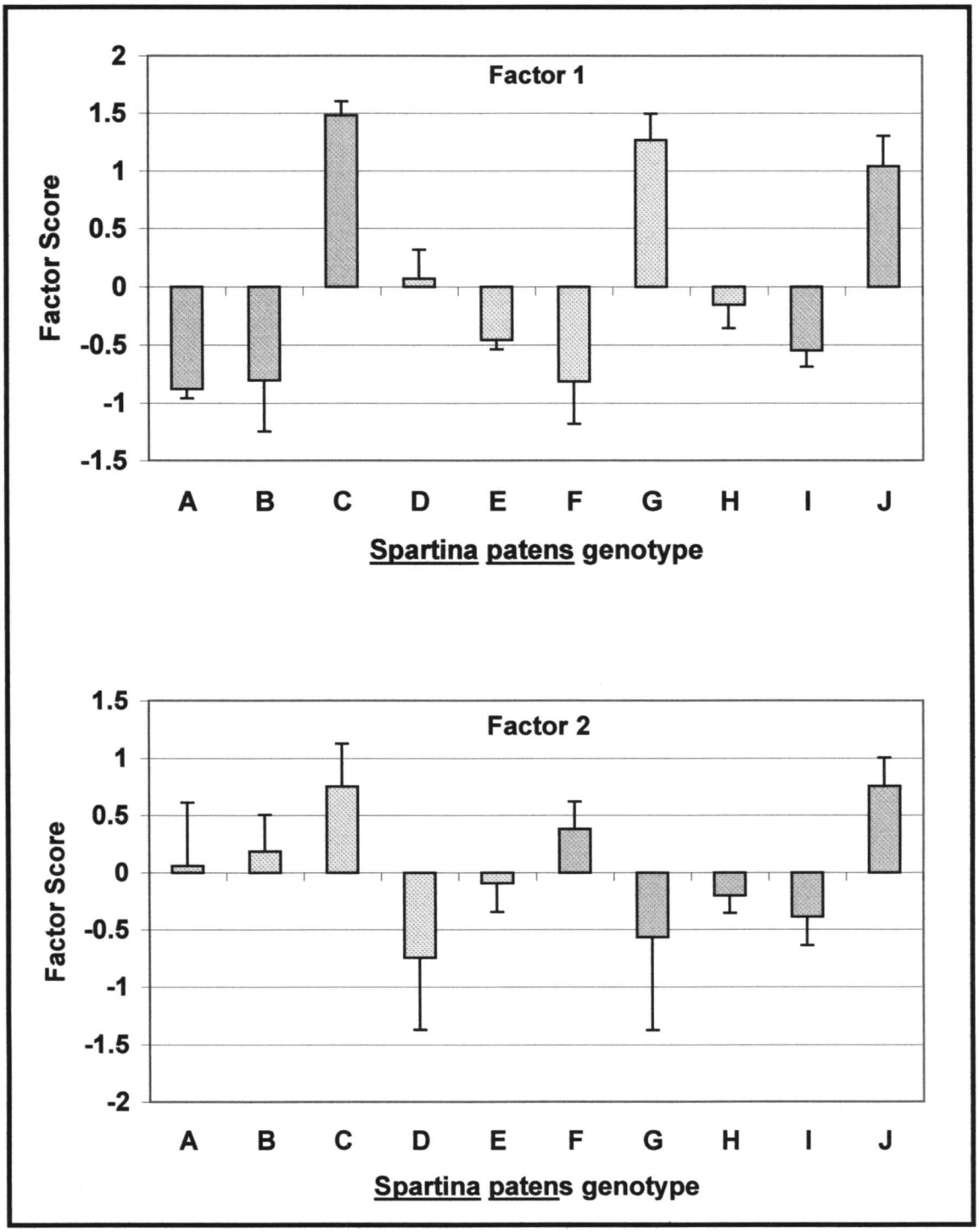

Figure 10. Mean (± standard error) factor 1 scores (top panel) and factor 2 scores (bottom panel) of oiled (5 L oil m^{-2}) Spartina patens genotypes. High factor 1 scores are indicative of high photosynthesis and biomass with very little tissue death and high aboveground biomass regrowth when oiled. High factor 2 scores are indicative of low amounts of residual oil remaining after six months (n=5).

Spartina alterniflora

Similar to S. patens, oiling resulted in a highly significant ($P<0.01$) reduction of net CO_2 assimilation rate in S. alterniflora, which was evident within one month after oiling and persisted through the third month (Figure 11). However, unlike S. patens, differences between S. alterniflora genotypes in net CO_2 assimilation rate only approached statistical significance ($P=0.059$) one month following oiling (Figure 11). Similarly, genotype photosynthetic response to oiling expressed as a percentage of the control was also not significant (Table 2). Nonetheless, after three months of oiling, differences between genotypes in net CO_2 assimilation rate were highly significant. Also after three months, the interaction of genotype x treatment was significant, thereby indicating that the genotypes were not responding uniformly to oiling (Figure 11). Genotypes R, U, V, and Y displayed the highest rates of net CO_2 assimilation after three months, both in terms of actual treatment responses and also as a percentage of their control responses, which also indicated significant differences between genotypes (Figure 11; Table 2).

Water-use efficiencies did not display any significant effects one month after oiling. Three months after oiling there was a highly significant treatment effect and also a highly significant genotype x treatment interaction (Figure 12). Genotypes V and Y possessed some of the highest water use efficiencies, perhaps largely due to their high rates of photosynthesis in the third month (Figure 12).

Leaf expansion rate proved to be quite variable and not as sensitive to oiling as net CO_2 assimilation rate. One month after oiling, there were no significant effects of genotype or treatment on leaf expansion rate (Figure 13). After three months, a highly significant reduction in leaf expansion rate was evident with oiling, but genotype differences were still not significant unless analyzed as a percentage of the control response (Figure 13; Table 2). Genotypes S, U, and V all had oiled leaf expansion rates that were near or exceeded 100% of their control responses, whereas the other genotypes had leaf expansion rates that ranged from 32% - 76% of their control responses (Table 2).

Live biomass, total biomass, and the number of live and dead stems harvested three months after oiling displayed highly significant effects of both genotype and oiling (Figures 14, 15, and 16). The effect of genotype remained either significant or highly significant when analyzed as a percentage of the control response for all of these variables (Table 2). The effect of genotype on dead biomass only approached statistical significance ($P=0.07$), but was significant when expressed as a percentage of the control response (Figure 15; Table 2). Live biomass and live and dead stems also displayed significant interactions of genotype x treatment. This interaction can be explained by the outstanding performance of genotype R (and to a lesser extent genotype Z), which did not show any reduction of live biomass when oiled, but rather displayed a slight stimulation (Figure 14; Table 2). As was observed in S. patens, the number of live and dead stems generally paralleled the responses of live and dead aboveground biomass (Figure 14). Genotype R, which already had higher biomass per live stem than the other genotypes, showed some stimulation upon oiling, which resulted in a further increase in live stem weight (Figure 14). The proportion of dead biomass displayed highly significant genotype and treatment effects, as well as a highly significant genotype x treatment interaction (Figure 16). Genotypes Q, S, and X did poorest in this regard and had much

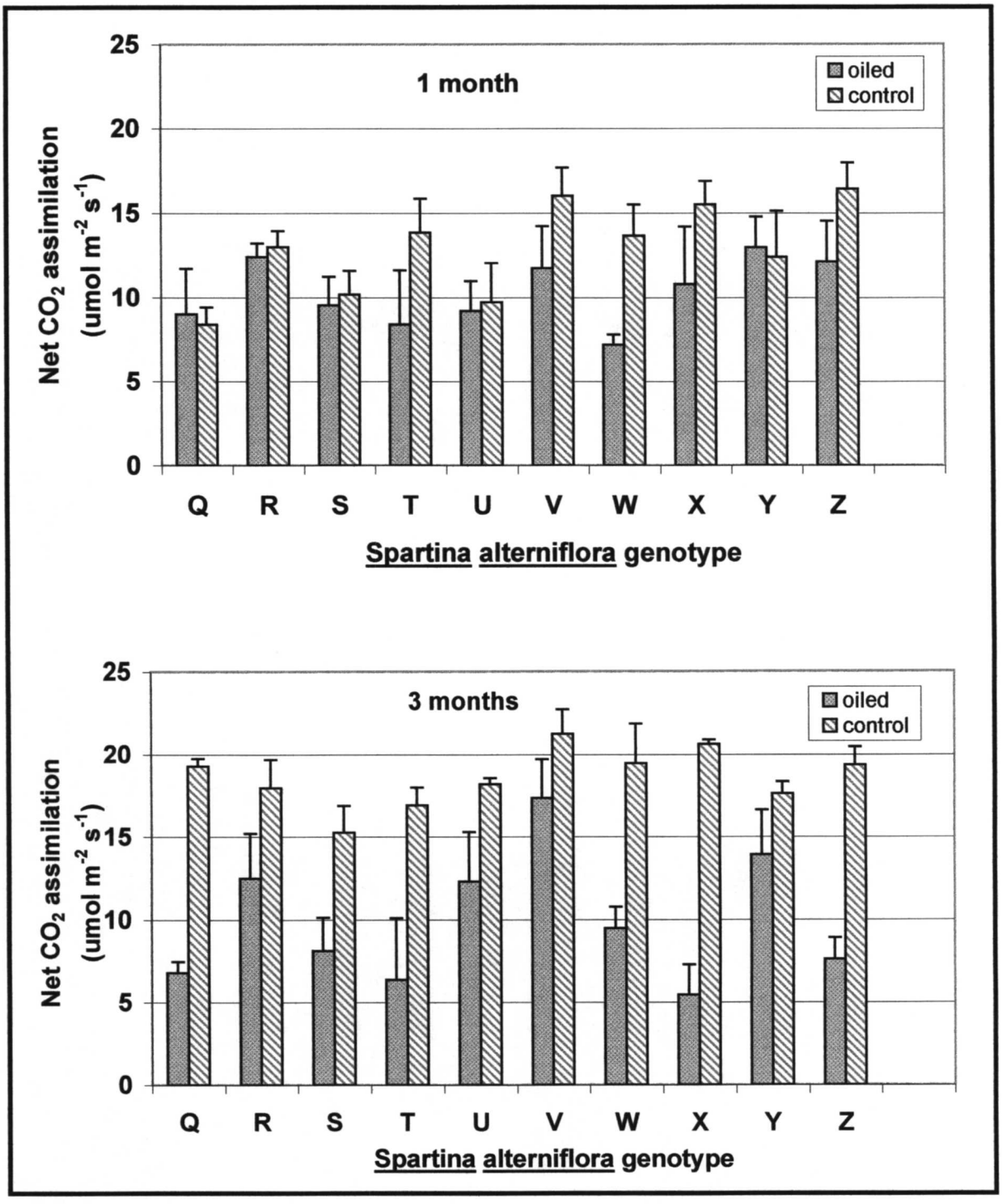

Figure 11. Mean (± standard error) net CO_2 assimilation rates (μmol m^{-2} s^{-1}) of Spartina alterniflora genotypes one month (top panel) and three months (bottom panel) after oiling at a rate of 8 L oil m^{-2} (n=5).

Table 2. Responses of Spartina alterniflora genotypes to oiling (8 L m^{-2}) expressed as percentages of the controls. Shown are mean responses and standard errors in parentheses (n=5). The probability of a genotype effect for each response is indicated as follows: NS = P > 0.05, * = P < 0.05, ** = P < 0.01, *** = P < 0.001.

Response	**Genotype**									
	A	B	C	D	E	F	G	H	I	J
Photosynthesis NS After 1 month	79.33 (20.11)	95.36 (6.38)	93.48 (16.61)	60.58 (23.25)	94.42 (18.57)	73.30 (15.59)	52.68 (4.48)	69.66 (21.89)	104.48 (14.40)	73.93 (14.36)
Photosynthesis * After 3 months	35.38 (3.48)	69.54 (15.21)	53.16 (13.58)	37.75 (21.68)	67.54 (16.26)	80.12 (10.85)	43.78 (5.98)	26.54 (8.74)	97.50 (19.0)	53.03 (9.31)
Leaf Expansion NS 1 month	48.96 (18.83)	75.16 (20.35)	135.70 (10.29)	69.50 (32.01)	122.68 (33.82)	121.65 (49.17)	109.56 (26.99)	96.94 (20.41)	56.14 (16.34)	88.48 (7.37)
Leaf Expansion ** 3 months	44.80 (13.82)	72.80 (17.12)	113.80 (10.07)	32.25 (18.87)	126.60 (27.0)	99.75 (12.44)	76.00 (12.15)	50.00 (13.98)	74.40 (7.39)	79.80 (22.34)
Live Stem *** Count	27.64 (5.88)	279.90 (24.95)	32.90 (11.98)	49.80 (19.49)	49.76 (9.81)	67.56 (13.57)	95.92 (17.59)	38.54 (18.65)	134.88 (29.19)	146.30 (16.45)
Live Biomass ***	24.34 (5.16)	246.30 (21.94)	28.94 (10.54)	43.83 (17.15)	40.38 (10.25)	59.46 (11.94)	84.44 (15.48)	33.88 (16.42	118.70 (25.70)	128.77 (14.48)
Dead Stem * Count	414.28 (88.64)	100.00 (31.62)	428.58 (59.76)	374.98 (131.64)	400.00 (167.33)	540.00 (40.0)	240.00 (24.50)	218.75 (106.74)	213.32 (80.00)	280.00 (105.83)
Dead Biomass *	190.68 (28.52)	52.34 (7.74)	177.54 (52.81)	104.37 (43.01)	100.00 (16.03)	204.68 (56.72)	67.28 (5.25)	221.50 (62.01)	88.78 (32.14)	161.2 (53.38)
Total Biomass **	46.15 (6.42)	78.01 (20.13)	52.60 (13.89)	52.74 (18.82)	83.35 (11.01)	77.55 (12.03)	72.47 (12.11)	48.40 (11.50)	71.88 (14.59)	160.38 (24.97)
Proportion Dead * Biomass/Total	503.54 (71.49)	152.44 (71.24)	509.02 (120.17)	401.37 (250.30)	749.92 (188.57)	563.38 (128.26)	178.86 (45.08)	565.62 (153.67)	150.38 (62.69)	299.78 (170.02)
Stem Count NS Regrowth	3.40 (3.40)	13.0 (7.51)	0 (0)	0 (0)	6.0 (2.95)	12.25 (7.22)	3.25 (3.25)	0 (0)	1.75 (1.75)	0 (0)
Biomass NS Regrowth	0.60 (0.6)	17.2 (12.44)	0 (0)	0 (0)	4.2 (2.46)	11.4 (5.27)	3.2 (2.52)	0 (0)	6.0 (5.06)	0 (0)

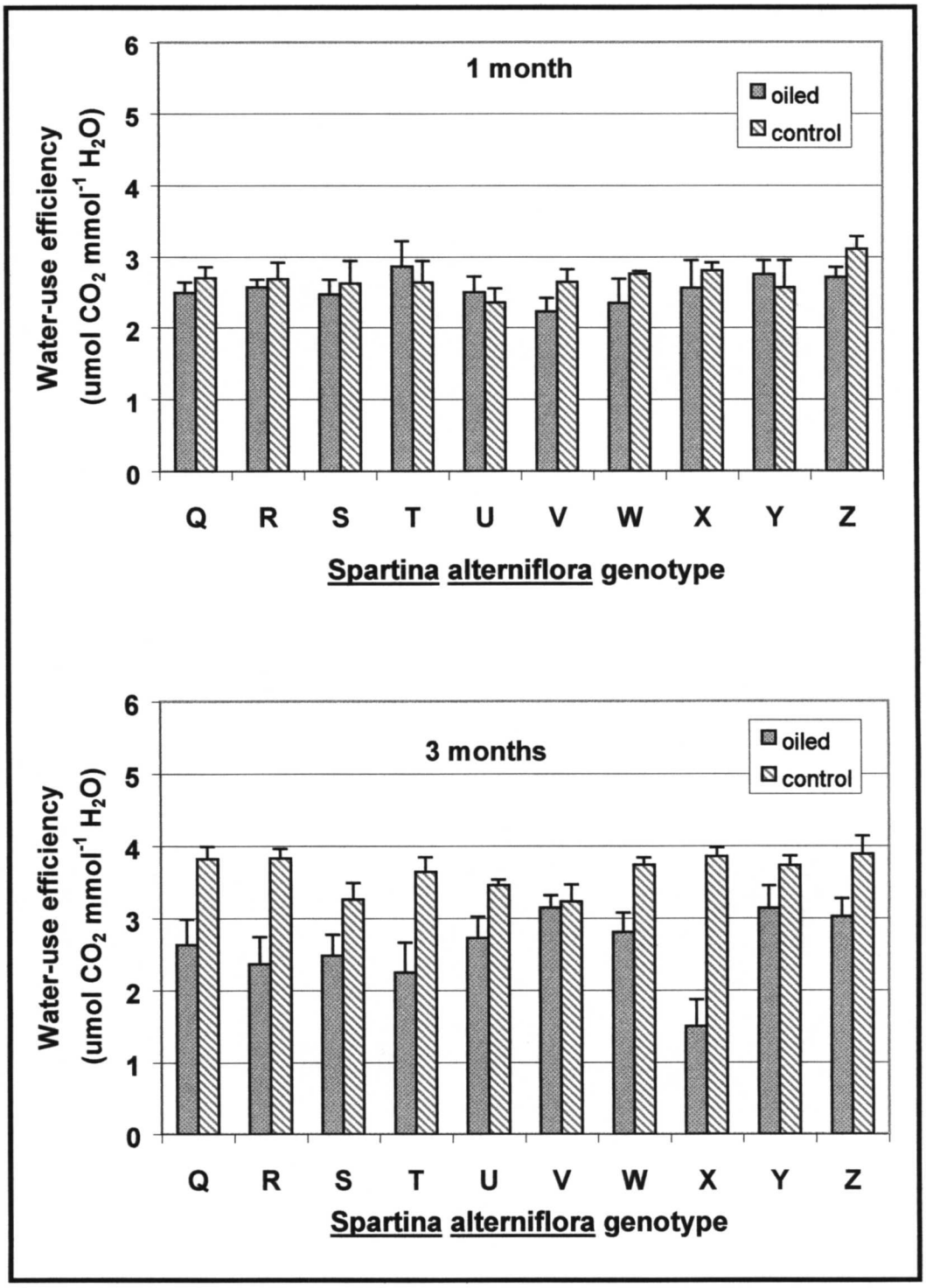

Figure 12. Mean (± standard error) water-use efficiencies (μmol CO_2 mmol^{-1} H_2O) of Spartina alterniflora genotypes one month (top panel) and three months (bottom panel) after oiling at a rate of 8 L oil m^{-2} (n=5).

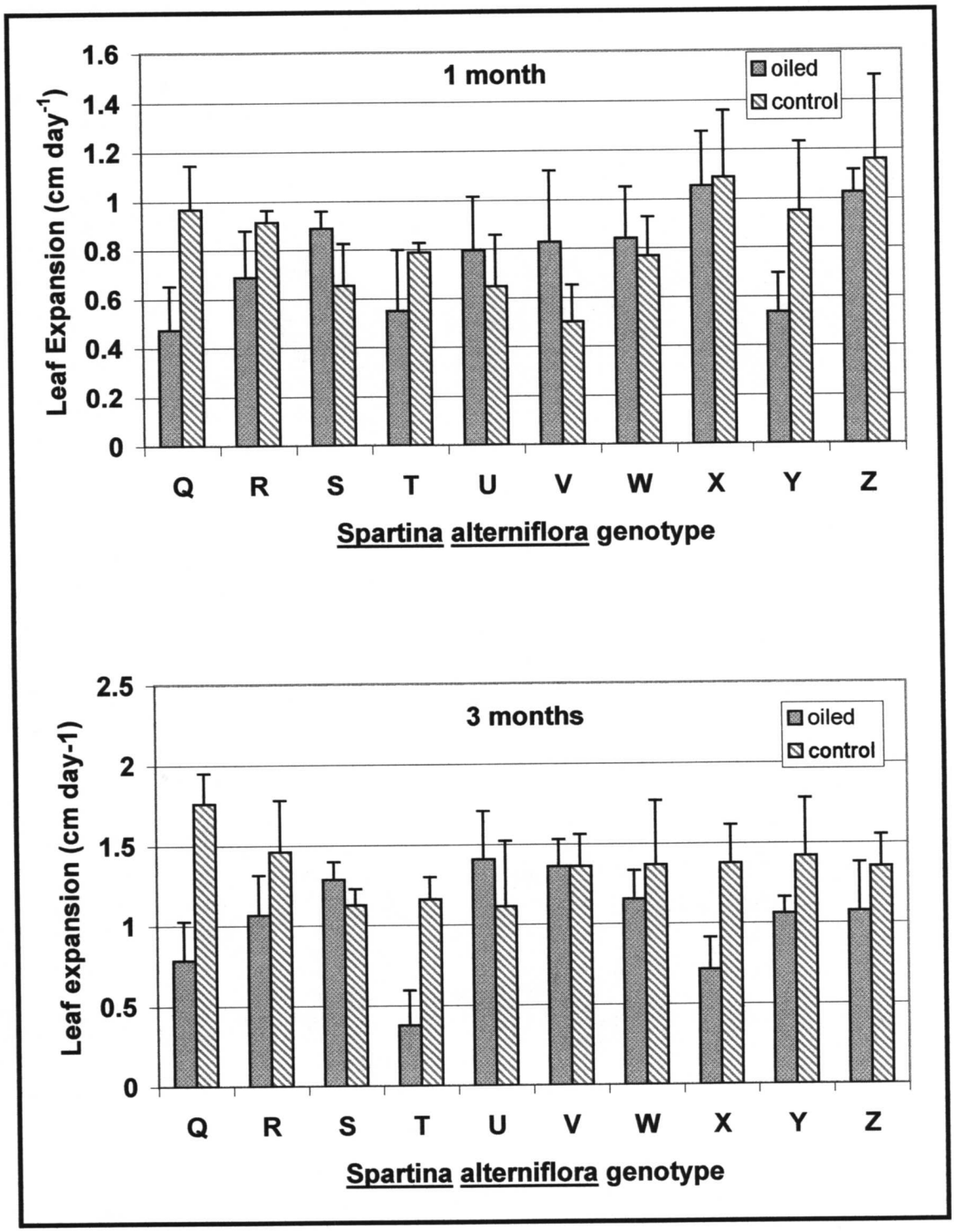

Figure 13. Mean (± standard error) leaf expansion rates (cm day^{-1}) of Spartina alterniflora genotypes one month (top panel) and three months (bottom panel) after oiling at a rate of 8 L oil m^{-2} (n=5).

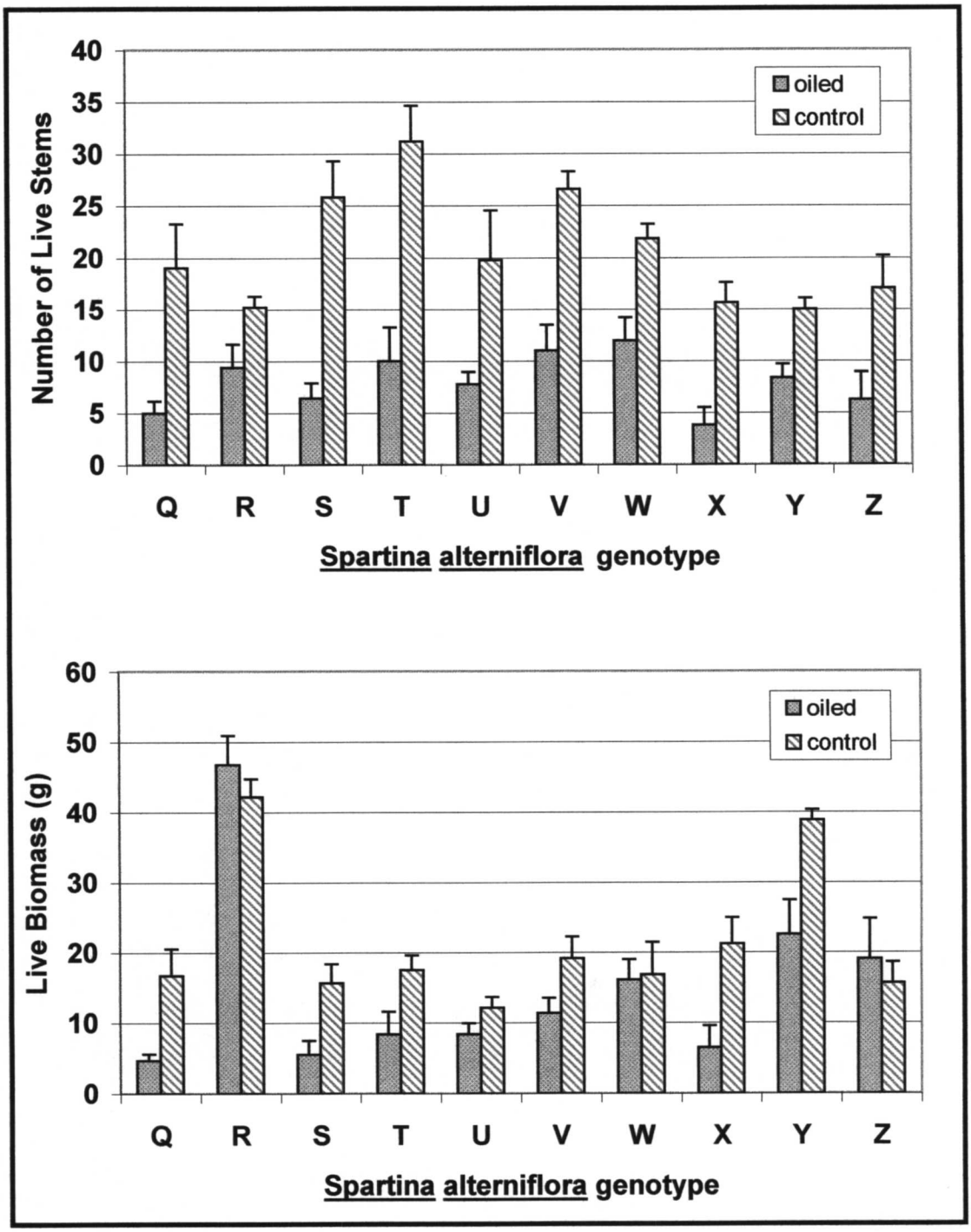

Figure 14. Mean (± standard error) number of live stems (# per pot; top panel) and live aboveground biomass (g per pot; bottom panel) of Spartina alterniflora genotypes three months after oiling at a rate of 8 L oil m^{-2} (n=5).

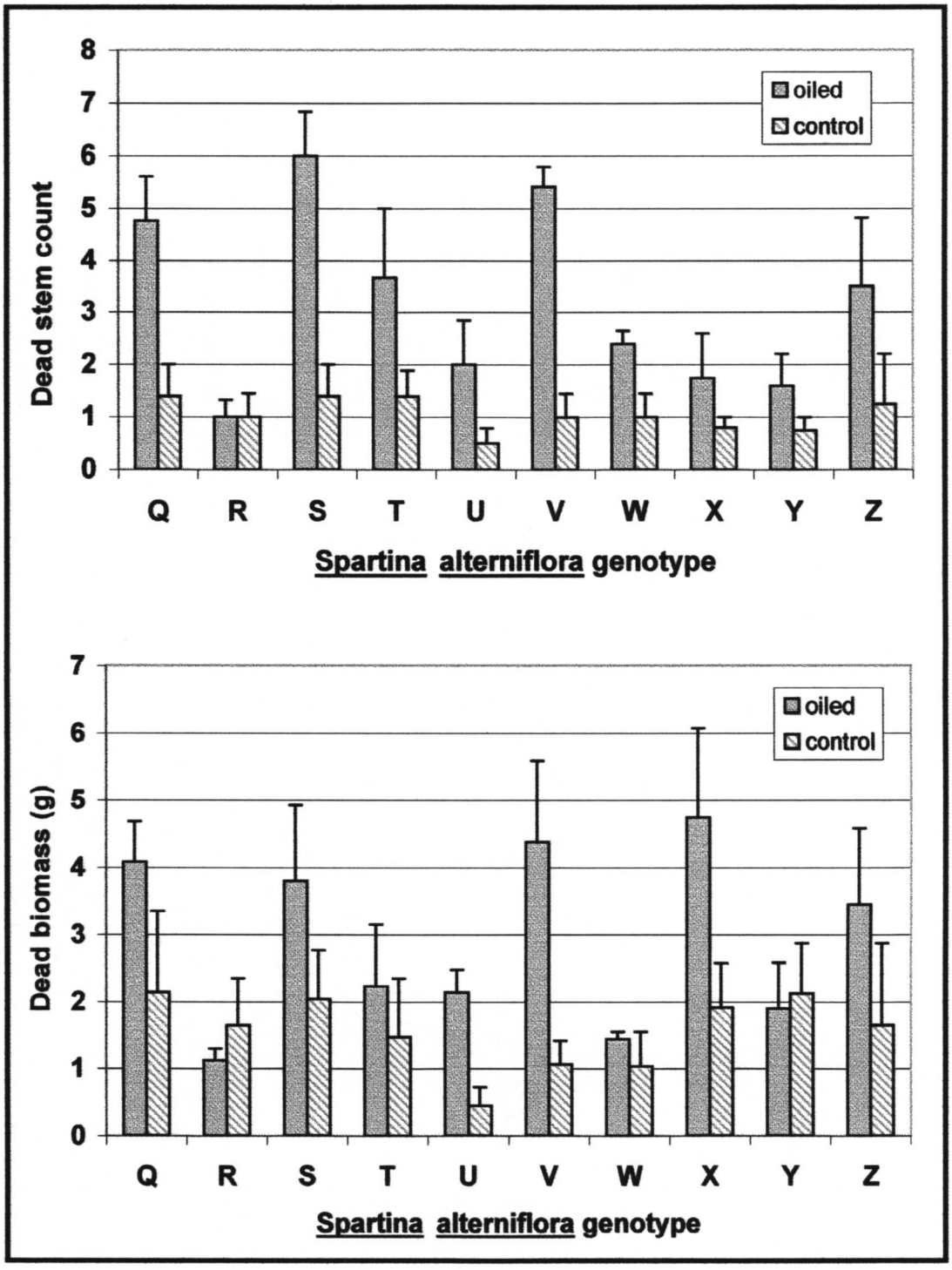

Figure 15. Mean (± standard error) number of dead stems (# per pot; top panel) and dead aboveground biomass (g per pot; bottom panel) of Spartina alterniflora genotypes three months after oiling at a rate of 8 L oil m^{-2} (n=5).

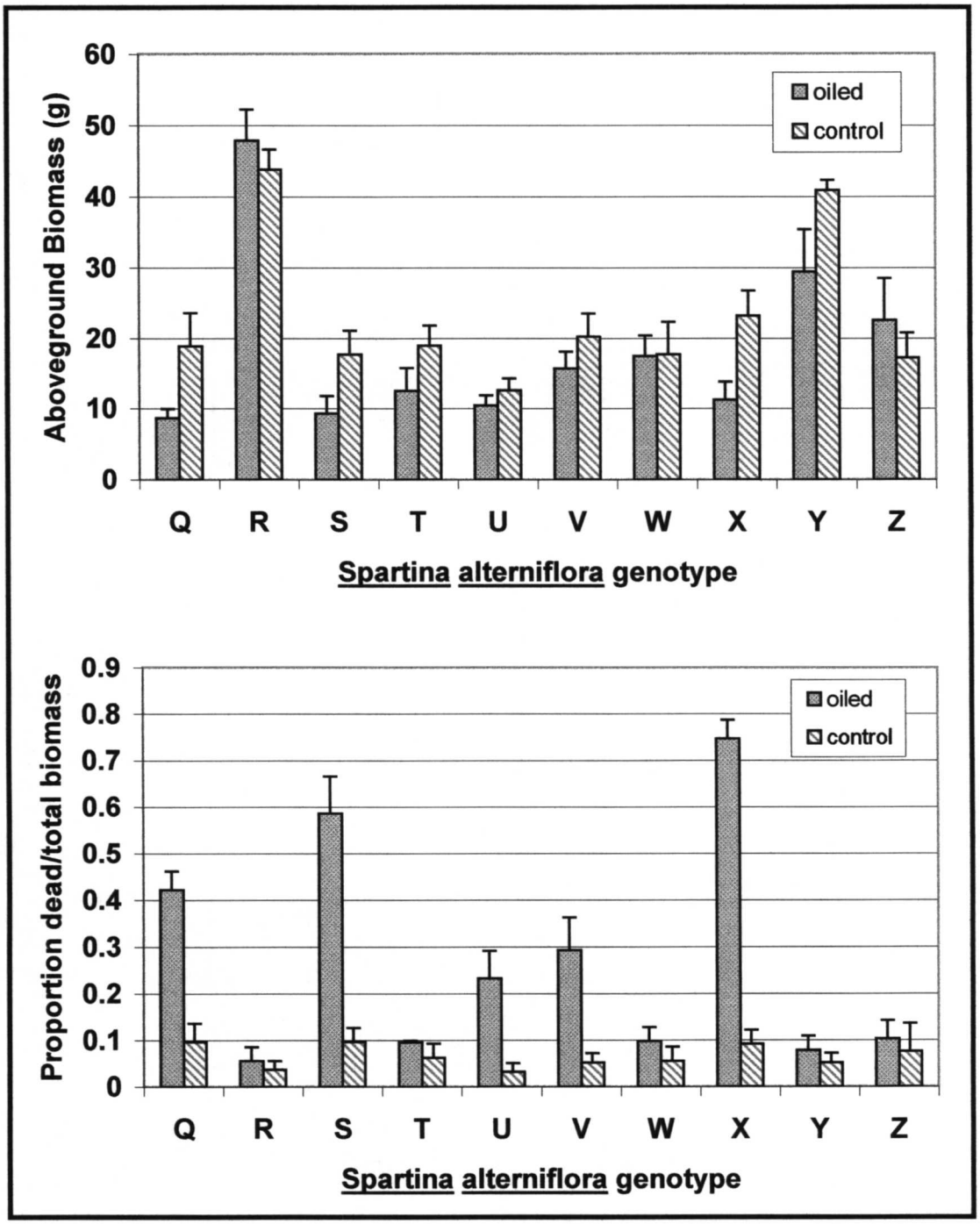

Figure 16. Mean (± standard error) total aboveground biomass (g per pot; top panel) and proportion of dead aboveground biomass (dead/total aboveground biomass) of Spartina alterniflora genotypes three months after oiling at a rate of 8 L oil m^{-2} (n=5).

larger proportions of dead aboveground tissue than the other genotypes (Figure 16). The analysis of the proportion of dead biomass expressed as a percentage of the control also showed significant genotype differences (Table 2). In addition to genotypes Q, S, and X, this analysis showed that genotypes U and V had some of the large values, but this was because of the relatively low amount of dead aboveground biomass in their controls (Figure 16; Table 2).

The amount of aboveground biomass regrowth (three months after the initial harvest) displayed a highly significant treatment effect of oiling, which drastically limited the amount of aboveground regrowth (Figure 17). There was also a significant effect of genotype, as well as a significant genotype x treatment interaction on aboveground biomass regrowth. The significant interaction resulted from the fact that four of the genotypes (S, T, X, and Z) failed to produce any new aboveground tissue in the oiled treatments, whereas genotypes R and V had substantial regrowth (Figure 17). In terms of regrowth expressed as a percentage of the control, there was also a highly significant effect of genotype. Genotypes R and V produced aboveground biomass regrowth in the oiled treatments that was 17.2% and 11.4%, respectively, of their control regrowth values (Table 2). Regrowth was also assessed in terms of the number of new stems produced after the initial harvest. In general, stem regrowth results were very similar to those of aboveground biomass regrowth (both in terms of actual response and percentage of control response) with genotypes R and V displaying the greatest regrowth and genotypes Q, U, W, and Y displaying measurable, but more limited, regrowth (Figure 17; Table 2).

The amount of residual oil remaining in the soil at the final harvest (six months after oiling) failed to display a significant effect of genotype (Figure 18). Values of residual oil ranged from lows of about 42 mg oil g^{-1} dry weight of soil in genotypes T and U to highs greater than 70 mg g^{-1} in genotypes V and X (Figure 18).

Results from the factor analysis of S. alterniflora genotype responses in the oiled treatment were similar to those obtained for S. patens. The clearest interpretation was again obtained when utilizing the same five plant growth response variables (photosynthesis (net CO_2 assimilation rate) at one month and three months, total aboveground biomass at the three month harvest, the proportion of dead aboveground biomass at the three month harvest, and the amount of aboveground biomass regrowth that occurred through the oiled soil) in addition to the amount of residual oil remaining in the soil at the end of the study. This six variable model resulted in a two factor solution that accounted for 58.8% of the variation in these variables (Figure 19). The first factor accounted for 41.1% of the variation and was dominated by a high negative loading of proportion of dead aboveground biomass (-0.812) and large positive loadings of photosynthesis at three months (0.752) and total aboveground biomass (0.721). The amount of regrowth biomass and photosynthesis at one month also had fairly large positive loadings of 0.654 and 0.554, respectively (Figure 19). As was the case for S. patens, this first S. alterniflora factor is also best described as a plant growth/regrowth factor, but with slightly more emphasis on the aboveground tissue at the time of oiling being resistant to tissue death. Therefore, S. alterniflora genotypes with high factor 1 scores would be classified as having high rates of photosynthesis and growth, with very minimal tissue death when oiled, and also relatively high rates of new aboveground biomass production. The ANOVA that was run on these factor scores detected highly significant differences between genotypes, with genotypes R, V, and Y having the highest factor 1 scores (Figure 20). The second factor accounted for an additional 17.7%

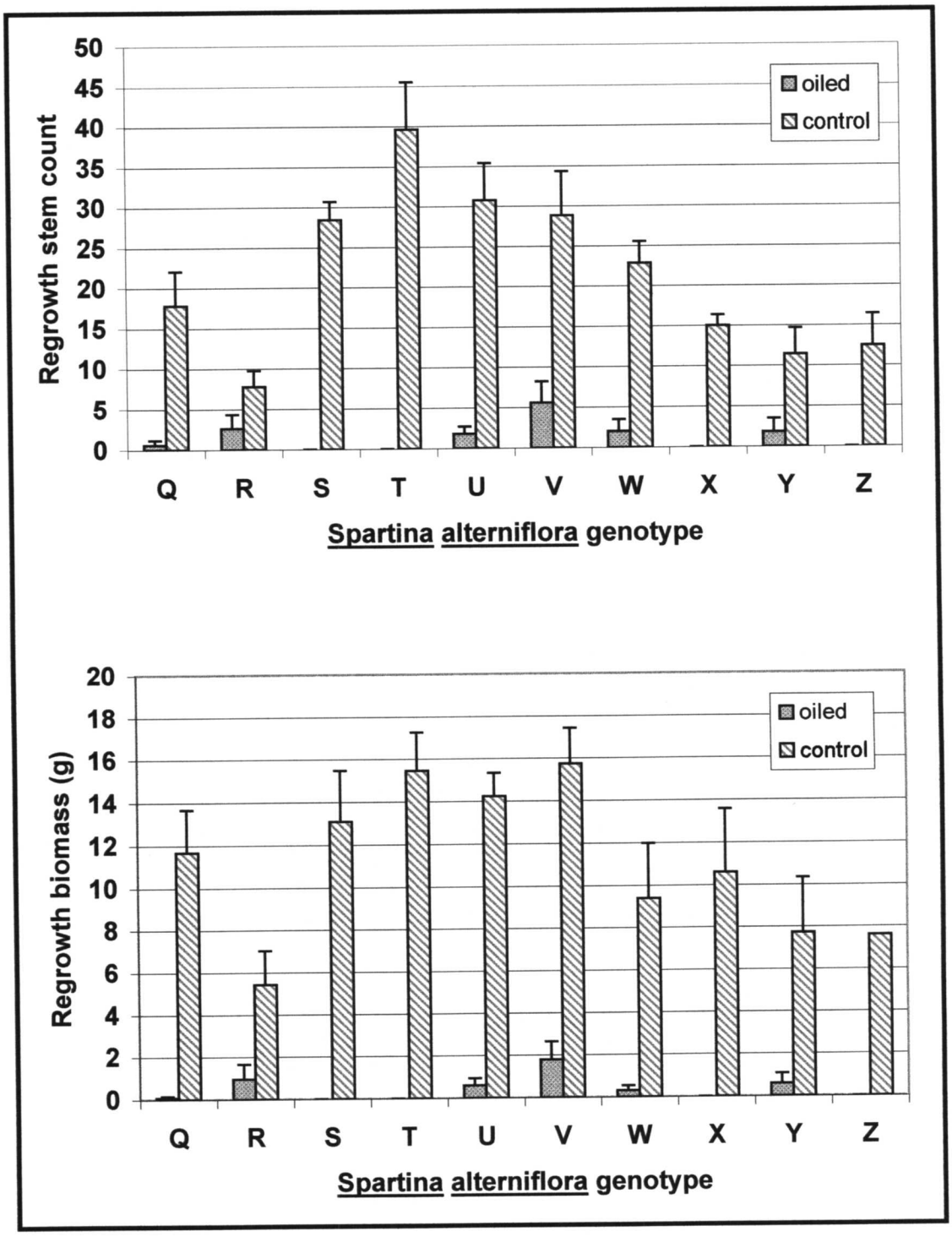

Figure 17. Mean (± standard error) number of regrowth stems (# per pot; top panel) and aboveground biomass regrowth (g per pot; bottom panel) of Spartina alterniflora genotypes three months after the initial harvest (n=5).

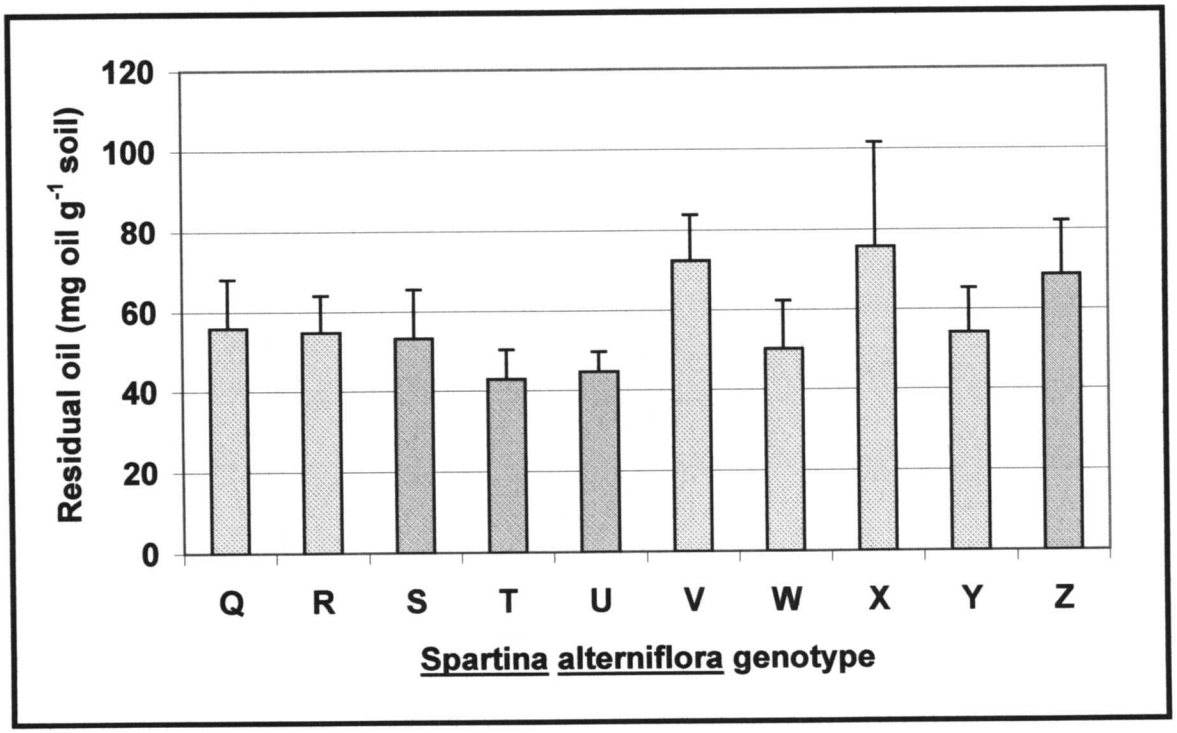

Figure 18. Mean (± standard error) residual oil (mg oil g^{-1} soil) remaining in the soil of Spartina alterniflora genotypes six months after oiling at a rate of 8 L oil m^{-2} (n=5).

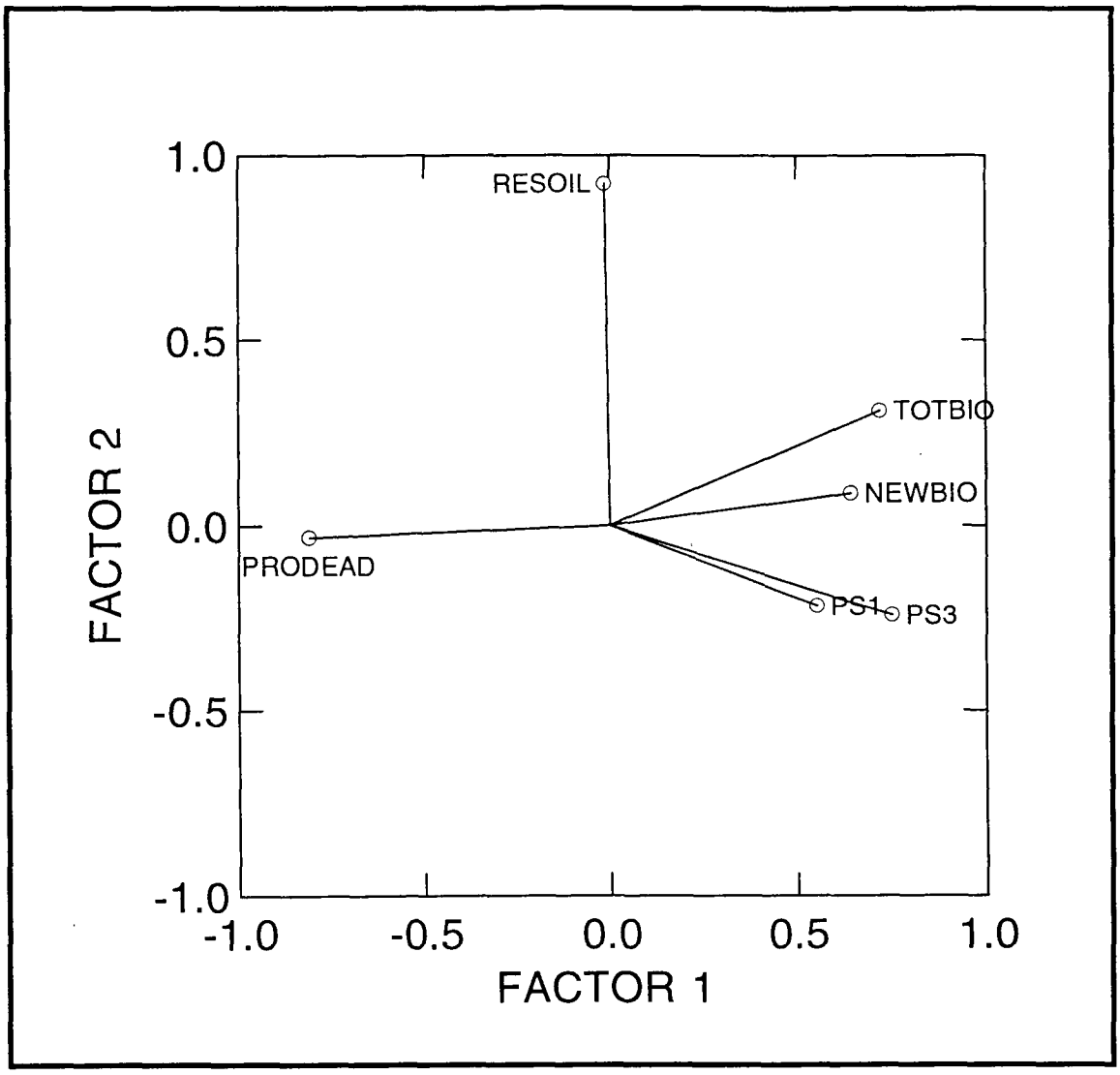

Figure 19. Factor loadings plot resulting from a factor analysis of residual oil and five plant response variables of oiled Spartina alterniflora genotypes. Abbreviations are as follows: PS1=photosynthesis (net CO_2 assimilation rate) one month after oiling, PS3=photosynthesis three months after oiling, TOTBIO=total aboveground biomass three months after oiling, PRODEAD=proportion of dead aboveground biomass three months after oiling, NEWBIO=amount of aboveground biomass regrowth between three and six months after oiling, and RESOIL=residual oil in the sediment six months after oiling (n=5).

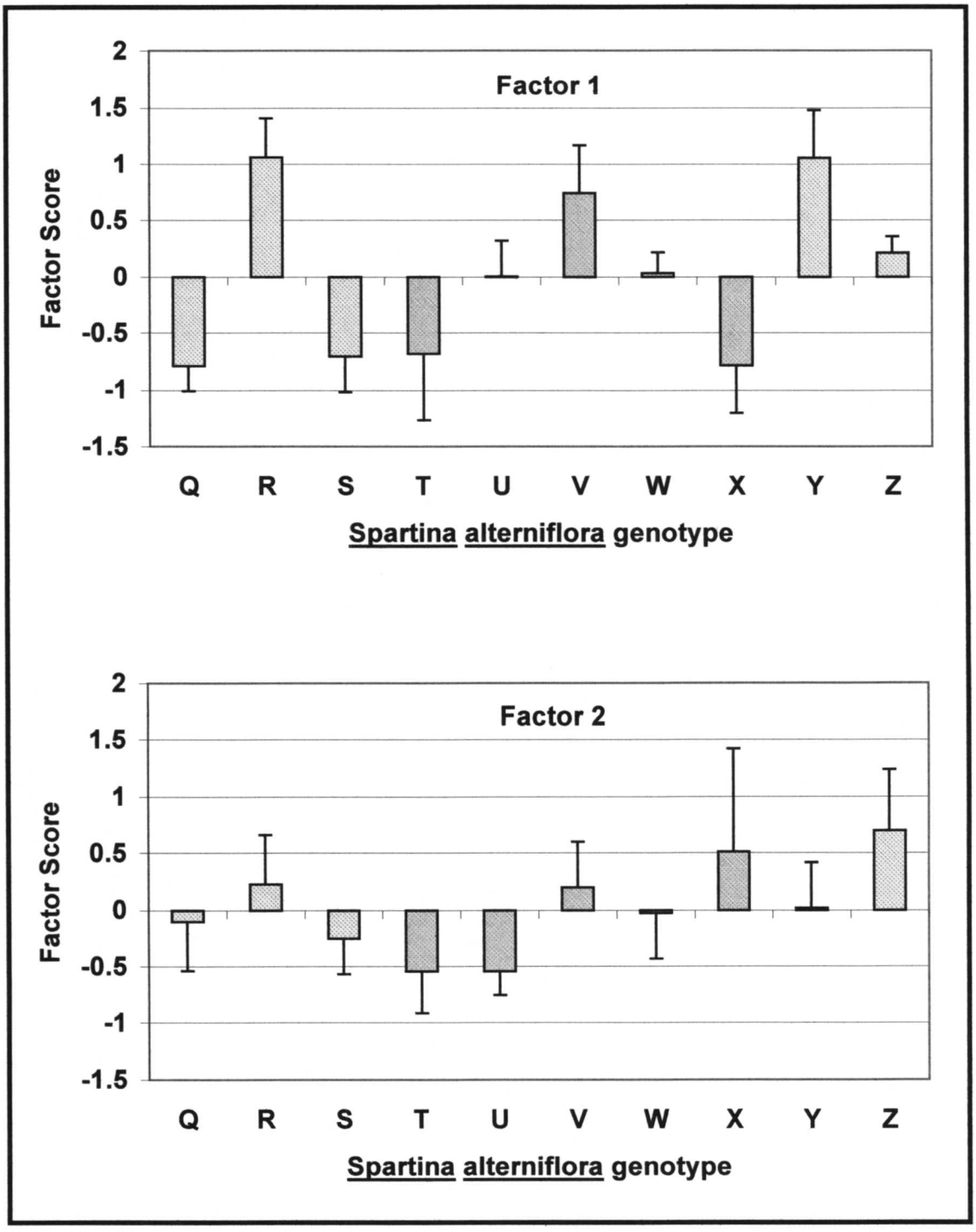

Figure 20. Mean (± standard error) factor 1 scores (top panel) and factor 2 scores (bottom panel) of oiled (8 L oil m^{-2}) Spartina alterniflora genotypes. High factor 1 scores are indicative of minimal tissue death, high photosynthesis and biomass, with relatively high aboveground biomass regrowth when oiled. High factor 2 scores are indicative of high amounts of residual oil remaining after six months (n=5).

of the variation and is described solely by a high positive loading of residual oil. Therefore, genotypes of S. alterniflora that have low (not high) factor 2 scores would be classified as genotypes whose presence resulted in less residual oil remaining at the end of the study. However, there was a tremendous amount of variation within genotypes in this factor 2 response and the ANOVA of genotype effects proved to be insignificant (P=0.774; Figure 20).

DISCUSSION

The results from this study have provided valuable, first-time information on intraspecific variation in oil tolerance in two dominant, wide-spread marsh grasses. In both Spartina patens (brackish marsh dominant) and Spartina alterniflora (salt marsh dominant) significant intraspecific variation to oiling was observed in a number of plant responses. Of the ten genotypes assessed in each species, several genotypes could be identified that displayed superior oil tolerance in terms of single and multiple plant-growth responses to oiling. This is a very exciting finding that has important ecological and applied value.

Although there has been considerable research on intraspecific variation in agricultural species for identifying those genotypes or varieties best suited for particular stressful environments (see Blum 1988 and references therein), researchers have only recently begun to seriously investigate the possibility of exploiting natural genetic variation to the environmental stresses present in coastal plant communities (Seliskar 1995). The ability to identify superior, stress-tolerant vegetation is anticipated to be of great value when selecting planting stocks to be used in marsh creation and restoration projects where stresses (both natural and anthropogenic) are known to be present, or are likely to occur. Much of the research to date on intraspecific variation in coastal plant species has focused on the natural environmental stresses of salinity and, to a lesser extent, flooding (see Seliskar 1995; Hester et al. 1996; Lessmann et al. 1997; Hester et al. 1998 and references therein). Of the coastal grass species, S. patens has been the most intensively investigated for intraspecific variation in salinity tolerance, which is indeed present across populations, or subpopulations, of this species (Silander 1979; Silander and Antonovics 1979; Pezeshki and DeLaune 1991; Seliskar 1995; Hester et al. 1996). Intraspecific variation in salinity tolerance in S. alterniflora has also been documented (Pezeshki and DeLaune 1995; Seliskar 1995; Hester et al. 1998). Similarly, Lessmann et al. (1997) have reported significant population variation in flood tolerance in both S. patens and S. alterniflora. Together, these studies have demonstrated that intraspecific variations in response to the natural stresses of salinity and flooding exist in these two wide-spread species, which should prove useful in selecting superior planting stocks for specific marsh restoration and creation needs.

Prior to this study, essentially no research had been conducted that assessed intraspecific variation to an anthropogenic stress, such as oiling. The results of this study clearly show that both S. patens and S. alterniflora display significant intraspecific variation to oiling and that superior genotypes can be identified that are more tolerant to oiling and have greater rates of regrowth and overall plant production than other genotypes. Not only is this finding valuable from a restoration perspective, but it may also help to explain some of the widely-divergent effects that oiling has been reported to have in similar wetland plant communities. For example, Mendelssohn et al. (1990) reported that a relatively minor spill of South Louisiana crude oil (0.28 L m^{-2}) in a Louisiana brackish marsh dominated by a mixture of S. patens and S. alterniflora resulted in a 64% reduction of live vegetative cover within three months, whereas DeLaune et al. (1979) reported that the addition of up to 8 L m^{-2} of Louisiana crude oil failed to reduce aboveground biomass in a S. alterniflora dominated Louisiana salt marsh

after four and sixteen months. Some of these observed differences may have been due to underlying genetic variation in oil tolerance. This is, however, difficult to assess because many factors have been identified that can affect the severity of oil impact on marsh vegetation. Factors such as the type and amount of oil spilled, water level (or tidal amplitude), degree of oiling to aboveground tissues, extent of penetration of the oil into the soil, soil organic matter content, season, species affected, and cleanup activities can all drastically influence the severity of an oil impact on marsh vegetation (Webb et al. 1985; Alexander and Webb 1987; Baker et al. 1993; Mendelssohn et al. 1990; Lin and Mendelssohn 1996). In the above comparison, Mendelssohn et al. (1990) state that oiling of the leaf tissue (due to high water levels) may have contributed to the severity of what would normally be considered a relatively low dosage spill. This is because leaf tissue of S. alterniflora (and other species) appears more sensitive to oiling than stem tissue (Crow 1974; Pezeshki et al. 1995). Nonetheless, the important point is that significant natural genetic diversity in oil tolerance has now been shown to exist across populations of S. patens and S. alterniflora and that this variation represents one more factor that needs to be considered, whether attempting to estimate or predict the severity of an oil spill, or explain observed differences in the severity of impact following a spill.

In this study, several oil-tolerant genotypes of S. patens and S. alterniflora displayed essentially no reduction in plant photosynthesis when oiled, while other genotypes displayed photosynthetic rates that were only about one-third those of the controls. In fact, some genotypes actually displayed a tendency toward greater rates of photosynthesis when oiled (Figures 1 and 11). The assessment of suitable short-term indicators of plant stress is a research area that has received considerable attention (see Mendelssohn and McKee 1992; Ewing et al. 1995; Larcher 1995 and references therein). Ewing et al. (1995) reported that net CO_2 assimilation rate (photosynthesis) appeared to be a more sensitive indicator of salinity stress in S. patens than leaf expansion rate. In their study, Ewing et al. (1995) were able to detect salinity stress sooner using net CO_2 assimilation rate than was possible using leaf expansion rate or other indicators. This agrees with the results of our study, where we generally found that leaf expansion rate was not as sensitive in detecting oil stress as net CO_2 assimilation rate. This was especially apparent in S. patens, which did not display a significant effect of oiling until the third month, when many of the oiled genotypes actually had higher leaf expansion rates than their controls (Figure 3). Lin and Mendelssohn (1996) also reported significant reductions in S. patens photosynthesis within one month of soil oiling, but this occurred at dosages greater than that used in this study (16 and 24 L m^{-2}).

Spartina alterniflora leaf expansion rates in our study similarly did not display a reduction with oiling in the first month, but did by the third month (Figure 13). However, even in the third month after oiling, S. alterniflora genotype differences were not detected in leaf expansion rate unless expressed as a percentage of the control, whereas genotype differences in net CO_2 assimilation rate were detectable from both the actual responses and these responses expressed as a percentage of the control (Table 2; Figure 11). Also using South Louisiana crude oil, Mendelssohn et al. (1993b) reported a reduction in S. alterniflora photosynthesis, but at oiling rates greater than the 8 L m^{-2} of our study (16 and 24 L m^{-2}). Lin and Mendelssohn (1996) did not observed a significant reduction of net CO_2 assimilation rate in S. alterniflora relative to controls until oiling at a rate of 24 L m^{-2} and, even then, not until after three months. Photosynthesis in S. alterniflora is reported to be reduced much more rapidly when the oil makes contact (either partially or

fully) with the leaf surface rather than just with the soil and root zone (Pezeshki and DeLaune 1993).

As was observed with leaf expansion rate, water-use efficiency (ratio of CO_2 assimilated per water lost) was also not a sensitive indicator in detecting oil stress or genotype differences. The only significant differences detected in water-use efficiency were in S. alterniflora in the third month after oiling, but even then these differences were largely driven by differences in net CO_2 assimilation rate (Figures 11 and 12). This finding is interesting since petroleum hydrocarbons can affect plants in a number of ways that may alter their water balance and respiration. Some of the these effects include disruption of plant water and ionic relations (McCown and Deneke 1972; Gilfillan et al. 1989), hindered nutrient uptake (McCown and Deneke 1972), toxicity to cells because of membrane disruption from lipophillic hydrocarbons in the oil (Prendeville and Warren 1977), and reduced gas exchange between the atmosphere and the soil (Ranwell 1968; Cowell 1969; Stebbing 1970) or between the atmosphere and the plant itself when leaf tissue is oiled (Webb 1994; Pezeshki et al. 1995).

Long-term plant stress response is most frequently assessed through the harvesting and partitioning of biomass into various components because plant production and allocation patterns ultimately reflect an integrated response to environmental stress (Fitter and Hay 1987; Larcher 1995; Bazzaz 1996). The value of partitioning biomass into various components was evident in both species' growth responses. In S. patens, total aboveground biomass (after three months of oiling) was not significantly affected by oiling, although significant oiling effects were evident in the amounts of live and dead aboveground biomass, as well as in the proportion of dead aboveground biomass (Figures 4, 5, and 6). Furthermore, for both species, differences in genotype performance generally became more lucid in the partitioned biomass variables, especially the proportion of dead aboveground biomass, which resulted in significant genotype effects in S. alterniflora that were not evident from dead aboveground biomass alone (Figures 15 and 16).

The amount of regrowth through the oiled soil proved to be a very critical indicator of a genotype's sensitivity and ability to asexually reproduce following an oil spill. In our study, all of the S. patens genotypes produced new stems through the oiled soil, although the extent of success differed significantly between genotypes (Figure 7). This finding generally agrees with the findings of Lin and Mendelssohn (1996), who reported significant decreases in the regrowth of S. patens through oiled sediment as oiling rates increased from 0 to 4 L m^{-2} and again from 4 to 8 L m^{-2}. The amount of regrowth at 8 L m^{-2} was so low that it was reported as not being significantly different from the zero regrowth that occurred in their higher oiling rates (Lin and Mendelssohn 1996). The oiling rate used in our study of S. patens (5 L m^{-2}) severely affected regrowth and allowed for good genotype separation without prohibiting at least minimal regrowth in the weakest genotypes. Conversely, the oiling rate used in our study of S. alterniflora (8 L m^{-2}) totally prevented any regrowth in four of the ten genotypes. Nonetheless, the six genotypes that did produce new stems through the oiled soil still displayed tremendous genotypic variation in this response (Figure 17). This observed sensitivity of emerging shoots of S. alterniflora to oiled sediment agrees with the findings of other studies. Mendelssohn et al. (1993b) reported significantly reduced regrowth of S. alterniflora through oiled sediment at an oil application rate of 4 L m^{-2}, although (as stated above) an application rate of greater than 8 L m^{-2} was required to significantly

reduce net photosynthesis. Furthermore, Lin and Mendelssohn (1996) reported that the emergence of new shoots of S. alterniflora through oiled sediment was extremely limited (and not different from zero regrowth) at an oiling level of 8 L m^{-2}. Therefore, as evidenced in both species in our study, but particularly in S. alterniflora, plants may survive an initial oiling of the soil surface, but experience a tremendous loss of regrowth potential as newly emergent shoots and leaf tissue come into contact with the oiled sediment.

Assessing regrowth potential through oiled sediment is an important consideration when evaluating oil tolerance in wetland vegetation since the continued (or renewed) production of new shoots is essential in maintaining marsh surface integrity. If no new growth occurs for an extended period of time following oiling, then sediment instability and erosion of the marsh surface may occur, resulting in a loss of marsh surface elevation and pond formation. Mendelssohn et al. (1993a) reported that a decrease in marsh surface elevation was believed to be a contributing factor in the failure of certain areas of Louisiana brackish and salt marsh to revegetate following aboveground tissue death after an oil spill. Several studies have found marsh elevational differences to have a significant impact on plant establishment and productivity (Mendelssohn and McKee 1988; Burdick and Mendelssohn 1990; Wilsey et al. 1992). Therefore, it may be highly desirable to have superior, oil-tolerant vegetation available that can be utilized to revegetate oiled marshes before substantial erosion of the marsh substrate occurs. It is possible that oil-tolerant genotypes may be directly planted in marshes impacted by minor spills, or planted following initial cleanup efforts in marshes impacted by major spills. Similarly, if oil tolerant vegetation has already been established in a restored marsh, then the probability of marsh loss resulting from future oil spills may be greatly reduced.

In addition to preventing erosion of the marsh substrate, the establishment of oil-tolerant vegetation at a spill site may aid in bioremediation by accelerating the oxidative degradation of residual oil via oxygen transport from the atmosphere through the leaves and stems and into the roots and rhizosphere, where the oxygen may diffuse into the oiled soil and potentially accelerate the degradation process (Armstrong 1978; Smirnoff and Crawford 1983). It was disappointing that significant differences between genotypes were not detected in their ability to degrade oil when analysed with univariate statistics. However, it must be kept in mind that the time frame of our study was relatively short, with the initial harvest of aboveground tissue taking place three months after oiling, and the final harvest of regrowth shoots occurring at six months after oiling. Also, there was considerable variation between replicates that was likely due to spatial variation in the distribution of the residual oil in the soil (Figures 8 and 18). In S. patens two of the three most oil-tolerant genotypes (C and J) were, in fact, associated with less residual oil, and had significantly greater oil degradation factor scores than the other genotypes (Figures 8 and 10). In S. alterniflora, there was less evidence of a trend associating the more oil-tolerant genotypes with less residual oil. However, it is likely that in the long term (and under field conditions) greater rates of oil degradation would be associated with those genotypes that are less stressed and have greater rates of plant productivity since air transport through the stems, leaves and roots of marsh vegetation is well-documented (Armstrong 1978; Smirnoff and Crawford 1983; Seliskar 1985), which should facilitate the degradation of oil. This may be especially important in marshes subject to a high incidence of oil pollution since chronic oil pollution has been implicated in causing the

development of anaerobic conditions that may also inhibit vegetative regrowth (Ranwell 1968; Cowell 1969).

Factor analysis proved indispensable in identifying genotypes in both species that displayed a suite of desirable plant responses when oiled. Univariate analyses showed that there were genotypes within each species that performed significantly better when oiled than others in terms of a number of single plant responses. However, to truly assess and test for significant genotype differences in performance across multiple variables, a multivariate approach, such as factor analysis, must be utilized (Johnson and Wichern 1988). The goal of factor analysis is to reduce the data from numerous variables into a fewer number of factors that still account for much of the information (variation) contained in the original variables. Initially, all the plant response variables plus the residual oil variable were included in the factor analysis of each species. Subsequent factor analyses were then conducted which systematically removed variables. Ideally, we wanted our final factor solution to consist of only a few factors, and for each factor to have variable loadings that would be consistent with either highly desirable or highly undesirable plant traits in terms of oil tolerance and oil degradation. The most suitable factor solutions were obtained for both species when utilizing the following variables: net CO_2 assimilation rate at one and three months, total aboveground biomass, the proportion of dead aboveground biomass, biomass regrowth through the oiled soil, and residual oil in the soil. Both species had a two factor solution that accounted for 72% of the variation in the S. patens data and 58.8% of the variation in the S. alterniflora data (Figures 9 and 19).

For both species, genotypes with significantly greater factor 1 scores were identified and are considered to be the most oil-tolerant genotypes. When oiled, these genotypes display higher photosynthesis, higher biomass accumulation with little tissue death, and higher regrowth of new stems than the other genotypes. For S. patens, these were genotypes C, G, and J (Figure 10), and for S. alterniflora, genotypes R, V, and Y (Figure 20). This finding is extremely encouraging because it identifies several genotypes within each species that truly display a suite of desirable, oil-tolerant characteristics. Factor 2 scores were associated with residual oil in both species. In S. patens, factor 2 had a large negative loading of residual oil, whereas in S. alterniflora, factor 2 had a large positive loading of residual oil. As a result, for S. patens, large factor 2 scores would be associated with low amounts of residual oil, whereas for S. alterniflora, small (not large) factor 2 scores would be associated with low amounts of residual oil. In neither species was an overall effect of genotype on factor 2 scores detected. However, in S. patens, a linear contrast did show that genotypes C and J had significantly larger factor 2 scores than the other genotypes, implying that genotypes C and J may, in fact, possess a greater ability to degrade oil than the other genotypes (Figure 10). Future research is needed to assess the long-term performance of selected genotypes to oiling with regard to sustained productivity and potential genotype differences in oil degradation.

CONCLUSIONS

This study has provided several findings of interest to state and federal agencies involved in oil spill response and the restoration and creation of wetlands. This project has provided first-time information on the amount of intraspecific variation in oil tolerance in two wide-spread marsh plant species that are found throughout the Gulf and Atlantic Coasts of the United States. In both Spartina patens (brackish marsh dominant) and Spartina alterniflora (salt marsh dominant) significant intraspecific variation was documented in a number of plant responses to oiling. Plant photosynthetic response (net CO_2 assimilation) was found to be an excellent short-term indicator of oil stress that displayed significant variation between genotypes, thereby facilitating the identification of superior, oil-tolerant genotypes to a much greater extent than was possible using leaf expansion rate. Partitioning plant aboveground biomass into live and dead components and, especially, assessing the proportion of dead to total aboveground biomass proved very useful in discerning significant genotype differences in longer-term response variables to oiling.

Factor analysis confirmed that several oil-tolerant genotypes (three in each species) could be identified that displayed suites of desirable plant responses when oiled that were superior to the other genotypes. These superior responses when oiled included the ability to maintain high photosynthetic rates over time, high plant production with limited tissue death, and high regrowth potential of new shoots through the oiled soil. Genotype differences in oil degradation potential, based on residual oil remaining in the soil six months after oiling, were not detected with univariate statistics in either species. However, in S. patens, a linear contrast did reveal that two of the three oil-tolerant genotypes were associated with significantly greater oil-degradation factor scores, indicating that these genotypes may possess a greater potential to degrade oil than other genotypes. Longer term studies, and more intensive sampling to reduce spatial heterogeneity of residual oil in the soil, may be necessary to elucidate genotype differences with regard to oil degradation potential.

The significant amount of intraspecific variation to oiling observed in S. patens and S. alterniflora in this study has several implications. Some of the reported variation in the severity of oiling in brackish and salt marshes may be at least partially explained by natural genetic variation in these dominant species. Intraspecific variation to oiling is, therefore, one more factor that should be considered when oil spill impacts are predicted or assessed in coastal plant communities. Also, the results of this study have shown that natural genetic variation in response to oiling can be exploited in the identification of superior, oil-tolerant genotypes. Oil-tolerant genotypes of these dominant grasses may be transplanted into severely oiled marshes, thereby re-establishing vegetation more rapidly, lessening the potential of wetland loss, and possibly accelerating the bioremediation of the site. A potential future application of this research may be planting oil-tolerant genotypes of S. patens and S. alterniflora in areas that historically have a high incidence of oil spills or oil leakage, thereby lessening the severity of impact that may occur in other genotypes.

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The Department of the Interior Mission

As the Nation's principal conservation agency, the Department of the Interior has responsibility for most of our nationally owned public lands and natural resources. This includes fostering sound use of our land and water resources; protecting our fish, wildlife, and biological diversity; preserving the environmental and cultural values of our national parks and historical places; and providing for the enjoyment of life through outdoor recreation. The Department assesses our energy and mineral resources and works to ensure that their development is in the best interests of all our people by encouraging stewardship and citizen participation in their care. The Department also has a major responsibility for American Indian reservation communities and for people who live in island territories under U.S. administration.

The Minerals Management Service Mission

As a bureau of the Department of the Interior, the Minerals Management Service's (MMS) primary responsibilities are to manage the mineral resources located on the Nation's Outer Continental Shelf (OCS), collect revenue from the Federal OCS and onshore Federal and Indian lands, and distribute those revenues.

Moreover, in working to meet its responsibilities, the **Offshore Minerals Management Program** administers the OCS competitive leasing program and oversees the safe and environmentally sound exploration and production of our Nation's offshore natural gas, oil and other mineral resources. The MMS **Royalty Management Program** meets its responsibilities by ensuring the efficient, timely and accurate collection and disbursement of revenue from mineral leasing and production due to Indian tribes and allottees, States and the U.S. Treasury.

The MMS strives to fulfill its responsibilities through the general guiding principles of: (1) being responsive to the public's concerns and interests by maintaining a dialogue with all potentially affected parties and (2) carrying out its programs with an emphasis on working to enhance the quality of life for all Americans by lending MMS assistance and expertise to economic development and environmental protection.