

1988

## **The Relationship Between Root Anatomic and Metabolic Responses to Soil Waterlogging in the Coastal Grass *Spartina Patens*.**

David Maaloe Burdick  
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**The relationship between root anatomic and metabolic responses  
to soil waterlogging in the coastal grass *Spartina patens***

Burdick, David Maaloe, Ph.D.

The Louisiana State University and Agricultural and Mechanical Col., 1988

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THE RELATIONSHIP BETWEEN ROOT ANATOMIC AND  
METABOLIC RESPONSES TO SOIL WATERLOGGING  
IN THE COASTAL GRASS *SPARTINA PATENS*

A DISSERTATION

Submitted to the Graduate Faculty of the  
Louisiana State University and  
Agricultural and Mechanical College  
in partial fulfillment of the  
requirements for the degree of  
Doctor of Philosophy

in

The Department of Marine Sciences

by

David Maaloe Burdick

B.S., Hobart College, 1977

December 1988

To Funi,  
for  
her devotion  
and inspiration,  
and for  
providing motivation,  
fondly

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## ABSTRACT

*Spartina patens* is a perennial grass that dominates dune, swale and marsh habitats in coastal Louisiana. Plant roots require oxygen for respiration and normal root function, and one of the most serious problems wetland plants encounter is the development of anaerobic conditions in the soil. The remarkable success of *S. patens* in habitats that occupy distant positions on a soil waterlogging gradient suggests either genetic differentiation or phenotypic adaptation among these populations. Field and greenhouse investigations were conducted to: 1) identify anatomic and metabolic soil waterlogging responses in *Spartina patens*, 2) examine the relationship between these responses, and finally 3) compare differences among dune, swale, and marsh populations with respect to soil waterlogging responses.

In the field, root aerenchyma (an internal gas-space system that allows for root aeration) increased with increasing soil waterlogging from dune to marsh habitats. Although aerenchyma volume was greatest in roots of marsh plants (50%), it supplied insufficient oxygen to fully support aerobic respiration, as indicated by relatively high alcohol dehydrogenase (ADH) activities in these roots. Roots from dune, swale, and marsh habitats responded to episodes of relatively greater soil waterlogging with changes in metabolism that resulted in increased malate and lactate concentrations and was accompanied by decreases in the adenylate energy charge ratio (AEC; energy status).

In the greenhouse, aerenchyma development in new and preexisting roots was stimulated by flooding. Increases in soil waterlogging led to increased root ADH activities and malate concentrations, while AEC ratio decreased. Time course measurements showed that development of aerenchyma in flooded roots was followed by

a decline in ADH activity, indicating aerenchyma reduced capacity for anaerobic fermentation. The relationship between anatomic and metabolic responses indicated that aerenchyma is important, but of limited utility in relieving hypoxic conditions in roots. In *Spartina patens*, metabolic adaptations may be essential for root survival under conditions of either severe or rapidly increasing soil waterlogging.

An experiment using plants repropagated four years in a common environment showed genetic differences in flooding responses among population sources. However, these differences may not have been physiologically significant since no differences in flooding tolerance were found among populations.

## INTRODUCTION

Wetland plants inhabit environments that are subject to varying degrees and frequencies of flooding. Unlike most plants which are damaged by flooding, wetland plants are adapted to survive flooding and long term soil waterlogging. The problems that wetland plants must overcome include root oxygen deficiencies and exposure to soil toxins, such as hydrogen sulfide, and are reviewed in Chapter 1. This first chapter is a literature review emphasizing the connection between changes in root processes and growth responses that are associated with soil waterlogging.

One of the most important problems wetland plants face is the development of anaerobic conditions in the soil. In their treatise on survival in anaerobic environments, Hochachka and Somero (1973) outline three approaches organisms use to endure life in anaerobic environments. In the context of plant survival in flooded soils, 1) the avoidance strategy entails securing a supply of oxygen independent of the soil environment (e.g. the development of aerenchyma, an internal gas space system that allows for oxygen diffusion from the atmosphere to below ground organs); 2) the compensatory strategy involves an acceleration of anaerobic fermentation (notably ethanol fermentation) to provide energy for survival under anaerobic conditions; and 3) the exploitative strategy depends on changes in root metabolism that enhance cell function under anaerobiosis through the generation of alternate end products and coupling of metabolic paths.

Plant roots require oxygen for respiration and the maintenance of normal root function. It appears that root growth and other oxidative processes that are poorly understood require a greater oxygen concentration than does the support of aerobic

respiration (Jackson and Drew 1984, Saglio et al. 1984, Atwell and Greenway 1987). Under drained conditions, oxygen required by the roots is obtained from the soil atmosphere via radial oxygen diffusion across the epidermis through the root (Luxmore et al. 1970a). In wetland soils devoid of oxygen, plants must convey oxygen from above-ground organs through the internal paths of aerenchyma to their roots for continued growth and aerobic respiration (Luxmore et al. 1970b, Armstrong 1979, Saglio et al. 1983). This is interpreted as an avoidance strategy. Some investigators have proposed that the aerenchyma provision in wetland roots exceeds the aerobic and growth requirements of the root system (Webb and Armstrong 1983). They believe the large amount of gas-space in these roots is an adaptation for detoxification of the root rhizosphere through oxidation of soil toxins (Armstrong 1972, 1979), or for reducing the number of respiring cells while maintaining structural support (Williams and Barber 1961).

Alternatively, changes in root metabolism could enhance survival in anaerobic habitats. Observing what they interpreted to be examples of the exploitative strategy, McManmon and Crawford (1971) published a metabolic theory of flooding tolerance. Decreasing reliance on alcoholic fermentation by switching to other fermentations (e.g., malate) enabled plants to tolerate soil waterlogging better than intolerant plants that mainly relied on an acceleration of ethanol fermentation. However, work by other investigators usually includes a discussion of how their findings contradict McManmon and Crawford's hypothesis (Keeley 1979, Smith and ap Rees 1979, Jackson and Drew 1984). At present, it is generally believed that flood tolerance in hypoxic roots of wetland plants is conferred through an acceleration of ethanol fermentation. Increases in alcohol dehydrogenase activity, the enzyme responsible for catalyzing the reaction that produces ethanol, occurs in roots of many flood-tolerant plants subject to root anaerobiosis (Smith and ap Rees 1979, Mendelssohn et al. 1981, Jackson and Drew 1984). It is believed that



the ATP produced during ethanol fermentation provides enough metabolic energy to enable the roots to survive anaerobiosis (Jackson and Drew 1984, Roberts et al. 1984; Saglio et al. 1988).

The results of several investigators (Keeley 1979, Saglio et al 1983, Drew et al. 1985) indicate that anatomic and metabolic adaptations to flooding tolerance act in combination. Others have concluded that a particular set of adaptations is primarily responsible for flooding tolerance in the species they have studied (Tripeppi and Mitchell 1984, Armstrong 1971, Pearson and Havill 1988). The work in the following chapters compares these types of adaptations in a common wetland grass of coastal Louisiana.

*Spartina patens* Aiton (Mulh.) is a perennial grass that dominates the coastal vegetation of Louisiana. Populations of *S. patens* may dominate dune, swale, and especially marsh habitats. Soil waterlogging is a major environmental parameter that differs greatly across the three habitats and is responsible for many of the species zonation patterns along elevational gradients in and around wetlands (Vince and Snow 1984). The remarkable success of *S. patens* in habitats that occupy distant positions on the soil waterlogging gradient suggests either genetic differentiation or plastic phenotypic adaptations expressed differentially among these populations. Genetic differences among different populations growing along similar gradients have been found for *Spartina patens* (Silander and Antonovics 1979), *Agrostis* (Ahmad and Wainwright 1974), and *Nyssa sylvatica* (Keeley 1979). In this study, responses to soil waterlogging by the three populations of *Spartina patens* were examined in the field and are reported in Chapter 2. Anatomic and metabolic responses to both spatial and temporal increases in soil waterlogging were found (Burdick and Mendelssohn 1987 Chapter 2), but these inferences were based on correlative associations. Therefore, a series of experiments were performed under controlled conditions to firmly establish relationships between soil waterlogging and *Spartina patens* responses. Chapter 3 examines the development of

aerenchyma in new and previously existing roots under drained and flooded conditions. Metabolic responses of roots to short-term increases in soil waterlogging were monitored in Chapter 4. Finally in Chapter 5, the relationship between anatomic and metabolic responses to soil waterlogging is examined. This dissertation also addresses the question of ecotypic differences among *Spartina patens* populations from dune, swale, and marsh habitats with respect to flooding adaptations and tolerance to flooding. These were examined in the field (Chapter 2) and with plants repropagated and grown in a common environment for several years prior to initiating the flooding experiment reported in Chapter 5. In summary, these investigations were devised to: (1) identify anatomic and metabolic soil waterlogging responses in *Spartina patens*, (2) examine the relationship between these responses, and finally (3) compare differences among the three populations with respect to soil waterlogging responses. Since the chapters are manuscripts prepared for specific books and journals, subtle differences in format exist.

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CHAPTER 1.  
THE RELATIONSHIP OF SOIL PARAMETERS AND ROOT FUNCTION TO  
PRIMARY PRODUCTION IN PERIODICALLY INUNDATED SOILS<sup>1</sup>

INTRODUCTION

Hydrology is the dominant forcing function in wetland ecosystems (Gosselink and Turner 1978). Either directly, or through its direct effect on soil waterlogging, the dynamic hydrologic environment controls soil physicochemical status, sedimentation rates, salinity, nutrient cycling, decomposition, and faunal and microfloral activities. Thus soil waterlogging controls the interaction of soil and root processes which, in turn, influence growth (Figure 1.1). Although wetland vegetation is highly productive, the roots of these plants often experience chemically reduced conditions in the soil that are severe, lack of oxygen, and toxic compounds as a result of soil waterlogging. This review emphasizes the linkage between growth responses and waterlogging-induced changes in root processes of wetland vegetation. While not exhaustive, the examples in Tables 1-5 illustrate the major soil-root interactions which influence growth.

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<sup>1</sup> Portions of this chapter were published as part of "The relationship of soil parameters and root metabolism to primary production in periodically inundated soils" published in: *The Ecology and Management of Wetlands, Volume 1: The Ecology of Wetlands*, edited by D. D. Hook et al., and published by Croom Helm, London.

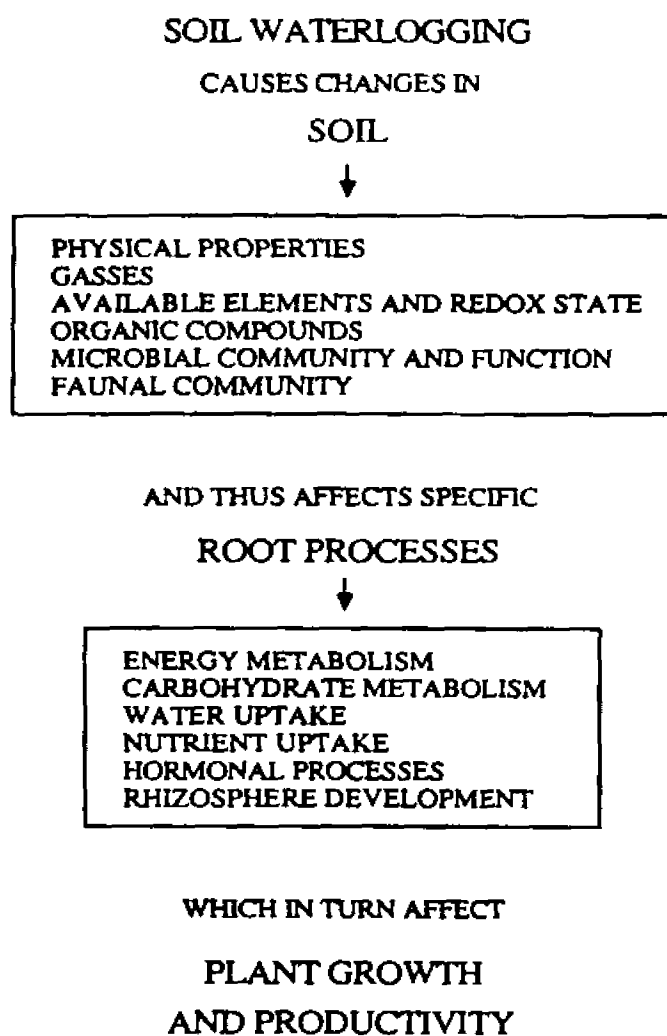


Figure 1.1: Changes in the soil environment due to soil waterlogging affect specific root processes which in turn affect plant growth

## ENERGY METABOLISM

Although this topic has received attention in several reviews (Pradet and Bomsel 1978, Davies 1980, Pradet and Raymond 1983, Hook 1984, Jackson and Drew 1984), much of the research concerned with the impacts of waterlogging on root energy metabolism has focused on a few agricultural species. In experiments with maize and rice, decreasing supplies of oxygen below the critical oxygen pressure (COP: the oxygen concentration below which aerobic respiration is reduced) result in a depression of the root energy status (Saglio et al. 1983) as indicated by the adenylate energy charge ratio (AEC: the ratio of phosphorylated adenine nucleotides to the total adenine nucleotide pool). However, the AEC of seeds, roots, and rhizomes of more waterlogging-tolerant species placed in anoxia partially rebounds after initially declining, apparently due to an increase in alcoholic fermentation (Pradet and Bomsel 1978, Monk and Braendle 1982, Rumpho et al. 1984). Furthermore, often drastic reductions in root AEC occur when the capacity to accelerate ethanol fermentation does not exist (Roberts et al. 1984a) or is eliminated by inhibitors or a lack of substrate (Saglio et al. 1980, 1983).

Declines in root and rhizome energy charge ratios in wetland species have been associated with root hypoxia in laboratory experiments (bullrush: Monk and Braendle 1982; rice: Saglio et al. 1984; river birch: Tripepi and Mitchell 1984a) and under natural field conditions (*Spartina*: Mendelssohn et al. 1981). As was found for agricultural species in the laboratory, decreases in root AEC associated with decreases in oxygen availability to the root (measured as decreases in soil redox potential) were partially reversed with increases in alcoholic fermentation (measured as ADH activity) in *Spartina alterniflora* (Mendelssohn et al. 1981, Mendelssohn and McKee 1986). No research was found that showed a direct or a strong correlative link between root hypoxia and growth in wetland species via energy metabolism (Table 1.1), but Monk and Braendle (1982) do



Table 1.1: Growth responses to changes in energy and carbohydrate metabolism due to soil waterlogging of selected wetland plants.

Species	Treatment	Change in metabolism <sup>a</sup>	Change in growth or survival	Reference
Rice	N <sub>2</sub> /sand culture	PDC and ADH increase at 0% [O <sub>2</sub> ]	No change in root or shoot	Wignarajah et al. (1976)
Rice	N <sub>2</sub> /added glucose	Anaerobic respiration with added glucose	Increased survival time	Vartepatian et al. (1977)
Rice	4 cm H <sub>2</sub> O over seeds	ethanol increases in solution	No root growth	Avadhani et al. (1978)
Rice	N <sub>2</sub> for 48 hrs	Pasteur effect with pure ethanol fermentation	No growth but tips viable	Bertani et al. (1980)
Rice	Several [O <sub>2</sub> ]	ADH increases if [O <sub>2</sub> ] < 10%	Shoot decrease if [O <sub>2</sub> ] < 10%	Bertani and Brambilla (1982)
Rice and <i>Echinochloa</i> complex	Seeds or whole seedlings in several [O <sub>2</sub> ] up to 7 days	Glycolysis and pentose-phosphate paths important; CO <sub>2</sub> + EtOH produced but Pasteur Effect absent delay and depression in respiration	No root growth but survival	Rumpho and Kennedy (1981) Rumpho and Kennedy (1983) Kennedy et al. (1983)
3 Wetland species	Rhizomes in 0% O <sub>2</sub> and several [CO <sub>2</sub> ]	Respiration reduced	Retained viability	Crawford (1982b)
13 Wetland species	Flooding in sand for 1 month	Control of metabolic rate	Growth continues and plants survive	Crawford (1966, 1967)
<i>Glyceria maxima</i>	Several [O <sub>2</sub> ]	Carbon metabolism changes	Biosynthesis for growth stops at 2% [O <sub>2</sub> ]	ap Rees and Wilson (1984)
<i>Spartina alterniflora</i>	Eh controlled for 20 days	ADH rises with falling Eh	No change in root or shoot	DeLaune et al. (1984)

Table 1.1 (continued)

Species	Treatment	Change in metabolism <sup>a</sup>	Change in growth or survival	Reference
5 salt marsh species	100µM Sulfide	O <sub>2</sub> uptake reduced in all species 2/4 species with low metallo-enzyme activities	1 species showed no effects, 1 showed decreased root growth, 3 had lower growth and chlorosis	Havill et al. (1985)
<i>Pinus contorta</i>	Anoxic hydroponics	Glycolytic control of ethanol production	Growth continues in leading shoots	Crawford + Baines (1977)
<i>Pinus contorta</i>	Flooding	Changes in root [sugar]	Shoot growth sustained	Vester and Crawford (1978)
<i>Nyssa sylvatica</i>	Flooding 3 populations	ADH activity + malate levels rise in tolerant population	Roots replaced but equal production	Keeley (1977, 1979)
5 tropical tree species	Flooding 1 month	5 had Pasteur Effect; but carbohydrate metabolisms differ	3/5 species continued shoot growth	Joly and Crawford (1982)
<i>Betula nigra</i>	N <sub>2</sub> gas 18 days	AEC falls after 6 days; ADH increases	Survival with leaf chlorosis cytochrome oxidase activity falls	Tripepi and Mitchell (1984a)
<i>Acer rubrum</i> and <i>Betula nigra</i>	Flooding 1 month	Capacity for aerobic respiration falls	No change in <i>A. rubrum</i> ; <i>B. nigra</i> survives, but growth decreases	Tripepi and Mitchell (1984b)

Note: <sup>a</sup>, PDC = pyruvate decarboxylase; ADH = alcohol dehydrogenase; AEC = adenylate energy charge ratio

suggest that the upwardly stabilized AEC observed after two days in bullrush rhizomes under anaerobic treatment (supported by accelerated anaerobic metabolism) can sustain slow shoot growth as observed by Crawford (1982b). Other research linking energy metabolism with anoxia includes reversible and irreversible damage of the mitochondrial membranes involved in aerobic respiration (Vartapetian et al. 1977, 1985), the coupling of carbon and nitrogen paths to glycolysis by means of NAD cycling (Kennedy et al. 1983, Garcia-Novo and Crawford 1973), and the decrease in energy from ATP hydrolysis available to drive cellular processes (Roberts et al. 1984b) under anaerobiosis.

## CARBOHYDRATE METABOLISM

Changes in root carbohydrate metabolism due to waterlogging or anoxia are mainly caused by oxygen deprivation (reviewed by Davies 1980, Crawford 1982 a,b, Hook 1984, Jackson and Drew 1984), but can also be affected by increased CO<sub>2</sub> concentrations in the root (Zemlianuthin and Ivanov 1978, Crawford 1982b, Chang et al. 1983). A decline in root oxygen availability disrupts the flow of photosynthate from shoot to root (Schumacher and Smucker 1985) which is necessary to support root respiration (Vartapetian et al. 1978). The loss of solute translocation may result from the effect of low oxygen concentrations in the root on energy metabolism, since this is an energy dependent process, but evidence to the contrary is reviewed by Kozlowski and Pallardy (1984). During root anoxia, decreases in substrate availability for carbohydrate metabolism have been implicated in: changes in mitochondrial ultrastructure (Vartapetian et al. 1985); decreases in anaerobic metabolism and failure to maintain ATP levels (Saglio et al. 1980); and decreased growth and viability following the anaerobic episode (Webb and Armstrong 1983). More fundamentally, lack of oxygen results in cessation or severe

restriction of the tricarboxylic acid cycle (Jackson and Drew 1984, Kennedy et al. 1983) since NAD, which is reduced in this path, cannot be reoxidized and recycled once the cytochrome system has ceased to operate.

All wetland species examined to date possess the ability to ferment ethanol, and some species accelerate glycolysis under hypoxia (Pasteur Effect) to maintain ATP levels (Table 1.1, rice: Bertani et al. 1980, river birch: Tripepi and Mitchell 1984a). The accumulation of ethanol may be toxic (Crawford and Zochowski 1984, but see Jackson et al. 1982). Wetland species that cannot easily dispose of ethanol when it accumulates in thickened roots, rhizomes, and germinating seeds seem to have developed the ability to control glycolysis so that ethanol accumulation is limited (Crawford 1982a,b). This ability has been observed in waterlogging-tolerant trees of temperate climates (Crawford 1976), and may be thought of as a dormancy period lasting until the hypoxic stress has passed (Raymond and Pradet 1980).

Mineral deficiencies caused by soil flooding may reduce activities of enzymes involved in carbohydrate metabolism that require specific metal cofactors for activation. Zinc is a cofactor for alcohol dehydrogenase (ADH), which converts acetaldehyde to ethanol. ADH activity in flooded rice roots, believed to support increased fermentation during root hypoxia, was depressed if Zn availability was decreased (Moore and Patrick 1988).

## WATER UPTAKE

The maintenance of photosynthetic rates, crucial to continued growth and productivity, depends upon high stomatal conductance which provides the necessary CO<sub>2</sub> for fixation. Paradoxically, waterlogging often produces plant symptoms similar to those seen under drought conditions: closing of the stomates resulting in decreased CO<sub>2</sub> uptake and

transpiration, declining plant water potential, and even wilting (for recent reviews, see Jackson 1983, Kramer 1983, Jackson and Drew 1984). These symptoms occur once waterlogging reduces water uptake at the root-soil interface, a manifestation of increased root resistance or decreased root permeability (Kramer 1983).

Waterlogging-tolerant species often show few or no symptoms of drought stress upon waterlogging or anaerobiosis (rice: Tomar and Ghildyal 1975; *Quercus palustris* : Black 1984). Root permeability seems to be maintained either through internal oxygen supplies (Tomar and Ghildyal 1975) or a favorable energy balance due to metabolic adaptation. No work has been performed on the water relations of wetland herbs or grasses that shows whether root permeability to water uptake decreases with the cessation of aerobic metabolism of waterlogged plants, or whether permeability can be maintained with the energy derived from an accelerated glycolysis. In plants lacking roots adapted to waterlogging (aerenchymatous and/or adventitious roots), either depressed or elevated plant water potential is usually noted in waterlogged treatments, accompanied by stomatal closure and increased root resistance to water uptake (Kozlowski and Pallardy 1984).

Water relations research on wetland species has been performed primarily on woody angiosperms and gymnosperms. In plants tolerant of waterlogging, significant declines in several aspects of root and shoot growth, as well as changes in development, have been ascribed to soil waterlogging by some workers, while others have found no or minor responses (Table 1.2). Ultimately, long-term survival depends upon continued aeration of the root system, either through anatomical modification or adventitious roots growing in the inundated soil or water, so that root resistance to water uptake is reduced and hormonal communication between root and shoot is restored (Holder and Brown 1980, Jackson and Drew 1984, Reid and Bradford 1984, Tsukahara and Kozlowski 1985). In his review of waterlogging tolerance in trees, Gill (1970) stressed the importance of length, intensity and seasonality of flooding and of tree age and conditioning with regard

Table 1.2: Effects of flooding on water uptake and associated growth responses of selected wetland plants.

Species	Treatment	Change in water relations	Change in growth	Reference
<i>Populus deltoides</i>	Seedlings flooded 4 weeks then drained 1 week	Decrease in transpiration without decrease in turgor pressure	Photosynthesis falls 50% but shoot growth continues	Regehr et al. (1975)
5 tree species	Seedlings flooded 5 weeks	Stomatal closure in 3 days, but without leaf water stress; 2 tolerant species affected less than other 3	Root growth inhibited; leaves senesce in 3 less tolerant species	Pereira and Kozlowski (1977)
<i>Fraxinus pennsylvanicus</i>	Seedlings flooded 5 weeks	Stomatal closure; plants making adventitious roots opened stomates in 15 days	Reduced growth in roots, stem and leaves; leaf death occurred	Sena-Gomes and Kozlowski (1980b)
<i>Platanus occidentalis</i>	Seedlings flooded 5 weeks	Stomatal closure; stem C <sub>2</sub> H <sub>4</sub> leads to adventitious root production	Reduced growth in roots, stem, and leaves; root death; fall in root/shoot ratio	Tang and Kozlowski (1982)
3 conifer species	Seedlings flooded 4 days	None in flood tolerant species; transpiration 50% and xylem pressure potential falls in other 2 species	None in flood tolerant species; Photosynthesis falls 50%, and shoot growth falls within 5 hours in other 2 species	Zaerr (1983)
3 <i>Pyrus</i> species	Seedlings in liquid media gassed with N <sub>2</sub> for about 5 weeks	Stomatal closure in 1 week, root conductivity fell at 3 weeks in all species; leaf water stress occurred in least tolerant species	Slight chlorosis in 2 tolerant species; wilting, defoliation, and root death in least tolerant species	Andersen et al. (1984)
<i>Acer saccharinum</i>	3- and 12-month-old seedlings flooded 4-8 weeks	Transpiration falls w/stomatal closure Increased water use efficiency in year old seedlings when flooded	Reduced root, stem and leaf biomass; Photosynthesis falls in 3 month old seedlings	Petersen and Bazzaz (1984)

Table 1.2 (continued)

Species	Treatment	Change in water relations	Change in growth	Reference
<i>Quercus palustris</i>	Flooded in the dormant season	None	None	Black (1984)
	flooded near end of growing season	Stomatal closure: day 5+6, recovery: day 10, soil-plant water resistance normal when measured on day 14	Autumn leaf senescence 14 days early	
	2 years continuous flooding	None in leaf conductance or xylem water potential	In 2 <sup>nd</sup> year: less flowers; acorns abort; autumn leaf senescence 14 days early	
<i>Salix nigra</i> and <i>S. exigua</i>	Flooded 36 hrs. with lights on	Leaf conductance and water potential fell w/in 36 hrs.	Wilting	Dionigi et al. (1985)
<i>Liquidambar styraciflua</i>	3 year old seedlings flooded 9 days	Decrease in transpiration w/o decrease in xylem pressure potential	Photosynthesis reduced 77%	Pezeshki and Chambers (1985)

to which species were most tolerant to waterlogging. Coutts (1981) extended these concepts using Sitka spruce seedlings to hypothesize different mechanisms inducing stomatal closure depending upon length and seasonality of waterlogging.

## NUTRIENT AVAILABILITY

### *Nitrogen*

Waterlogging often increases nitrogen uptake in wetland species (bottomland hardwood trees: Hosner and Leaf 1962; rice: Islam and Islam 1973; general: Kozlowski and Pallardy 1984), but sometimes soil waterlogging is associated with no change (rice: Jugsujinda and Patrick 1977), or an inhibition of nitrogen uptake (bottomland hardwood trees: Dickson et al. 1972; *Spartina*: Morris 1984). In a comparison of four species that form a natural topographic zonation pattern in periodically waterlogged dune slacks, nitrogen uptake and translocation were inhibited by soil waterlogging in all the species except those normally occupying the lowest sites, and the inhibition was in order of increasing elevation (Schat 1984; Table 1.3). Nitrogen uptake and assimilation is believed to be an energy-requiring process (Luttge and Higinbotham, 1979). In both wetland and agricultural species, the effect of low oxygen availability on ATP production was believed to be the key factor in reducing nitrogen uptake (Drew and Sisworo 1979, Morris and Dacey 1984, Schat 1984). Besides a lack of oxygen, increased salinity or sulfide levels in waterlogged soils may interfere with nitrogen uptake (Garcia-Novo 1976, Morris 1984) or allocation (Cavalieri and Huang 1981).



Table 1.3: Flooding effects on nutrient, salinity and mineral uptake and associated growth responses in selected wetland plants.

Species	Treatment	Change in uptake	Growth response	Reference
Rice	Flooding vs. alternate flooding and draining	N uptake falls, especially if more than 1 alternation	Growth and grain yield fall with number of alternations	Patrick et al. (1967)
Rice	Aerobic and anaerobic hydroponic treatments pH=5,6,7,8	Anaerobic: fall in Zn, rise in Fe at pH 5, equal for P, $\text{NH}_4^+$	Root and shoot growth fall at pH 5	Jugsujinda and Patrick (1977)
8 salt marsh species	Flooding and salinity factorial	None	No synergistic effects	Cooper (1982)
4 dune slack species	Flooding and submerging	N P K uptake reduced in 3 species	Magnitude of growth decrease in order of natural zonation	Schat (1984)
4 salt marsh species	Species and $\text{S}^{-2}$ distribution in marsh	Change in $\text{S}^{-2}$ uptake not measured	Only <i>Salicornia</i> colonizes and grows in high $\text{S}^{-2}$ areas	Ingold and Havill (1984)
	0.1mM $\text{S}^{-2}$ hydroponics	Change in $\text{S}^{-2}$ uptake not measured	Growth falls in all but <i>Salicornia</i> .	
5 salt marsh species	Flooding, salinity, and soil factorial (silt or peat)	No change in Na metabolism	Growth increased due to flooding	Snow and Vince (1984)
15 salt marsh species	Flooding-salinity factorial	None	Growth increases in 2 marsh species; equal for 13 species	Rozema et al. (1985a)
Fresh marsh	P addition in plots in wet and dry years	P uptake increases in plot without added P in wet year	Production increases in plots without added P in wet year	Bayley et al. (1985)

Table 1.3 (continued)

Species	Treatment	Change in uptake	Growth response	Reference
<i>Erica tetralix</i> and <i>E. cinerea</i>	Flooding 8 weeks	Uptake of Fe and Mn greater in <i>E. cinerea</i>	No growth; root and plant death greater in <i>E. cinerea</i>	Jones and Etherington (1970)
<i>Empetrum nigrum</i>	Observations in wet and dry years: [Al] lower in dry year	Aluminium availability and uptake higher in wet year	Increased abundance in dry year ([Al] lower)	Bell and Tallis (1974)
<i>Pinus taeda</i>	Seedlings flooded media pH 4-5	Fe,Mn,Zn,Na uptake in shoot increased	Reduced growth	Hook et al. (1983)
<i>Pinus taeda</i>	Flooding-phosphorus factorial	Flooding: P uptake decreased P addition: P uptake increased	Reduced growth Increased growth	McKee et al. (1984)

### *Phosphorus*

Phosphorus availability usually increases with waterlogging of the soil (Gambrell and Patrick 1978, Ponnaperuma 1984). A large-scale flooding event in a Florida freshwater marsh mimicked the effect of phosphorus fertilizer application on shoot biomass and concentration of P in the tissues (Table 1.3), "presumably due to the release of P from" the waterlogged soil (Bayley et al. 1985). On the other hand, waterlogging has led to decreases in P uptake in species relatively intolerant to waterlogging (Drew and Sisworo 1979, Schat 1984), perhaps due to root deterioration or an energy requirement of this process. In addition, it has been suggested that the precipitation of P by Fe (made more available under waterlogged conditions) reduces P uptake and biomass accumulation of *Pinus taeda* (McKee et al. 1984, McKevlin et al. unpublished manuscript). Among relatively waterlogging-tolerant species, no differences in phosphorus uptake were shown between aerobic and anaerobic treatments in rice (Jugsujinda and Patrick 1977) or drained and waterlogged treatments in *Samolus valerandi* (Schat 1984; Table 1.3).

### *Potassium and Calcium*

The availability of  $\text{Ca}^{+2}$  and  $\text{K}^{+}$  generally increases in waterlogged soils (Ponnampereuma 1984), but their uptake by intolerant species decreases with waterlogging (Kozlowski and Pallardy 1984, Sojka and Stolzy 1987). On the other hand, the species most tolerant of waterlogging in Schat's experiment (1984) showed no changes in uptake of these cations in waterlogged culture. This supports the position of Kozlowski and Pallardy (1984) who assert  $\text{K}^{+}$  and  $\text{Ca}^{+2}$  absorption in waterlogged environments poses no problems for waterlogging tolerant species. In periodically waterlogged saline environments, however, relatively high  $\text{Na}^{+}$  concentrations pose problems for  $\text{K}^{+}$  and  $\text{Ca}^{+2}$  uptake for herbs and grasses (Rozema et al. 1985a). In contrast, stimulation of

potassium uptake by NaCl in waterlogging-tolerant mangrove species has been documented (Wainwright 1984).

*Other minerals and toxic effects*

Aerenchymatous waterlogging-tolerant plants that are not waterlogged or are waterlogged less than is normal for that species have been reported to show signs of iron-deficient chlorosis (Eleuterius and Caldwell 1981, Rozema et al. 1985b) through a mechanism discussed by Schat (1984). Zinc deficiencies can occur in rice paddies under certain conditions as discussed by Kozłowski and Pallardy (1984), and may limit anaerobic ethanol fermentation in roots (Moore and Patrick 1988).

Under waterlogged conditions, root discrimination regarding uptake of some minerals and compounds is often lost, leading to the accumulation of unwanted substances or toxic quantities of essential elements (Armstrong 1975, Crawford 1982a). This usually occurs in plants intolerant of waterlogging. For example, in comparative studies of wetland versus mesophytic species and ecotypes, essential elements and metals such as Fe, Mn, Mg, B, and Li have been found to accumulate to toxic levels in the less waterlogging-tolerant plants (Jones and Etherington 1970, Wu 1981, Hodson et al. 1981, Rozema et al. 1985b). Recently, these findings have been extended to include distributional patterns across salt marshes (Rozema et al. 1985b). Similarly, species colonizing coastal mudflats without competition have been shown to overcome high concentrations of sulfide that are toxic to their competitors (Ingold and Havill 1984, Thibodeau and Nickerson 1986).

## HORMONES

Both Jackson (1988) and Reid and Bradford (1984) have recently reviewed the effect of soil waterlogging on hormonal processes. While much research shows that hormonal changes are caused by flooding, and the responses seen are characteristic of hormone imbalance, few growth responses to flooding can be ascribed to specific hormone actions (Crawford 1982a, Reid and Bradford 1984). There seems to be agreement that imbalances in hormones due to waterlogging generally inhibit both root and shoot growth in various ways in agricultural species (the mechanisms remain unknown), but in wetland species hormonal responses to soil waterlogging have only been shown to promote growth.(Table 1.4).

Soil waterlogging results in stem hypertrophy and lenticel formation, adventitious rooting, and submergence-promoted growth in wetland plants (Jackson and Drew 1984, Kozlowski 1984, Table 1.4). Even though auxin and ethylene usually act antagonistically to control growth, both have been implicated in the control of these flooding adaptations. However, ethylene alone seems mainly responsible for flooding-induced aerenchyma formation (Kawase 1979, Rozema et al. 1985b). Both auxin and ethylene are found to increase in plant stems in response to flooding. The elimination of rapid gas exchange between the root and soil atmosphere and the development of root hypoxia following flooding are currently needed to explain environmental control over auxin and ethylene accumulation (Jackson and Drew 1984, Reid and Bradford 1984).

Adventitious roots originate along the stem as replacements for those roots which have ceased to function under waterlogged conditions. They are stimulated to grow by indoleacetic acid (IAA) (Wample and Reid 1979), ethylene (Drew et al. 1979, Jackson 1983), or a combination of both (Jackson and Drew 1984, Tang and Kozlowski 1984). Of six tree species tested by Tang and Kozlowski (1984), the five that were most flood

Table 1.4: Internal hormonal responses to soil flooding and hormone application to wetland plants and their effects on growth.

Species	Treatment	Hormonal changes	Growth response	Reference
Rice	C <sub>2</sub> H <sub>4</sub> (ethylene) applied to roots	Not measured	Root growth peaks at 1-10 ppm C <sub>2</sub> H <sub>4</sub>	Smith and Robertson (1971)
Rice	Flooding then draining	C <sub>2</sub> H <sub>4</sub> increased	Stems elongated then lodged, reducing yield	Rose-John and Kende (1984)
<i>Callitriche platycarpa</i>	Flooding	Stem C <sub>2</sub> H <sub>4</sub> increased	Extension growth response saturated	Musgrave et al. (1972)
<i>Sagittaria pygmaea</i> <i>Potamogeton distinctus</i>	Flooding	Stem C <sub>2</sub> H <sub>4</sub> increased	Extension growth	Suge and Kusanagi (1975)
<i>Hydrocharis morsus-ranae</i> <i>Regnellidium diphyllum</i> <i>Ranunculus sceleratus</i>	C <sub>2</sub> H <sub>4</sub> and IAA applied to roots	Not measured	Extension growth from both hormones but by different mechanisms	Cookson and Osborne (1978)
<i>Ranunculus sceleratus</i>	C <sub>2</sub> H <sub>4</sub> applied to roots	Not measured	Extension growth	Samarakoon and Horton (1984)
	petiole submergence	C <sub>2</sub> H <sub>4</sub> increased	Extension growth	
<i>Picea sitchensis</i> and <i>Pinus contorta</i>	C <sub>2</sub> H <sub>4</sub> in hydroponics (28-110 ppm) for 3 weeks	Not measured	Growth reduced 40% at 28 ppm C <sub>2</sub> H <sub>4</sub> treatment	Sanderson and Armstrong (1980)
6 tree species	Flooding	C <sub>2</sub> H <sub>4</sub> increased in all but least tolerant species	Growth and survival greatest in species producing the most adventitious roots	Sena Gomes and Kozlowski (1980a) Tang and Kozlowski (1984)

tolerant increased ethylene levels and produced adventitious roots, and these abilities were found to be directly related to flooding tolerance (Table 1.4). Adaptations of flood-tolerant plants may influence the effect of soil waterlogging on ethylene action and plant response. For example, Tang and Kozlowski (1984) found no ethylene accumulation in an intolerant tree to which IAA was applied, while application of IAA to two flood-tolerant trees resulted in increased ethylene levels.

Although there is some evidence that ethylene endogenously and exogenously produced during flooding can inhibit root growth of flood-tolerant plants (Sanderson and Armstrong 1980), some results have questioned this effect (Jackson et al. 1984). On the other hand, relatively low ethylene levels were consistent in stimulating root growth in rice (Smith and Robertson 1971, Jackson and Drew 1984). At Present, the only conclusive evidence linking a hormonal flooding response with a shoot growth response is that of rapid extension growth in stems of submerged wetland plants (Table 1.4). Rapid extension of stems enables leaves of submerged plants to reach the water surface quickly. Ethylene seems largely responsible for the rapid extension growth, but other hormones (especially auxin) or CO<sub>2</sub> may enhance its action (Musgrave et al. 1972, Suge and Kusanagi 1975, Cookson and Osborne 1978).

Abscissic acid (ABA) levels have been found only recently to increase with flooding, and the mechanism is yet to be elucidated (Reid and Bradford 1984). Drew (1983) has suggested that ABA release "is caused by a brief water stress in leaves, reflecting the early transient decrease in root conductivity" that often occurs upon waterlogging. However, it is not known how water stress could cause ABA increases. Similar to its occurrence and function in drought stress, ABA is thought to cause stomatal closure in order to conserve water and delay falls in plant water potential (Reid and Bradford 1984). Since ABA has been shown to increase root permeability (Glinka 1980), declines in root ABA due to

flooding may be a cause, rather than a result, of reduced water uptake leading to reduced photosynthesis.

## ROOT RHIZOSPHERE

Perhaps the most significant long-term adaptation of wetland species to soil waterlogging is the development of an internal gas path from the root to the shoot that assumes the role of the external gas diffusion path once provided by the soil atmosphere (Hook and Scholtens 1978, Armstrong 1979). As well as providing for aerobic root respiration, the oxygen may also diffuse out of the root into the surrounding soil, creating a microzone around the root termed the oxidized rhizosphere (Armstrong 1979). This layer reverses many of the negative effects soil waterlogging has upon roots, from detoxifying reduced phytotoxins such as sulfides (Carlson 1980; Thibodeau and Nickerson 1986) to supplying mycorrhizal symbionts with oxygen (Read and Armstrong 1972, Keeley 1980).

Although few reports link a change in root rhizosphere with a growth response (Table 1.5), the development of aerenchymatous roots allows or enhances continued growth and survival of wetland plants during waterlogging episodes (Hook and Brown 1973, Armstrong 1979, Keeley 1979). In an experiment comparing waterlogging stress in two willow species that exhibit topographic zonation in a drainage basin subject to periodic long-term river flooding, the species that flourished in areas of lower elevation was able to oxidize its rhizosphere to a greater extent (Dionigi et al. 1985). Similarly, Thibodeau and Nickerson (1986) showed that the distribution of two sympatric mangrove species depended upon their ability to oxidize the sediment around their roots (Table 1.5). Plants may also benefit from rhizosphere oxidation by associating with burrowing animals



Table 1.5: Flooding effects on rhizosphere and associated growth responses in selected wetland plants.

Species	Treatment	Change in rhizosphere	Growth response	Reference
5 hardwood species	Seedlings flooded about 5 weeks	Oxidized only by secondary roots of <i>Nyssa aquatica</i>	Greatest growth in <i>Nyssa aquatica</i>	Hook and Brown (1973)
<i>Nyssa sylvatica</i>	Flooded 1 year with mycorrhizal inoculum	Endomycorrhizal association	Increased growth	Keeley (1980)
4 dune slack species	Flooding with <i>Juncus</i> for 10 weeks	Oxygen persists in soil	Increased number of leaves; root + leaf survival enhanced	Schat (1984)
<i>Salix nigra</i> and <i>S. exigua</i>	Field: observation; Lab: flooding 7 weeks	Not measured Eh fall less with <i>Salix nigra</i>	<i>S. nigra</i> occupies sites of lower elevation Leaf AEC drops in <i>S. exigua</i>	Dionigi et al. (1985)
<i>Spartina alterniflora</i>	En- and exclosures to control <i>Uca</i> density	More burrows with greater <i>Uca</i> density	Growth increases with <i>Uca</i> density	Bertness (1985)
<i>Avicennia germinans</i> and <i>Rhizophora mangle</i>	S <sup>-2</sup> and species distributions in field	More oxidized in <i>Avicennia</i> areas due to pneumatophores	<i>A. germinans</i> colonizes high S <sup>-2</sup> areas but <i>R. mangle</i> cannot	Thibodeau and Nickerson (1986)

(Bertness 1985) or with other plants that produce an extensive oxidized rhizosphere (Rozema et al. 1985a). Such associations with *Juncus maritimus* allowed increased root survival and shoot growth in several species compared with similarly waterlogged plants in monoculture (Schat 1984).

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CHAPTER 2.  
WATERLOGGING RESPONSES IN DUNE, SWALE,  
AND MARSH POPULATIONS OF *SPARTINA PATENS*  
UNDER FIELD CONDITIONS<sup>1</sup>

ABSTRACT

Soil waterlogging responses were examined in three *Spartina patens* populations along a steep flooding gradient in coastal Louisiana. Root anatomy and physiological indicators of anaerobic metabolism were examined to identify and compare flooding responses in dune, swale, and marsh populations, while soil physicochemical factors were measured to characterize the three habitats. Soil waterlogging increased along the gradient from dune to marsh habitats and was accompanied by increases in root porosity (aerenchyma). Aerenchyma in marsh roots was apparently insufficient to provide enough oxygen for aerobic respiratory demand, as indicated by high root alcohol dehydrogenase activities and low energy charge ratios. Patterns of root metabolic indicators suggest that dune and swale roots generally respired aerobically, while anaerobic metabolism was important in marsh roots. However, in each population, relatively greater soil waterlogging was accompanied by differences in enzyme activities leading to malate accumulation. In dune and swale roots under these circumstances, depressed adenylate energy charge ratios may have been the result of an absence of increased ethanol fermentation. These trends suggest that: (1) Aerenchyma formation was an important, albeit incomplete, long-term adaptation to the prevalent degree of soil waterlogging. (2) All populations adjusted root metabolism in response to a relative (short-term) increase in soil waterlogging.

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## INTRODUCTION

The broad range of coastal habitats exploited by the perennial angiosperm *Spartina patens* (Aiton) Mulh. is well documented on the East and Gulf Coasts of North America (Chabreck 1972, Silander 1976). In Louisiana, *S. patens* can be the dominant macrophyte in dune, swale, and marsh habitats of barrier systems and in mainland brackish marshes (Chabreck 1972, Monteferrante et al. 1982). The remarkable ability of this species to compete successfully in diverse environments suggests either genetic differentiation or plastic phenotypic adaptations expressed differentially among the populations. Silander (1976) reported morphological differences resulting from ecotypic differentiation in three North Carolina *S. patens* populations in field and culture experiments. Physiological differences among clones from dune, swale, and marsh populations were demonstrated by Silander and Antonovics (1979) using survival and growth responses to environmental factors such as salinity, nutrients, and drought stress. However, they did not investigate the response of these populations to soil waterlogging, a major environmental variable that differs among the three habitats.

Oxygen diffusion in the soil decreases 10,000-fold with flooding, and aerobic soil respiratory processes quickly exhaust the soil oxygen (Gambrell and Patrick 1978). Sediment anoxia resulting from soil waterlogging can severely limit aerobic root respiration (Saglio et al. 1983) which is essential for plant growth (Crawford 1978) and nutrient uptake (Drew and Sisworo 1979; Schat 1984). Zonation of plants in coastal habitats is thought to be strongly influenced by flooding frequency and duration, and the resultant pattern of species is thought to reflect, at least in part, the different species' abilities to avoid or tolerate sediment anoxia as it increases along the flooding stress gradient (Chapman 1978, Armstrong 1975, Schat 1984, Vince and Snow 1984).

Anatomical, morphological and physiological responses to waterlogging stresses have been documented for many species (Crawford 1982, Jackson and Drew 1984, Kozlowski 1984), but much disagreement exists regarding the adaptive value of these responses. Such responses in *Spartina alterniflora* have been reported to vary with soil redox potential (Eh) in the field (Mendelssohn et al. 1981, Mendelssohn and Postek 1982). However, similar responses by *S. patens* to the steep flooding gradient between dune, swale, and marsh habitats have not been studied. The objective of this research was to compare anatomical and metabolic responses of three such *S. patens* field populations to soil waterlogging. We found differences among populations in root anatomy and physiology attributable to soil waterlogging. Moreover, root metabolic indicators suggest that metabolic responses to hypoxia appear to occur with both spatial and temporal increases in soil waterlogging.

## METHODS

### *Study sites*

Dune, swale, and marsh habitats dominated by *Spartina patens* were chosen in August 1983. Soil for physicochemical parameters and plant leaves and roots for physiological analyses (described below) were collected from five replicate sites within each habitat in August 1983 and May and July 1984. Sampling for root specific gravity (a measure of root porosity) was performed in the same areas during October and November 1985. The dune and swale communities were located on Elmer's Island (Lat. 29° 11' 00" N, Long. 90° 03' 40" W), a barrier spit system on the Louisiana coast. Dune *S. patens* plants were sampled along the primary dune ridge, while swale samples

were collected from a transect 15 meters landward from, and parallel to, the dune ridge. Plants on the dune ridge are not subject to saltwater inundation unless the area is overwashed by storms. The swale area, defined by the topographical depression immediately landward of the dune, is reached by high tides only rarely.

The marsh habitat was located 15 km NNW of Elmer's Island (Lat. 29° 17' 50" W) in a mainland salt marsh (Chabreck 1972). Marsh samples were collected along a zone 2 m wide and parallel to the bayou, 4 m from the bank. Relative to mean sea level, the marsh environment was lowest in elevation. Standing water was often present on the marsh surface, and the area was inundated irregularly by high tides.

#### *Soil environment*

Soil samples were collected with aluminum tubes 5 cm diameter to a depth of 30 cm and placed in plastic bags. After removal of as much air as possible, the bags were sealed and refrigerated up to four weeks prior to analysis. Moisture content was determined gravimetrically and other analyses were performed on 1:1 (w/v) soil:water extractions following one hour of shaking and subsequent filtration through Whatman #2 Ashless paper. Aliquots for specific determinations were stored frozen until the day of analysis. Nitrate-nitrite and ammonia were measured on the extracts with an Auto Analyzer unit (AA2), using EPA Method 353.2 (Colorimetric, Automated Cadmium Reduction) and EPA Method 350.1 (Colorimetric, Automated Phenate), respectively (U. S. Environmental Protection Agency 1979). Sodium and the essential elements K, Ca, P, Mg, Mn, Fe, Zn, and Cu were measured in soil extracts of the May and July samples using a Fisher inductively coupled plasma argon emission spectrometer (Atom Comp Series 800) with appropriate standards.

Redox potential was measured in the field at a depth of 15 cm using platinum electrodes (checked for accuracy with quinhydrone prior to use), and a calomel electrode as a reference (+244mV was added to the meter reading to arrive at Eh). The electrodes were allowed to equilibrate 20 min before measuring Eh. In dune and swale habitats, distilled water was poured on the soil to provide conductance through the soil portion of the circuit when needed.

#### *Root specific gravity*

The specific gravity of the whole root system which included three morphologically distinct root types (white unbranched; yellow to gray unbranched to slightly branched; and highly branched), was calculated by determining the live proportion of each root type, measuring the specific gravity of each type, and summing these three specific gravity estimates after weighting by the proportion of occurrence for each root type. Dune and swale roots were collected in the field, wrapped in wet paper towels, placed in plastic bags, and kept on ice up to 48 h. Marsh *S. patens* roots were very fragile, so whole plant-soil sections were transported to the laboratory where the roots were excised carefully. Specific gravity was determined by weighing a 25 ml pycnometer filled with water (W), the roots (R), and the roots in the pycnometer (P), and applying the formula:  $R/(W + R - P)$ . When grown under both flooded and drained conditions, *S. patens* root specific gravity was negatively correlated with root porosity ( $r = -0.99$ ) measured using the pycnometer method of Jensen et al. (1969). These two measures were related linearly by:  $\text{root porosity} = 1.026 - 0.969 \times \text{root specific gravity}$  [derived in Chapter 3]. Thus root specific gravity was a good predictor of root porosity in *S. patens*.

### *Physiological measurements*

Healthy leaf and root tissues (green leaves; white to light brown, turgid roots) were collected for determination of adenylate energy charge ratio ( $AEC = [ATP + 0.5 ADP]/[ATP + ADP + AMP]$ ; Atkinson 1971). Tissue was cut into 1 cm segments and frozen with 20 ml deionized water in liquid nitrogen, then stored on dry ice up to two days until placed in a freeze-drier upon return to the laboratory. Mendelssohn and McKee (1981) showed that the AEC ratios measured in similarly collected samples and stored for one day on dry ice prior to lyophilization were not significantly different from those of fresh tissue. Freeze-dried samples were ground through a size 60 mesh sieve in a Wiley mill and 0.05 g tissue was extracted in 10 ml boiling buffer (pH 7.4 1 mM EDTA with 5% polyvinylpolypyrrolidone) for 30 s. The AEC assay was performed on the supernatant following centrifugation at 4° C and 20000 gravities according to Mendelssohn and McKee (1985). ATP was quantified through its light-yielding reaction with firefly lantern complex (FLE-50 Sigma Chemical Co.) counted on a Beckman model LS100C liquid scintillation counter.

Root tissue collected in August for enzyme assays was frozen in liquid nitrogen and kept on dry ice for 48 h. It was then weighed and extracted in 17mM HEPES buffer (pH 8.0) with 5% PVPP in a tissue homogenizer for 30 s. The extracts were centrifuged at 4° C, 20000 gravities for 30 min and assays for alcohol dehydrogenase (ADH; EC 1.1.1.1), malate dehydrogenase (MDH; EC 1.1.1.27), phosphoenolpyruvate carboxylase (PEP-C; EC 4.1.1.31), and malic enzyme (ME; EC 1.1.1.40) were performed at 30° C as follows: ADH modified from John and Greenway (1976) using 14mM HEPES, 5.4 mM  $MgCl_2$ , 0.13 mM NADH, 0.1 ml enzyme extract and 0.4 mM acetaldehyde for the reaction mixture; MDH according to Rumpho and Kennedy (1981); PEP-C and NADP-ME modified from Smith and ap Rees (1979) for aerated *Ranunculus* with changes in

reaction mixture for PEP-C as 35 mM MOPS, 3.0 mM-MgCl<sub>2</sub> at pH 7.4, and changes in reaction mixture for ME as 37 mM MOPS at pH 7.4. Enzyme activities, which were linear with concentration of root extract, were measured when the disappearance of NADH (NADPH for ME) was linear with time. Recovery of internal standards of ADH and MDH added prior to extraction was high in root tissue from field-collected clones grown in the greenhouse under both drained (ADH: 100%; MDH: 95%) and flooded (ADH: 88%; MDH: 94%) conditions. Hence, there was little indication of inhibition, or differential inhibition due to soil waterlogging, in the extracts of these enzymes.

In May and July, one aliquot of roots per sample was handled as above for PEP-C and ME assays, while dehydrogenase assays were performed on freeze-dried root material ground as described earlier in a Wiley mill and extracted in Tris pH 7.4 buffer with 0.3% dithiothreitol (Cleland's reagent) by vortexing for 30 s. In order to make comparisons between months, the activities of the dehydrogenases analyzed in August were adjusted for the effects of freeze drying on enzyme activity and protein content (higher in freeze-dried tissue) of the extract (correction factors: 3.27 for ADH; 1.66 for MDH), obtained from two greenhouse experiments (ADH n = 8; ADH and MDH n = 12).

In the field, about 0.5 g root tissue for metabolite analysis was placed in liquid nitrogen, 10 mls of 8% perchloric acid was added, and the sample was immediately placed on dry ice. The samples were kept frozen until acid neutralization with ca. 1 ml 5 M dipotassium carbonate. The extract was then centrifuged at 14000 gravities for 10 min, and the supernatant was used for malate, lactate and ethanol analyses according to the methods of Gutmann and Wahlefeld (1974a, b) and Brent and Gutmann (1974), respectively, except 82 mM hydrazine-hydrate and 1.74 mM NAD were used for the malate and lactate incubations.

### *Statistical analyses*

Analysis of variance (ANOVA; PROC GLM), regression (PROC REG), canonical correlation (CC; PROC CANCORR) and principal component analysis (PCA; PROC FACTOR), were performed using the SAS statistical analysis package (SAS Institute, Inc. 1982). One observation with missing soil moisture data and one observation with a univariate outlier were removed prior to multivariate analyses. Fixed effects models were used to analyze the data with ANOVA, and significant effects were examined with least significant differences (LSD). All correlations and differences reported are significant at the 5% level unless otherwise indicated.

## RESULTS

### *Environment*

Soil moisture increased from dune to marsh habitats (Table 2.1), reflecting the differences in elevation and inundation regimes of the three environments. The Eh of the marsh sediment was lower than that of the dune and swale, but no difference was observed between the Eh of the dune and swale habitats (Table 2.1). Soil moisture and Eh varied as a function of sampling date: moisture was greatest in August (Hurricane Alicia passed and 11.3 cm of rain fell within seven days of sampling; Galliano, LA; NOAA 1983) and least in May (no rain during week prior to sampling; NOAA 1984), and was inversely correlated with redox potential at 15 cm ( $r = -0.84$ ).

Total inorganic nitrogen, measured as nitrate plus ammonia, was greatest in the marsh and dune soils and least in the swale soils (Table 2.2). Sodium was always highest in marsh soils but never greater than 3000 ppm. There were no significant differences in the

Table 2.1. Soil moisture and Eh, leaf AEC and root metabolic indicators by month in dune, swale and marsh populations (habitats) of *Spartina patens* and overall month and population (habitat) means (collected in 1983 and 1984)

		POPULATION <sup>1</sup>									POPULATION <sup>2</sup>			MONTH <sup>2</sup>		
		DUNE			SWALE			MARSH			Dune	Swale	Marsh	August	May	July
		Aug.	May	July	Aug.	May	July	Aug.	May	July						
Moisture	(% wet wt <sup>-1</sup> )	4.7 <sup>a</sup>	0.5 <sup>c</sup>	3.6 <sup>b</sup>	15.3 <sup>aa</sup>	6.9 <sup>b</sup>	15.3 <sup>a</sup>	79.9 <sup>a</sup>	75.7 <sup>b</sup>	73.4 <sup>b</sup>	2.9 <sup>c</sup>	12.5 <sup>ba</sup>	76.3 <sup>a</sup>	33.3 <sup>aa</sup>	27.7 <sup>c</sup>	30.8 <sup>b</sup>
Eh	(mV)	129 <sup>c</sup>	445 <sup>a</sup>	285 <sup>b</sup>	279 <sup>a</sup>	277 <sup>a</sup>	354 <sup>a</sup>	-154 <sup>b</sup>	15 <sup>a</sup>	-25 <sup>a</sup>	286 <sup>a</sup>	304 <sup>a</sup>	-55 <sup>b</sup>	85 <sup>b</sup>	246 <sup>a</sup>	205 <sup>a</sup>
Leaf AEC		.70 <sup>b</sup>	.80 <sup>a</sup>	.69 <sup>b</sup>	.67 <sup>b</sup>	.79 <sup>a</sup>	.65 <sup>b</sup>	.73 <sup>aaa</sup>	.81 <sup>a</sup>	.75 <sup>a</sup>	.73 <sup>b</sup>	.71 <sup>b</sup>	.76 <sup>aaa</sup>	.70 <sup>baa</sup>	.80 <sup>a</sup>	.70 <sup>b</sup>
Root AEC		.68 <sup>ba</sup>	.87 <sup>a</sup>	.86 <sup>a</sup>	.73 <sup>b</sup>	.81 <sup>a</sup>	.81 <sup>a</sup>	.77 <sup>a</sup>	.74 <sup>a</sup>	.77 <sup>a</sup>	.80 <sup>aa</sup>	.78 <sup>ab</sup>	.76 <sup>b</sup>	.72 <sup>ba</sup>	.81 <sup>a</sup>	.81 <sup>a</sup>
ADH	( $\mu\text{mols g}^{-1}$ dry wt hr <sup>-1</sup> )	141 <sup>a</sup>	162 <sup>a</sup>	147 <sup>a</sup>	300 <sup>a</sup>	166 <sup>ab</sup>	71 <sup>b</sup>	1606 <sup>b</sup>	2554 <sup>a</sup>	441 <sup>c</sup>	150 <sup>b</sup>	179 <sup>b</sup>	1533 <sup>a</sup>	682 <sup>b</sup>	961 <sup>a</sup>	220 <sup>c</sup>
PEP-C	( $\mu\text{mols g}^{-1}$ f wt hr <sup>-1</sup> )	2.18 <sup>a</sup>	.80 <sup>ab</sup>	.09 <sup>b</sup>	6.46 <sup>a</sup>	2.27 <sup>ab</sup>	.31 <sup>b</sup>	.20 <sup>b</sup>	2.66 <sup>a</sup>	.31 <sup>b</sup>	1.02 <sup>b</sup>	3.01 <sup>a</sup>	1.06 <sup>b</sup>	2.95 <sup>a</sup>	1.91 <sup>a</sup>	.24 <sup>b</sup>
MDH	( $\mu\text{mols g}^{-1}$ dry wt hr <sup>-1</sup> )	14516 <sup>a</sup>	936 <sup>c</sup>	4500 <sup>b</sup>	7153 <sup>a</sup>	900 <sup>b</sup>	1145 <sup>b</sup>	14424 <sup>a</sup>	3634 <sup>b</sup>	7507 <sup>b</sup>	6651 <sup>a</sup>	3066 <sup>b</sup>	8522 <sup>a</sup>	12031 <sup>a</sup>	1823 <sup>c</sup>	4384 <sup>b</sup>
ME	( $\mu\text{mols g}^{-1}$ f wt hr <sup>-1</sup> )	2.52 <sup>b</sup>	3.75 <sup>b</sup>	8.09 <sup>a</sup>	1.30 <sup>a</sup>	2.80 <sup>a</sup>	2.06 <sup>a</sup>	1.72 <sup>c</sup>	5.34 <sup>b</sup>	9.06 <sup>a</sup>	4.79 <sup>a</sup>	2.05 <sup>b</sup>	5.37 <sup>a</sup>	1.85 <sup>c</sup>	3.96 <sup>b</sup>	6.41 <sup>a</sup>
EtOH	( $\mu\text{mols g}^{-1}$ f wt <sup>-1</sup> )	.08 <sup>b</sup>	1.41 <sup>a</sup>	.09 <sup>b</sup>	.00 <sup>b</sup>	1.19 <sup>a</sup>	.17 <sup>b</sup>	.20 <sup>b</sup>	1.03 <sup>a</sup>	.37 <sup>b</sup>	.53 <sup>a</sup>	.45 <sup>a</sup>	.53 <sup>a</sup>	.09 <sup>b</sup>	1.21 <sup>a</sup>	.21 <sup>b</sup>
Malate	( $\mu\text{mols g}^{-1}$ f wt <sup>-1</sup> )	9.70 <sup>a</sup>	1.90 <sup>b</sup>	1.56 <sup>b</sup>	4.21 <sup>a</sup>	1.71 <sup>b</sup>	0.71 <sup>b</sup>	2.57 <sup>a</sup>	.36 <sup>b</sup>	.65 <sup>b</sup>	4.39 <sup>a</sup>	2.21 <sup>b</sup>	1.19 <sup>b</sup>	5.49 <sup>a</sup>	1.32 <sup>b</sup>	.98 <sup>b</sup>
Lactate	( $\mu\text{mols g}^{-1}$ f wt <sup>-1</sup> )	1.59 <sup>a</sup>	.79 <sup>b</sup>	.04 <sup>c</sup>	1.02 <sup>a</sup>	.71 <sup>a</sup>	.10 <sup>b</sup>	1.07 <sup>a</sup>	1.26 <sup>a</sup>	.22 <sup>b</sup>	.81 <sup>a</sup>	.61 <sup>a</sup>	.85 <sup>a</sup>	1.23 <sup>a</sup>	.92 <sup>b</sup>	.12 <sup>c</sup>

<sup>1</sup> n=5 unless (\*) then n=4, or (\*\*) then n=3. Superscripts denote significant differences between monthly means within each population (habitat) (P<.05)

<sup>2</sup> n=15 unless (\*) then n=14, or (\*\*) then n=13. Superscripts denote significant differences between populations (habitats) or months (P<.05)



Table 2.2. Soil mineral concentrations ( $\mu\text{g g}^{-1}$  dry soil) in *Spartina patens* habitats.  
 Values with different letter superscripts differ significantly ( $P < .05$ ;  $n = 10$  unless otherwise indicated)

	HABITAT		
	DUNE	SWALE	MARSH
MINERAL			
Nitrogen <sup>1</sup>	1.19a*	.70b**	2.17a*
Phosphorus	.55a	.29a	.52a
Potassium	4.0b	4.7b	74.4a
Sodium	35b	15b	1983a
Calcium	4.6b	2.5b	73.1a
Magnesium	4.4b	2.7b	122.8a
Manganese	.05b	.04b	.91a
Iron	.17b	.11b	.57a
Zinc	.03b	.02b	.07a
Copper	.02a	.01b	.02a

<sup>1</sup>  $\text{NH}_4^+ + \text{NO}_3^- + \text{NO}_2^-$

\*  $n = 15$

\*\*  $n = 14$

water extractable phosphorus content of soils between habitats, while Ca, K, Mg, Mn, Fe, and Zn concentrations were significantly greater in the marsh soil than in the dune and swale soils (Table 2.2). Copper concentrations in dune and marsh were similar, and greater than swale Cu concentrations.

#### *Root specific gravity*

Significant differences in the specific gravity of root systems were found between all three populations (Table 2.3). Dune roots possessed the highest specific gravity (lowest porosity), while marsh roots had the lowest specific gravity (highest porosity). Habitat differences in soil moisture and Eh, measured when roots for specific gravity were collected (Table 2.3), were similar to those found earlier (Table 2.1).

#### *Physiological variables*

The capacity for alcoholic fermentation as measured by in vitro ADH activity was always greater in marsh roots than in dune or swale roots (Table 2.1). Ethanol, produced by the ADH catalyzed reaction, and ADH activity were positively correlated in the roots of the marsh population ( $r = 0.60$ ), but low levels of ethanol ( $< 2 \mu\text{moles g}^{-1} \text{ f wt}$ ) were measured in all samples (Table 2.1).

Malate concentration was least in roots of marsh plants and greatest in roots of dune plants (Table 2.1). In the relatively dry, more aerobic soil conditions of May and July, plant roots from all habitats had low malate concentrations ( $< 2 \mu\text{moles g}^{-1} \text{ f wt}$ ). When subjected to greater soil waterlogging in their respective habitats, as observed in August, malate increases were found in all populations (Table 2.1).

Table 2.3. Soil moisture, Eh and root system specific gravity of three *Spartina patens* field populations collected in 1985. Superscripts denote significant differences ( $P < .05$ ;  $n=5$ )

		POPULATION		
		DUNE	SWALE	MARSH
SOIL				
Moisture	(%wet wt)	3.53 <sup>c</sup>	18.9 <sup>b</sup>	78.6 <sup>a</sup>
Eh 15 cm	(mv)	221 <sup>a</sup>	213 <sup>a</sup>	-177 <sup>b</sup>
ROOT				
Specific gravity		.784 <sup>a</sup>	.692 <sup>b</sup>	.561 <sup>c</sup>
Porosity <sup>1</sup>	(v/v)	.267 <sup>c</sup>	.355 <sup>b</sup>	.483 <sup>a</sup>

<sup>1</sup>Porosity determined from the relationship:  $\text{Porosity} = 1.026 - 0.969 * \text{Specific Gravity}$   
(from 35 *S. patens* samples)

The metabolic path to malate routes phosphoenolpyruvate to oxaloacetic acid through a carboxylation catalyzed by PEP-C and thence to malate via the enzyme MDH (Figure 2.1). Malate was positively correlated with MDH activity ( $r = 0.53$ ), and both increased with decreases in soil Eh and increases in soil moisture within dune and marsh habitats (Table 2.1). Malic enzyme can decarboxylate malate to pyruvate, which provides a substrate for aerobic respiration or, depending upon the availability of oxygen, for anaerobic fermentation to lactate or ethanol (Figure 2.1). When averaged over populations, malic enzyme activity was greatest in July and lowest in August, varying inversely with malate and lactate accumulation (Table 2.1). A reverse stepwise multiple regression analysis was used to model malate accumulation in each population. Approximately two-thirds of the variation in malate was described by MDH activity, Eh and malic enzyme activity (Dune: overall  $F = 7.93$ ,  $P = 0.0043$ ,  $R^2 = 0.684$ ; Swale: overall  $F = 8.84$ ,  $P = 0.0029$ ,  $R^2 = 0.707$ ; Marsh: overall  $F = 6.90$ ,  $P = 0.0070$ ,  $R^2 = 0.653$ ).

Lactate, another potential endproduct of anaerobic metabolism, is produced through a reduction of pyruvate by lactate dehydrogenase using NADH. Lactate accumulation was positively correlated with MDH ( $r = 0.41$ ) and ADH ( $r = 0.31$ ) activities and malate accumulation ( $r = 0.41$ ). Like ethanol, lactate concentrations were not found above  $2 \mu\text{moles g}^{-1} \text{ f wt}$  (Table 2.1).

Significant differences were found in adenylate energy charge ratios (AEC) of leaf and root tissues with respect to habitat and month sampled (Table 2.1). The highest mean leaf AEC for all habitats was found in May, the middle of the vegetative growth period for *S. patens*, while lower values were measured in August and July. When averaged over months, marsh leaf AEC was significantly greater than dune or swale leaf energy charge (Table 2.1). Root energy charge decreased along the gradient from dune to marsh

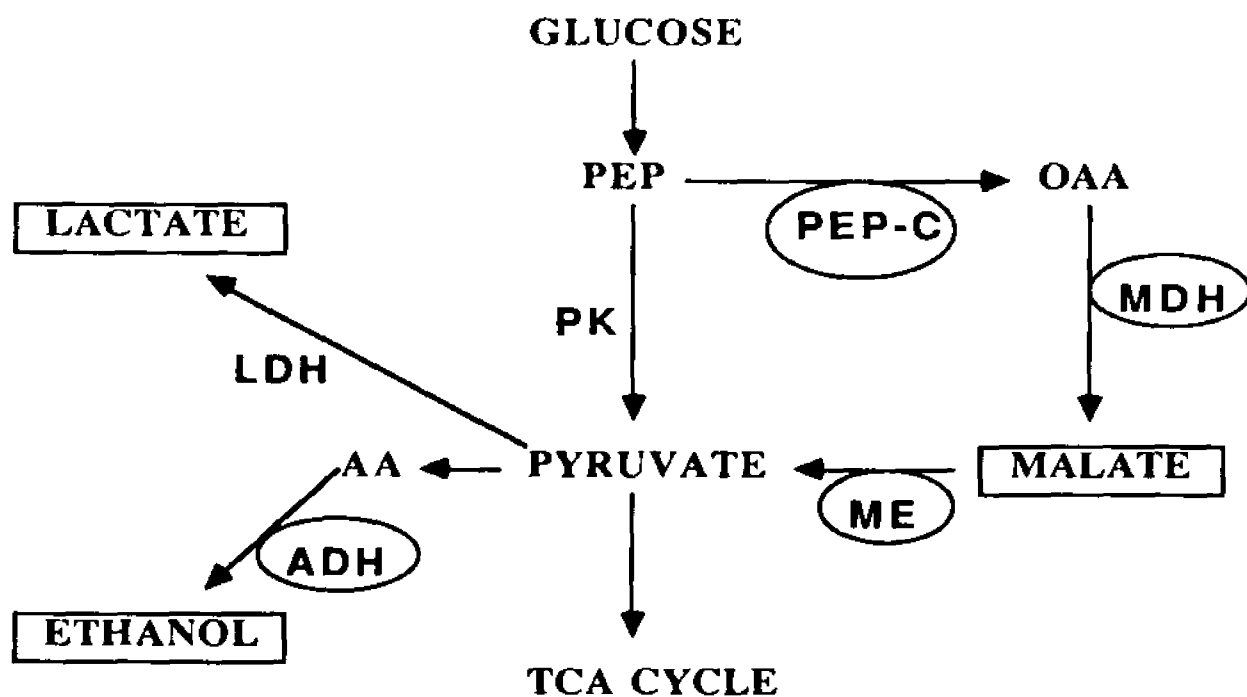


Figure 2.1. Several potential pathways of fermentation under hypoxia. Circled enzymes and metabolites in boxes were measured. PEP phosphoenol pyruvate; OAA oxaloacetate; AA acetaldehyde; PEP-C phosphoenol pyruvate carboxylase; MDH malate dehydrogenase; ME malic enzyme; PK pyruvate kinase; LDH lactate dehydrogenase; ADH alcohol dehydrogenase; TCA Cycle, tricarboxylic acid cycle

habitats following the degree of waterlogging determined from soil moisture and Eh (Table 2.1). The depressed root AEC observed in August coincided with the time of greatest soil waterlogging as indicated by the highest mean soil moistures and lowest soil Eh levels (Table 2.1).

#### *Multivariate analysis of physiological variables*

A canonical correlation (CC) (PROC CANCORR; SAS Institute, Inc. 1982) was performed between the root physiological (dependent) variables and environmental (independent) variables in order to associate patterns in metabolic indicators with soil waterlogging. The CC may be considered as an extension of multiple regression where more than one dependent variable is included. The environmental variables included habitat, with values assigned in order of decreasing elevation (dune = 1, swale = 2, marsh = 3), date of sampling, with values assigned in chronological order (August = 1, May = 2, July = 3), and two variables indicative of soil waterlogging: soil moisture and soil Eh. This procedure provides insight as to which of the environmental factors is most strongly associated with the metabolic state represented by the physiological variate. The squared canonical correlations of the first two pairs of environmental and physiological variates were high, accounting for 90% of their common variance (Table 2.4). In addition, a substantial portion (38%) of the variance of the physiological variables was explained by the two environmental variates in the redundancy analysis (Table 2.4).

After choosing an appropriate cutoff (correlations below 0.50 are excluded because they represent less than 25% of the variation in any given variable and are excluded), analysis focuses on interpretation of the canonical structure (the pattern formed within the variates and the relationship between the patterns in independent and dependent variates). Interpreting the size and sign of the correlation coefficients of the first variate pair, we

Table 2.4. Canonical correlation between environmental and root physiological variables. a. Canonical correlation and structure: squared canonical correlations, proportion of variance in common to the two variable sets that is explained by each variate pair and correlations of variables with complementary canonical variates. b. Redundancy analysis: percents of variance in each variable set explained by own variates and by their complementary canonical variates

a. Canonical Correlation and Structure:

		Variate Pairs	
		First	Second
Correlations of variables with the complementary canonical variate			
<u>Environmental</u>	habitat	-	.72
	sampling date	-.86	-
	soil moisture	-	.82
	soil Eh	-.55	-.63
<u>Physiological</u>	root AEC	-.51	-
	PEP-C	-	-
	MDH	.73	-
	ME	-	-
	ADH	-	.62
	malate	.50	-
	lactate	.75	-
	ethanol	-	-
Squared canonical correlation		.86	.81
% common variance explained by variate pair		.54	.36

Table 2.4 (continued)

b. Redundancy Analysis<sup>1</sup>:

	<u>Environmental Variates</u>			<u>Physiological Variates</u>		
	<u>First</u>	<u>Second</u>	<u>Total</u>	<u>First</u>	<u>Second</u>	<u>Total</u>
% variance within set explained by its own variate	35	52	87	30	15	45
% variance within set explained by its complementary variate	30	42	72	26	12	38

<sup>1</sup>Redundancy analysis determines the degree to which the canonical variates can predict the original variables (SAS Institute, Inc. 1982)



find that the earliest sampling date (August) and low soil Eh are associated with roots having relatively lower AEC, greater MDH activity, and elevated malate and lactate levels (Table 2.4). Thus the pattern of indicators in the first variate pair suggests that increased anaerobic metabolism leading to malate and lactate accumulation occurred in roots of all populations during August when soil waterlogging was greatest. The correlation coefficients of the second variate pair suggest that as soils become wetter and of lower Eh with declines in habitat elevation from dune to marsh, there is a corresponding increase in root ADH activity (Table 2.4).

In order to gain a clearer image of root metabolic patterns, the CC constraint of having the linear combinations of the physiological variables optimally correlated to the environmental canonical variate was avoided by performing a principal component analysis (PCA; SAS Institute, Inc. 1982) on the physiological variables alone. A three-factor unrotated solution yielded the most interpretable factor patterns and explained 69% of the variance in the physiological variables (Table 2.5). Variables within factor patterns were interpreted if more than 10% of their variance was explained by the factor ( $r = .3161$ ). Factor 1 was similar to the first canonical variate with both malate and lactate accumulation, but the enzyme pattern leading to malate accumulation (Figure. 2.1) is now more complete with PEP-C as well as MDH activity correlating positively, and ME exhibiting a negative correlation (Table 2.5).

Using the standardized scoring coefficients (Table 2.5), each observation may be scored for each factor pattern. A reverse stepwise regression, using these scores as dependent variables and the same independent variables used in the CC, was performed to find the best environmental variables to predict the scores for each factor (BMDP-P4M and GLM procedure; BMDP 1981). As with the first canonical variate, sampling date proved to be the best predictor of Factor 1 scores (Table 2.5), with the metabolic pattern

Table 2.5. Correlations of root physiological variables with principal component factors, standardized scoring coefficients, percent variance of the physiological variables explained by the factors and percent variance of the factors explained by environmental variables following reverse stepwise regressions

	FACTOR PATTERNS			STANDARDIZED SCORING COEFFICIENTS		
	Factors			Factors		
	1	2	3	1	2	3
Physiological variables						
ROOT AEC	-.77			-.27	-.11	-.03
PEP-C	.43		-.58	.15	.11	-.46
MDH	.73		.55	.26	-.12	.43
ME	-.42		.56	-.15	-.06	.44
ADH		.75	.48	.05	.52	.38
MALATE	.79			.28	-.20	-.11
LACTATE	.69	.48		.25	.33	-.01
ETHANOL	-.44	.70		-.16	.48	-.19
% Variance Explained by Each Factor (Total=69)	35	18	16			
Best Single Predictor	Month	Habitat or Moisture	Moisture			
% Variance Explained	63	19	46			
Best Second Predictor in a Two Variable Model	Eh	Month	Habitat			
Additional % Variance Explained	3	7	16			

exhibited by Factor 1 occurring in August, the sampling date of greatest soil waterlogging.

Factor 2 positively correlates high ADH activity with lactate and ethanol accumulation (Table 2.5), and may represent lactate-stimulated ethanol fermentation. This factor was best predicted by either habitat and month ( $R^2 = 0.26$ ), or soil moisture and month ( $R^2 = 0.26$ ). Low PEP-C, but high MDH, ME and ADH activities were correlated with Factor 3 which was best explained by moisture and habitat differences ( $R^2 = 0.62$ ). Generally marsh observations scored highly on Factors 2 and 3 which seem to represent a response to spatial increases in soil waterlogging and correspond to the second variate of the CC (Table 2.4).

## DISCUSSION

### *Soil waterlogging*

Soil Eh and moisture content, indicators of the degree of soil waterlogging, indicated that marsh plants were subject to greater waterlogging than dune or swale plants at all sampling dates (Table 2.1). Although the Eh of dune and swale soils were similar, soil moisture was always greater in the swale than in the dune (Table 2.1). This suggests that a gradient in soil waterlogging similar to the gradient in elevation existed across the three habitats.

When examined on a monthly basis, temporal differences in soil waterlogging were apparent. Soil Eh and moisture data from August indicate roots of all populations experienced a period of increased soil waterlogging (Table 2.1). In general, soil Eh

below 260 mV indicates oxygen deficiencies in the soil (Patrick 1981), which may be accompanied by root hypoxia (Saglio et al. 1984). Oxygen deficiency may even develop in roots growing in aerated soil, as demonstrated by Crawford (1976) and theorized by de Willigen and Van Noodwijk (1984).

#### *Root specific gravity*

Aerenchyma has been shown to develop with soil waterlogging in agricultural and wetland plants in greenhouse experiments (Yu et al. 1969; Kawase and Whitmoyer 1981; Smirnoff and Crawford 1983; Schat 1984) and in the field (Smirnoff and Crawford 1983). Root tissue collected from the three populations showed that the specific gravity of the root systems decreased (thus the amount of air space increased) from the dune to the marsh habitats (Table 2.3). In a laboratory study comparing three agricultural species, Webb and Armstrong (1983) concluded that "tolerance of anoxia in root tissue is largely a function of the degree of ventilation provided by the aerenchyma, and not due to metabolic adaptations which are of secondary importance." While we recognize the importance of aerenchyma development, this appears to be a long-term response, whereas the metabolic response observed in August may be a short-term response to soil waterlogging.

Marsh roots had a mean specific gravity corresponding to a porosity of  $49 \pm 5\%$ , which may indicate maximal aerenchyma formation. Armstrong (1979) suggested the maximum porosity achieved by wetland plant roots is 60%. Thus marsh soil may be so reduced that maximum aerenchyma development is not sufficient for completely aerobic respiration, and fermentation is required to supplement energy production for normal root processes. This possibility is consistent with the high levels of ADH and low AEC found in the roots of marsh plants which indicate aerobic respiration was limited (Mendelssohn

et al. 1981, Monk and Braendle 1982, Saglio et al. 1983, Mendelssohn and McKee 1987).

#### *Root physiological variables*

*ADH activity and ethanol concentration.* The consistently anoxic conditions measured in the marsh soils (Table 2.1) were associated with a ten-fold greater ADH activity in marsh roots than in dune or swale roots. This observation agrees with much of the experimental work showing that ADH activity increases with soil hypoxia under greenhouse or laboratory conditions in flood tolerant species such as rice (John and Greenway 1976, Pedrazzini and McKee 1984), *Glyceria*, *Ranunculus*, *Senecio* (Smith and ap Rees 1979) and *S. alterniflora* (DeLaune et al. 1984, Mendelssohn and McKee 1987). High ADH activities have been induced in flooded clones of dune and swale populations (Chapter 5), but ADH activity was never high in these habitats (Table 2.1), suggesting that ADH was not induced and aerobic respiration was the primary source of energy for root metabolism. Although ethanol concentration was positively correlated to ADH activity in marsh and swale habitats, root ethanol levels were always very low. Thus, ethanol was probably lost to the environment through passive diffusion as occurs in many wetland species (Davies 1980, Alpi and Beevers 1983, Barta 1984), and therefore posed no autointoxication problem.

*Alternative endproducts under anaerobiosis.* Greater soil waterlogging within each habitat was accompanied by increases in malate comparable to increases found in other wetland grasses due to waterlogging or hypoxia (Mendelssohn et al. 1981, Rumpho and Kennedy 1981, Mendelssohn and McKee 1987). The accumulation of malate is thought to be governed by the two enzymes involved in malate formation, PEP-C and MDH, as

well as malic enzyme which decarboxylates malate to pyruvate (Lance and Rustin 1984). Metabolic patterns in regressions, CC, and PCA analyses indicate that when *S. patens* is subject to greater than average reducing conditions in the soil, malate levels are higher via elevated PEP-C and MDH activities and depressed ME activities (Figure 2.1). Whether this malate accumulation has any physiological or ecological significance is unknown. Recent reviews (Davies 1980, Jackson and Drew 1984) have been unable to define a physiological or adaptive role for malate accumulation in waterlogged wetland plants but it may function in: (1) providing a metabolite pool of reduced carbon available to the TCA cycle once oxygen becomes less limiting; (2) storing reduced equivalents that cannot be oxidized until oxygen becomes more available and thus (3) recycling  $\text{NAD}^+$  for essential metabolism; (4) storing carbon dioxide; or (5) providing a source of reduced equivalents for nitrate assimilation (Crawford 1982, Lance and Rustin 1984).

Another alternative to the production and subsequent loss of ethanol is lactate fermentation (Figure 2.1), which can also result in ATP generation. Placing maize root tips under anoxia, Saglio et al. (1980) showed that an acceleration of glycolysis increased both ethanol and lactate levels (ethanol levels were six times greater than lactate levels) and restored root AEC. More importantly, lactate may serve a regulatory function in anaerobic root metabolism (Davies et al. 1974), especially since lactate production cannot maintain ATP levels longer than 10 minutes in hypoxic cells due to mortality from cytoplasmic acidosis (Roberts et al. 1984a). Roberts et al. (1984a) demonstrated that a short period of lactate production lowered cytoplasmic pH, and thus stimulated alcoholic fermentation in hypoxic maize root tips (for mechanism, see Davies 1980). However, those plants which secrete lactate from their roots may prevent acidosis, allowing continued production of both lactate and ethanol (Hoffman et al. 1986). In marsh roots where we presume alcoholic fermentation occurred, positive correlations of ADH activity

with both lactate and ethanol (Table 2.5, Factor 2) suggest stimulated ethanol fermentation through lactate-mediated acidosis under natural conditions.

*Adenylate energy charge.* Leaf AEC does not appear to be related to soil waterlogging but follows expected seasonal trends with high values associated with vegetative growth (May) in all habitats, and low values found during the reproductive phase (July and August) of the life cycle (McKee and Mendelssohn 1984). In addition, leaf AEC varied with population (Table 2.1), closely following the trends in available soil nitrogen (Table 2.2) which has also been shown to influence leaf AEC in *Spartina patens* (Mendelssohn and McKee 1985).

The gradient in habitat elevation accompanied by increasing soil waterlogging was reflected in decreasing root AEC from dune to marsh plants. Thus, the decline in root AEC is attributed to increasing root hypoxia. Decreases in oxygen availability to roots are accompanied by declining AEC, but the fall in AEC can be buffered by an acceleration of anaerobic fermentation (Mendelssohn et al. 1981, Saglio et al. 1983, Mendelssohn and McKee 1987). Mutant maize roots lacking an ADH isozyme were unable to accelerate ethanol synthesis and could not maintain ATP levels after a few minutes of hypoxia (Roberts et al. 1984b). Thus, the AEC of marsh roots might have been lower if the fall in AEC had not been buffered by a stimulation of ethanol fermentation, hypothesized from elevated ADH activities. In contrast, August dune roots experiencing soil waterlogging exhibited the lowest root AEC measured in this study, and this may have resulted from the lack of ADH induction. This and other work (Mendelssohn et al. 1981, Monk and Braendle 1982, Mendelssohn and McKee 1987) suggest that some threshold of hypoxia must be reached to restore AEC through ethanol fermentation; perhaps this threshold was not reached in the August dune roots.

*Paths of root respiration.* Spatial and temporal differences in physiological variables due to soil waterlogging are best discussed using multivariate analyses, though the results are correlative in nature and inferences drawn from them are not conclusive. For example, the metabolic changes leading to malate accumulation in August may be a response to some factor other than soil waterlogging. Experiments where substrate Eh and root porosity are carefully controlled are needed for substantive proof that these patterns are metabolic responses to hypoxia.

All three populations appeared to possess similar metabolic responses to temporal increases in soil waterlogging, including depressed root AEC, enzyme activities leading to malate accumulation, and malate and lactate accumulation (Table 2.5), even though the absolute degree of soil waterlogging differed among the habitats. We hypothesize that aerenchyma formed in response to the prevalent intensity of soil waterlogging found in each habitat allows the roots of each of the three *S. patens* populations to behave similarly in response to temporal increases in soil waterlogging. The second environmental variate of the CC (Table 2.4) and Factors 2 and 3 of the PCA (Table 2.5), which are thought to reflect a spatial waterlogging gradient, were correlated with ADH activity. We hypothesize that this represents a gradient of increasing reliance of root respiration on alcoholic fermentation from dune and swale roots to marsh roots where the prevalent degree of soil waterlogging is severe.

The univariate results and multivariate correlations indicate that: (1) *Spartina patens* roots of dune and swale populations primarily respired aerobically, and exhibited a high AEC when soils were well aerated. (2) The marsh roots, subjected to continual soil waterlogging, possessed a high capacity for alcoholic fermentation and a moderate AEC. (3) During a period of greater soil waterlogging, the AEC was depressed in dune and



swale roots, suggesting that root hypoxia reduced aerobic respiration, while in marsh roots alcoholic fermentation is believed to have continued and supported a moderate energy charge ratio. In all three populations, this temporal increase in waterlogging appears to direct glycolysis toward malate accumulation by means of elevated PEP-C and MDH and lower ME activities. Whether this occurs at the expense of alcoholic fermentation is unknown.

## CONCLUSIONS

*Spartina patens* thrives across a steep gradient of soil water and oxygen availability and responds to the ambient degree of soil waterlogging by inducing differential root porosity. In the marsh where soil waterlogging is severe, the upper limit of aerenchyma development may be realized, and root hypoxia is accompanied by a depression in the adenylate energy charge ratio. Induction of ADH activity was correlated with ethanol and lactate accumulation in marsh roots, indicating alcoholic fermentation may have been an important path of root respiration and, along with root porosity, an important long-term response to soil waterlogging.

Dune plants had the lowest root porosity, while swale root porosity was intermediate between dune and marsh. When soil waterlogging was minimal in these two habitats, the roots are thought to have respired aerobically. Root metabolic response to relatively greater soil waterlogging, illustrated by the multivariate analyses, was similar in all three populations, and indicated utilization of metabolic paths leading to malate and lactate accumulation. Thus it seems the long-term anatomical adaptation of root porosity could

not always fulfill the respiratory oxygen requirements of *S. patens* roots in any of the habitats studied, although the physiological significance of the temporal metabolic response leading to malate accumulation remains an enigma.

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CHAPTER 3.  
ROOT AERENCHYMA DEVELOPMENT IN *SPARTINA PATENS*  
IN RESPONSE TO FLOODING

ABSTRACT

Root aerenchyma, developed in response to flooding, was measured as specific gravity in previously existing (old) and newly developed (new) roots of *Spartina patens* in a 25-day greenhouse experiment. Root specific gravity was related to porosity (fractional volume of aerenchyma) by:  $\text{Porosity} = 1.026 - 0.969 \times \text{Specific Gravity}$ , over a wide range of specific gravities (0.5 to 0.9). The specific gravity of flooded roots (new and old) decreased from 0.78 to 0.58 between day 5 and 25, while the specific gravity of old roots that remained drained did not change. After 5 days growth, newly produced roots were found to have less aerenchyma than their older counterparts, but after 25 days the specific gravity of new roots equaled that of old roots. In addition, flooding increased mortality of preexisting roots and inhibited growth of new roots.

INTRODUCTION

*Spartina patens* (Aiton) Mulh., a coastal grass that grows along a gradient of soil moisture conditions from sand dunes to marshes, was shown to possess roots varying in specific gravity from 0.78 in a dune to 0.56 in a salt marsh (Burdick and Mendelssohn 1987 Chapter 2). A similar relationship between soil moisture and root porosity was demonstrated for *Senecio aquaticus* in Scottish wetlands (Smirnoff and Crawford 1983).



Formation of aerenchyma in wetland species not only reduces the number of respiring cells in a growing root, but also facilitates oxygen diffusion to aerate the remaining living tissues and detoxify reduced soil components in the rhizosphere (Williams and Barber 1961, Luxmoore, Stolzy, and Letey 1970, Armstrong 1972, Mendelssohn and Postek 1982). In some (Das and Jat 1977, Schat 1984), but not all wetland plant species (Smirnoff and Crawford 1983) grown in laboratory culture, flooding increases the amount of root aerenchyma.

A time course experiment was designed to test the hypothesis that the amount of aerenchyma developed in roots is a function of root age and soil drainage in *S. patens*. Specifically, I wished to determine if flooding increases aerenchyma development under controlled conditions, and if so, whether new roots increase aerenchyma more effectively than older roots as suggested by some studies (Luxmore and Stolzy 1969, Yu, Stolzy, and Letey 1969). The results are based on measurements of root specific gravity which have been used as a relative indicator of root aerenchyma along flooding gradients in the field (Iverson 1949, Burdick and Mendelssohn 1987 Chapter 2) and in laboratory culture (Schat 1984).

## MATERIALS AND METHODS

Clones were grown from *Spartina patens* collected from a salt marsh. Two to three plants, totalling five to seven culms, were planted in 2 L pots filled with sand and 8% (by weight) Crowley silt loam, and grown for eight weeks prior to initiating treatments.

At the beginning of the experiment, (treatment day 0), plants were removed from pots, dead roots were discarded, and live, intact roots were stained in a 0.25 g/l solution of neutral red for 10 minutes so that new root growth could be distinguished from

previously existing roots. Schumacher, Smucker, Eshel, and Curry (1983) report that roots exhibited no adverse effects with respect to respiration or growth rates when dyed at this concentration for five minutes. Roots were then rinsed with tap water, and the plants were carefully replaced in the sand-silt growth medium to minimize root damage. A nutrient solution (100 ml of a half-strength Hoagland's solution (Goss 1973) amended with  $\text{NH}_4\text{NO}_3$  to produce 20 mM nitrogen) was added on days 0 and 12. Harvest date (day 5, 15, and 25), flooding treatment (flooded, drained), and root age (new, old) were the main effects in a completely randomized design with a 3 X 2 X 2 factorial treatment arrangement. Four replicates of each treatment combination were arranged randomly in the greenhouse (24 pots in all). Effects were evaluated using analysis of variance (significance at the 0.05 level unless otherwise stated) with specific *a priori* hypothesis tests generated by linear contrasts (SAS Institute, Inc. 1985).

At harvest, the plants were washed of soil and the roots were carefully removed and rinsed. New, white roots were separated from previously existing roots that were stained pink. Samples of roots were wrapped in wet paper towels, enclosed in plastic bags and kept on ice. Three determinations of specific gravity per replicate were made from representative groups of structurally intact roots and the mean of these three was used in the subsequent analysis. Remaining live roots were blotted dry and weighed. Roots growing out of the bottom of pots through drainage holes and those above the soil in the flooded treatment were not used for specific gravity measurements but were included in root biomass determinations.

Specific gravity was calculated by weighing a 25 ml pycnometer filled with water, weighing 0.1 to 0.3 g roots (gently blotted dry on tissue paper), then placing the roots in the water-filled pycnometer and reweighing. Specific gravity (SG) was computed using the formula:  $\text{SG} = (r)/(p + r - p\&r)$ , where  $r$  = mass of roots,  $p$  = mass of water-filled pycnometer, and  $p\&r$  = mass of pycnometer with roots and water. For determinations

used to develop the relationship between root specific gravity and porosity, the roots were removed from the pycnometer after reweighing, ground with a mortar and pestle, and returned quantitatively to the pycnometer (using water to rinse the mortar) and reweighed as in Jensen et al. (1969). The formula for porosity is:  $\text{Porosity} = (p_{gr} - p_r) / (p + r - p_r)$ , where the symbols are as above and  $p_{gr}$  = mass of pycnometer with ground roots and water.

To test the effect of storage time following harvesting on root specific gravity, three time course experiments (with six to eight sampling periods and one to three samples per period) were conducted over 45 hours. Simple linear regressions of root specific gravity on time indicated there was no change in specific gravity with time in roots stored as described above (range for the three experiments:  $0.62 < P < 0.88$ ,  $0.03 < r^2 < 0.09$ ).

Roots were collected from a salt marsh population of *Spartina patens* for microscopic examination of aerenchyma. Roots were preserved in formalin acetic acid, dehydrated with a 14-step tertiary butyl alcohol series, embedded in paraffin, and sectioned at 7  $\mu\text{m}$ .

## RESULTS

A comparison of specific gravity with porosity measurements in both drained and flooded roots of *Spartina patens* is shown in Figure 3.1. The relationship obtained from simple linear regression ( $\text{Porosity} = 1.026 - 0.969 \times \text{Specific Gravity}$ ) suggests that roots with no lacunae would be slightly more dense (specific gravity = 1.06) than water. However, increases in porosity were matched by decreases in specific gravity (i.e., a 0.1 decrease in specific gravity indicated a 0.1 increase in porosity), since the null hypothesis,  $H_0$ : slope = -1 was not rejected ( $P=0.45$ ).

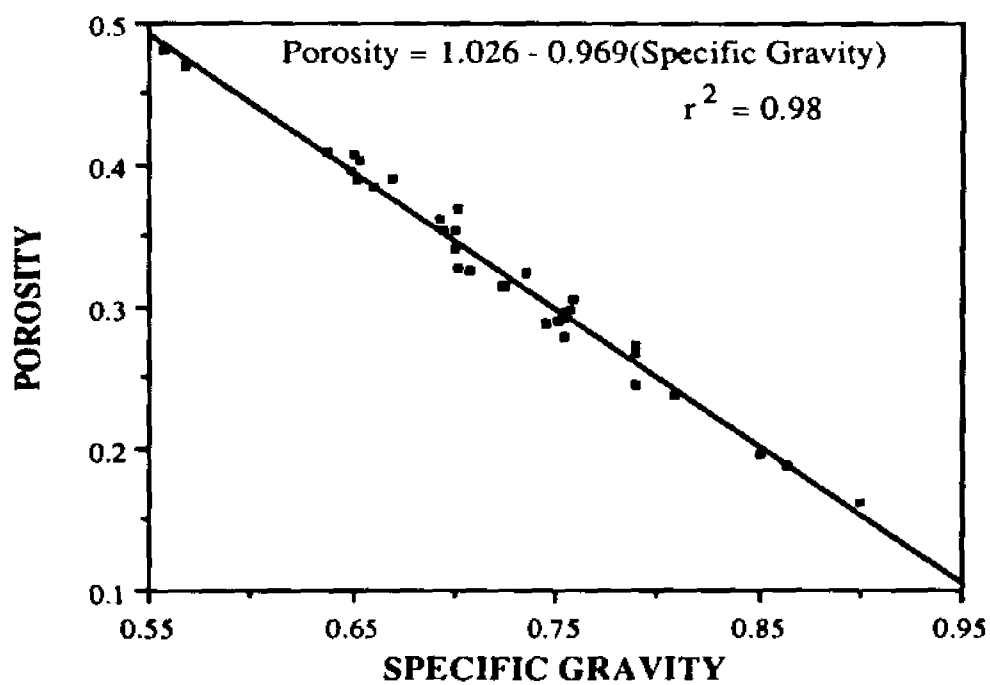


Figure 3.1. Root porosity (fractional volume of aerenchyma) as a function of specific gravity in *Spartina patens*.

Aerenchyma development in roots of *S. patens* induced by flooding was followed over 25 days (Fig. 3.2). Root specific gravity was significantly decreased by flooding treatment, root age, and date of harvest (Table 3.1). Flooding by root age and flooding by harvest date interactions were also significant. After five days of flooding, both new and old roots from flooded pots had statistically similar amounts of aerenchyma as roots from drained pots (Figure 3.2). At this time, new roots possessed greater specific gravity than old roots ( $P=0.0009$ ), but by day 25 the aerenchyma had increased in new roots such that no differences existed in specific gravity between new and old roots (Figure 3.2). Flooding decreased specific gravity of older roots ( $P=0.0002$ ), and since there was no flooding by root age interaction (Table 3.1), flooding reduced specific gravity to the same extent in new roots. In contrast, old roots from the drained treatment did not change specific gravity over the course of the experiment (Figure 3.2).

Root fresh weight depended upon all main effects and day by flooding and day by age interactions (Table 3.2). The fresh weight of new root tissue increased over time in both drained and flooded treatments, but more rapidly in drained plants ( $P=0.0001$ , Figure 3.3). The mass of live older roots was not measured on day 5, but there was no change between 15 and 25 days in either flooded or drained treatment. Older root fresh weight was greater in drained than flooded roots (Figure 3.3). On the day 15 harvest, some of the root systems in the flooded treatment exhibited new growth at the tips, but these root systems did not appear to be living when harvested (water-filled and translucent), and these roots (mean mass=0.39 g, standard error=0.15) were excluded from the biomass measurements. Thus it appears flooding not only inhibited new root growth, but also increased mortality in old roots.

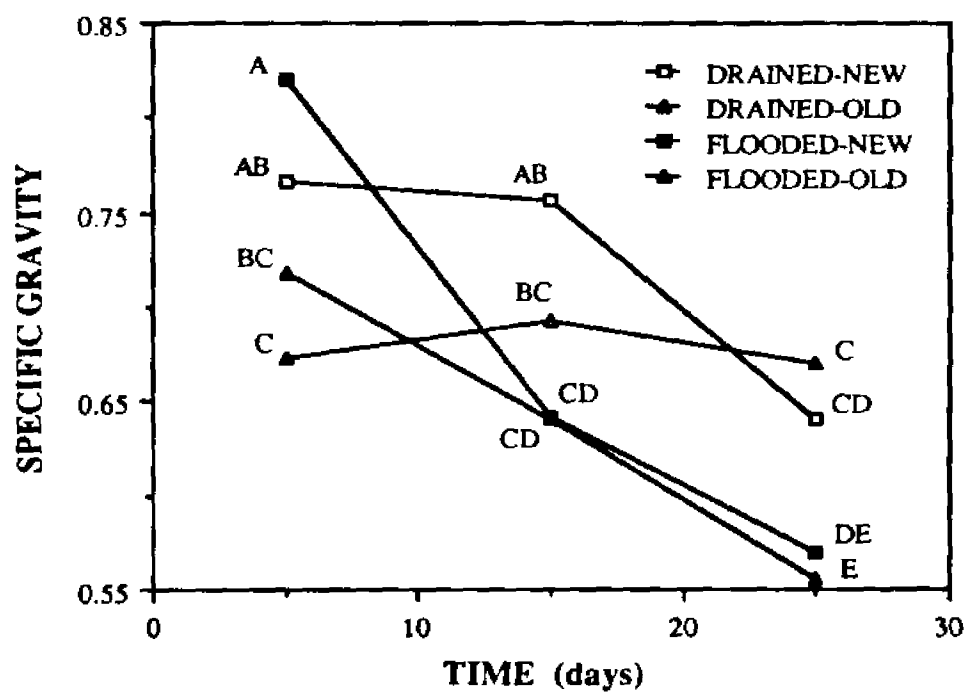


Figure 3.2. Changes in specific gravity with flooding treatment, harvest day and root age in *Spartina patens*. Different letter superscripts denote statistically different means (least significant difference = 0.0774).

Table 3.1. Sources in the analysis of variance of root specific gravity in *Spartina patens* with main effects of harvest day, flooding treatment, and root age and their interactions.

SOURCE	Degrees of Freedom	Sum of Squares	F Value	Probability of a Greater F
Harvest Day	2	0.1473	24.96	0.0001
Flooding Treatment	1	0.0213	7.22	0.0108
Root Age	1	0.0203	6.88	0.0127
Day x Flooding	2	0.0497	8.42	0.0010
Day x Age	2	0.0227	3.84	0.0307
Flooding x Age	1	0.0000	0.01	0.9306
Day x Flooding x Age	2	0.0058	0.97	0.3873
Error	36	0.1062		

Table 3.2. Sources in the analysis of variance of root biomass (fresh weight) in *Spartina patens* with main effects of harvest day, flooding treatment, and root age and their interactions.

SOURCE	Degrees of Freedom	Sum of Squares	F Value	Probability of a Greater F
Harvest Day	2	17.34	45.18	0.0001
Flooding Treatment	1	5.13	26.75	0.0001
Root Age	1	7.55	39.37	0.0001
Day x Flooding	2	1.43	3.71	0.0362
Day x Age	1	1.01	5.26	0.0290
Flooding x Age	1	0.29	1.53	0.2259
Day x Flooding x Age	1	0.11	0.56	0.4587
Error	30			



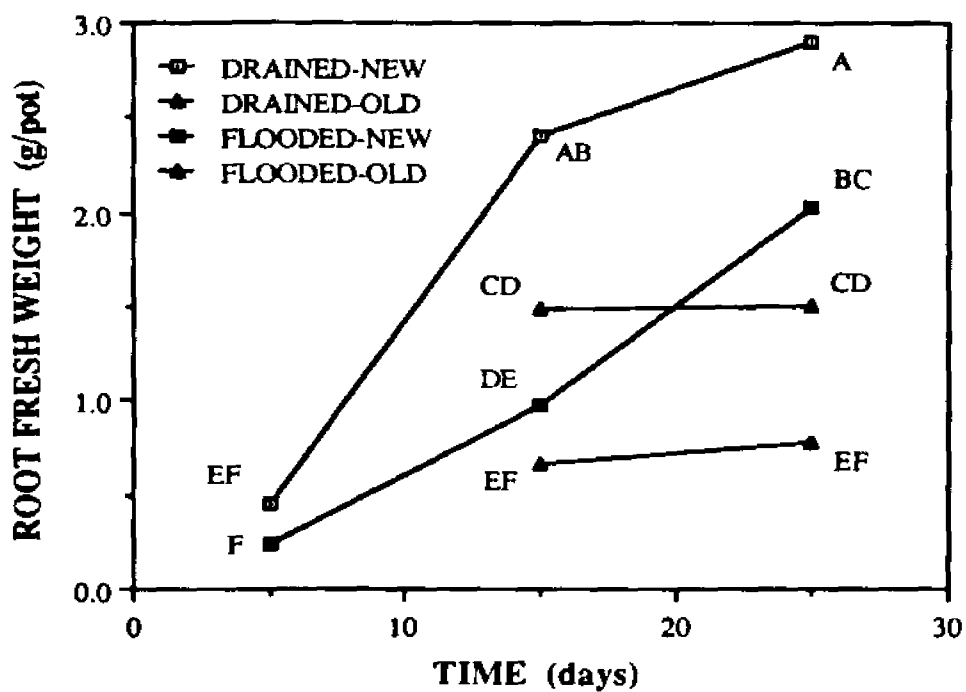


Figure 3.3. Changes in fresh weight of live *Spartina patens* root systems with flooding treatment, harvest day and root age. Different letter superscripts denote statistically different means (least significant difference = 0.627 g).

## DISCUSSION

### *Measurement method*

Jensen et al. (1969) reported that the large standard deviations of porosity measurements required large samples ( $\geq 1$  g) with many replicates ( $\geq 10$ ) to obtain reasonable estimates of root aerenchyma. They identified the cause of the variation as the determination of the numerator in their formula ( $p_{gr} - p_r$ , see MATERIALS AND METHODS), since this difference depends upon two measurements that are both subject to error. In addition they warned, "Constant temperature of the bottle contents throughout all weighings is also essential for accuracy." Although not a direct measure of the amount of aerenchyma, the determination of specific gravity using a pycnometer is preferred to porosity measurements for two reasons. First, specific gravity measurements require less tissue and shorter processing time, (I found that three samples yielded satisfactory precision for these experiments). Second, measurements of specific gravity are not subject to large errors from: (a) incomplete homogenization or loss of sample during transfer from the grinding vessel back to the pycnometer; (b) changes in temperature, since performance of the three specific gravity measurements requires less than five minutes; and (c) determination of the difference between two similar numbers in the numerator of the porosity formula. Use of specific gravity to obtain an estimate of root aerenchyma in *Spartina patens* is equally accurate (using the relationship in Figure 3.1) and more time efficient compared to the determination of root porosity using the method of Jensen et al. (1969).

### *Aerenchyma development*

As *Spartina patens* roots developed in sand culture under both drained and flooded conditions, their specific gravity decreased. In addition, the specific gravity of old as

well as new roots decreased under flooded conditions. Observation of root sections taken from field populations of *S. patens* showed that aerenchyma formed through schizogeny close to the root tip, and then primarily through lysigeny within a few cm of the root tip as described by McPherson (1939) for roots of *Zea mays*. McPherson (1939) found that aerenchyma would form in the cortex of both new and preexisting roots when maize plants with roots lacking aerenchyma were transferred from vigorously aerated to stagnant water culture. Although sand culture was used in this study, the results (Figure 3.2) agree with those of McPherson and extend to *S. patens* the observation that older roots, as well as new roots, can induce aerenchyma upon flooding. Furthermore, the extent of aerenchyma development in preexisting roots of *S. patens* was not surpassed by that in the newly formed roots (through 25 days), as occurred in adventitious roots of rice and maize (Luxmoore and Stolzy 1969). Results from another experiment (Chapter 5) showed that root aerenchyma development is complete (no further decreases in specific gravity were measured) sometime between 16 and 29 days of flooding.

Many factors affect aerenchyma formation, but their mechanism of action appears to occur through the development of low oxygen concentrations in the root tips as indicated by McPherson in 1939. Later work showed that the synthesis and accumulation of ethylene in hypoxic roots lead directly to aerenchyma formation (Drew, Jackson and Giffard 1979). Increased root respiration, either directly (elevated temperature) or through increased growth rates (elevated temperature, high light intensity), may lead to oxygen shortages and result in aerenchyma formation (Luxmoore, Sojka, and Stolzy 1972, Smirnoff and Crawford 1983). Recently, Konings and Verschuren (1980) reported that nitrogen deficiency may induce aerenchyma formation through cortical disintegration in roots of *Zea mays* grown in solution culture. A factorial experiment showed that both low nutrient levels and hypoxia increased aerenchyma in *Nardus stricta*, a rooted aquatic, but also resulted in no interaction between these effects (Smirnoff and

Crawford 1983). Since (1) both drained and flooded treatments were exposed to identical light and temperature conditions and were well fertilized, and (2) flooding did not stimulate, but inhibited, root growth (Figure 3.3), it appears the reduction in specific gravity in *S. patens* roots was due to increased aerenchyma as a result of oxygen deficits associated with flooding.

The ability of *S. patens* to develop aerenchyma in response to flooding may be important in maintaining flood tolerance and competitive ability in habitats at various positions on a soil waterlogging gradient. The intensity of waterlogging stress that affects wetland plants varies in time and space, and aerenchyma development has ecological implications in all but xeric habitats.

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CHAPTER 4.  
SOIL WATERLOGGING RESPONSES IN *SPARTINA PATENS* PRETREATED  
WITH FLOODED AND DRAINED CONDITIONS

ABSTRACT

Soil waterlogging responses were measured in roots of the flood tolerant grass *Spartina patens* following pretreatment with drained or flooded conditions in sand culture. The drained and flooded pretreatments generated differences in soil redox potential comparable with those of dune and marsh habitats, respectively. Root specific gravity indicated that flooded plants had twice the root aerenchyma volume of drained plants. After approximately four weeks of pretreatments, the following treatments were applied: drained for six days, flooded for three days, and flooded with 0.2% sucrose for three days. The soil redox potential (Eh) increased in response to draining and decreased with flooding treatments. Overall, root adenylate energy charge (AEC) ratio (a measure of the metabolic energy status) decreased, and root alcohol dehydrogenase (ADH) activity and malate concentration (indicators of anaerobic metabolism) increased with flooding stress.

In plants pretreated with flooded conditions, release from flooding stress for six days resulted in a four-fold decline in ADH activity and increased AEC ratios to levels exceeding those of all other treatments. Continued flooding and flooding with sucrose of preflooded plants, both led to high root ADH activities, and moderate AEC ratios. It appears, therefore, that aerenchyma formation in preflooded roots had not increased root aeration sufficiently to significantly reduce anaerobic metabolism (as measured by ADH activity) or improve the energy status (as indicated by AEC ratios) of the roots in the flooding treatment.

## INTRODUCTION

*Spartina patens* (Aiton) Mulh. is a grass inhabiting coastal environments that span a wide range of soil waterlogging conditions. A comparison of aerenchyma (gas space) and metabolism in roots of *S. patens* growing in three habitats (dune, swale, and marsh) in coastal Louisiana indicated that in the marsh habitat, where soil waterlogging was greatest, roots had almost twice as much aerenchyma and ten-fold greater alcohol dehydrogenase (ADH) activity (an indicator of anaerobic metabolism) than roots growing in the dune habitat (Burdick and Mendelssohn 1987 Chapter 2). In addition, temporal increases in soil waterlogging were accompanied by increases in malate concentrations, and decreases in adenylate energy charge ratios (AEC) from all habitats (Burdick and Mendelssohn 1987 Chapter 2). Metabolic responses such as these have been associated with anoxic stress in other wetland species (Keeley 1979, Smith and ap Rees 1979, Mendelssohn et al. 1981, McKee and Mendelssohn 1987). The purpose of this investigation was to observe metabolic responses to soil waterlogging under controlled conditions. To mimic the relative differences in soil waterlogging observed between marsh and dune environments, and in aerenchyma volume found between marsh and dune roots, plants were pretreated with flooded and drained soil conditions. Metabolic responses to changes in soil waterlogging were examined in these plants following three treatments: drained for six days, flooded for three days, and flooded with 0.2% sucrose for three days.



## MATERIALS AND METHODS

*Spartina patens* was propagated from plants collected from a salt marsh in Louisiana. Six to seven culms, composing two to three clonal groups, were planted in 2 L plastic pots with a mixture of coarse to fine sand. To encourage a minimum of aerenchyma formation in drained roots, plants were grown in a growth chamber under conditions of low temperature (24° C day/18° C night:12/12 h cycles), low light (300 to 700  $\mu\text{mol}/\text{m}^2/\text{sec}$  in the canopy edge and center, respectively) and high fertility. Nutrient solution (200 ml) was added once per week as a half-strength Hoagland's solution (Goss 1973) modified to contain 10 mM nitrogen as  $\text{NH}_4\text{NO}_3$  and 5.5 mM calcium using 5.0 mM  $\text{CaCl}_2$  and 0.5 mM  $\text{Ca}(\text{H}_2\text{PO}_4)_2$ . After plants were grown for 17 days under drained conditions, the pretreatment periods (used to generate roots with different amounts of aerenchyma) were begun. Half of the pots were immersed in 4 L buckets of half-strength nutrient solution (1.7 L), while the other pots remained drained. These conditions were continued 32 days for flooded plants and 29 days for drained plants. Flooded buckets were washed to remove algae and pots were reimmersed in fresh solution at days 17 and 32. The effects of the pretreatments on soil chemistry were monitored by measuring soil Eh at 10 cm depth every two weeks.

When treatments were begun, each treatment (drained, flooded, and flooded with 0.2% sucrose) was applied to six pots of each pretreatment. For the drained treatment, soil Eh was measured and the plants were harvested following six days of drained conditions. For the two flooded treatments, soil Eh was measured and plants were harvested after three days (following 32 days of drained and flooded pretreatments). The longer duration for the drained treatment was required to allow sufficient time for soil reoxidation. Culm number, length, and above-ground biomass were also measured. In addition, representative, live root samples were collected for specific gravity and

metabolic analyses as in Chapters 3 and 5, respectively. Remaining live roots were collected for below-ground biomass determination.

Root specific gravity was measured for three samples from each of the six replicates of the predrained then drained plants, and preflooded then flooded plants. Specific gravity (SG) was determined as described in detail in Chapter 3, using the formula:  $SG = r / (p+r-p\&r)$ , where  $r$  = mass of roots,  $p$  = mass of water-filled pycnometer, and  $p\&r$  = mass of pycnometer filled with both roots and water. Root porosity was calculated using the formula:  $Porosity = 1.026 - 0.969 \times (SG)$ , also discussed in Chapter 3. For determination of root biomass, root tissue used for specific gravity measurements was weighed and converted to dry weight using a wet weight to dry weight ratio ( $4.94 \pm 0.09$ , mean  $\pm$  standard error) that was determined for four root samples.

Root tissue for metabolic analysis was frozen in liquid nitrogen with ca. 20 ml deionized water to eliminate thawing during handling (Mendelssohn and McKee 1981), then freeze dried and ground in a Wiley mill (mesh size #40). Adenylates (ATP, ADP, and AMP) were extracted and converted to ATP as described earlier (Burdick and Mendelssohn 1987 Chapter 2). ATP concentration was quantified via the light-yielding reaction of ATP with firefly lantern extract (FLE-50, Sigma Chem. Corp.) on an integrating photometer (SAI Technology, model 3000) with a 10 s measurement and a 5 s delay. Adenylate concentrations (ATP, ATP + ADP, and ATP + ADP + AMP) were calculated from regressions of ATP standards that were mixed with the corresponding incubation buffers prior to measurement (Delistraty 1982).

Analysis of malate concentration was conducted as in Burdick and Mendelssohn (1987 Chapter 2), except that 0.02 g of freeze dried tissue was extracted in 3.0 ml of 11% perchloric acid by vortexing 30 s and waiting 0.5 h before neutralization with 5 M  $K_2CO_3$ . Analyses of ADH activity and total protein content are described in Chapter 5. Recoveries of added standards were  $95.9 \pm 2.8\%$  for ATP concentration ( $n=8$ ),  $100.2 \pm$

3.4% for ADH activity (n=6), and  $97.0 \pm 1.2\%$  for malate concentration (n=6), and exhibited no consistent trends with respect to pretreatments or treatments (means  $\pm$  standard error with sample size in parentheses). Conversion of internal adenylate standards (ADP and AMP) to ATP averaged  $94.2 \pm 1.7\%$  (n=4).

Treatments and pretreatments were assigned to randomly placed pots in a 6 x 6 matrix in a growth chamber. Statistically, this experiment had a completely randomized design with a two pretreatment by three treatment factorial treatment arrangement with six replicates. Analysis of variance (ANOVA) was performed using SAS software (SAS Institute, Inc. 1985). Root ADH activity required log transformation to reduce non-homogeneity of the error variance. Protected least significant differences between means of treatments were reported at the 0.05 level of significance. Correlation coefficients (SAS Institute, Inc. 1985) were reported if significant at the 0.05 level.

## RESULTS

### *Soil waterlogging conditions*

Before treatments were begun, the flooding pretreatment resulted in an initial soil Eh averaging 225 mV, indicative of slightly reducing conditions (Table 4.1). The Eh of the drained soil remained relatively high (375 mV), and was indicative of aerobic conditions. Final soil Eh depended upon soil waterlogging treatment as well as the pretreatment, but not their interaction (Table 4.2). The lack of a significant interaction ( $P=0.1458$ ) suggests that the effect of pretreatment was still present at the close of the experiment, with predrained soils having a higher Eh than preflooded soils (187 and 127 mV, respectively,  $LSD_{0.05} = 59$  mV). Treatment had the largest effect on final soil Eh (Table 4.2). Drained soil had the greatest Eh, flooded soil was slightly reduced, and pots

Table 4.1. Effects of drained and flooded pretreatments on soil conditions (measured before treatments were begun) and plant morphology and anatomy in *Spartina patens* (measured following treatments). Different letter superscripts denote significantly different means ( $P < 0.05$ ). Aerenchyma volume (porosity) was calculated from root specific gravity: Porosity =  $1.026 - 0.969(\text{SG})$ .

	PRETREATMENT	
	Drained	Flooded
Initial Soil Eh (mV)	375a	225b
Final # Stems	9.2a	8.8a
Culm Height (cm)	97.6a	99.4a
Stem Mass (g)	.49b	.60a
Aboveground Biomass (g)	4.39a	5.01a
Belowground Biomass (g)	.37a	.33a
Specific Gravity	.859a	.657b
Aerenchyma volume	.194b	.389a

Table 4.2. Analysis of variance of final soil redox potential (Eh; sum of squares  $\times 10^{-3}$ ), and ATP concentration, adenylate energy charge (AEC) ratio (sum of squares  $\times 10^3$ ), alcohol dehydrogenase (ADH) activity, protein concentration, and malate concentration in roots of *Spartina patens* with pretreatment, treatment, and interaction effects.

SOURCE	Degrees of Freedom	Sum of Squares	F Value	Probability of a Greater F
Soil Eh				
Pretreatment	1	32	4.36	.0453
Treatment	2	2559	172.31	.0001
Pretrt x Trt	2	31	2.05	.1458
Error	30	223		
Root ATP				
Pretreatment	1	24.0	36.17	.0001
Treatment	2	9.7	7.28	.0026
Pretrt x Trt	2	11.2	8.45	.0012
Error	30	19.9		
Root AEC				
Pretreatment	1	11.3	4.51	.0420
Treatment	2	21.8	4.35	.0219
Pretrt x Trt	2	16.5	3.30	.0507
Error	30	75.1		
Root ADH				
Pretreatment	1	0.036	2.48	.1258
Treatment	2	4.349	149.36	.0001
Pretrt x Trt	2	0.110	3.79	.0340
Error	30	0.437		
Root Protein				
Pretreatment	1	380	18.71	.0002
Treatment	2	1027	25.25	.0001
Pretrt x Trt	2	672	16.53	.0001
Error	30	610		
Root Malate				
Pretreatment	1	384	9.88	.0039
Treatment	2	1046	13.46	.0001
Pretrt x Trt	2	275	3.53	.0428
Error	30	1088		

flooded with sucrose solution had the lowest soil Eh, indicative of highly reduced conditions (Figure 4.1).

#### *Plant morphology and anatomy*

The flooding pretreatment increased stem weight, although there was no significant reduction in the number of stems or increase in aboveground biomass due to flooding (Table 4.1). There was also no significant difference between pretreatment means for belowground (root) biomass (Table 4.1). The flooding pretreatment resulted in decreased root specific gravity as compared to drained roots, indicating increased aerenchyma (two-fold greater volume) was formed in response to flooding (Table 4.1).

#### *Plant metabolism*

Root ATP concentrations and AEC ratios depended upon pretreatment and treatment effects (Table 4.2). The treatment effect of increased soil waterlogging led to significant reductions in root ATP concentrations and AEC ratios (Figures 4.2-4.3). For the pretreatment, preflooded plant roots (that had greater root aerenchyma volume) contained greater ATP and AEC levels than predrained roots after the treatment period (ATP: 3.25 and 4.88  $\mu\text{moles/g dry wt}$ , LSD 0.05 = 0.56; AEC ratio: 0.791 and 0.827, LSD 0.05 = 0.034; respectively). In addition, draining preflooded roots resulted in greater ATP and AEC levels (Table 4.3). The total adenine nucleotide concentrations (ATP + ADP + AMP) and ATP/ADP ratios were calculated, but these results are not reported since they followed trends similar to ATP and AEC levels.

Root ADH activity was calculated on a protein basis and displayed highly significant variation with respect to soil waterlogging treatment (Table 4.2). ADH activity increased more than five-fold in response to both flooding treatments (Figure 4.4). Root protein, which varied significantly with all effects in the model (Table 4.2), was greatest in

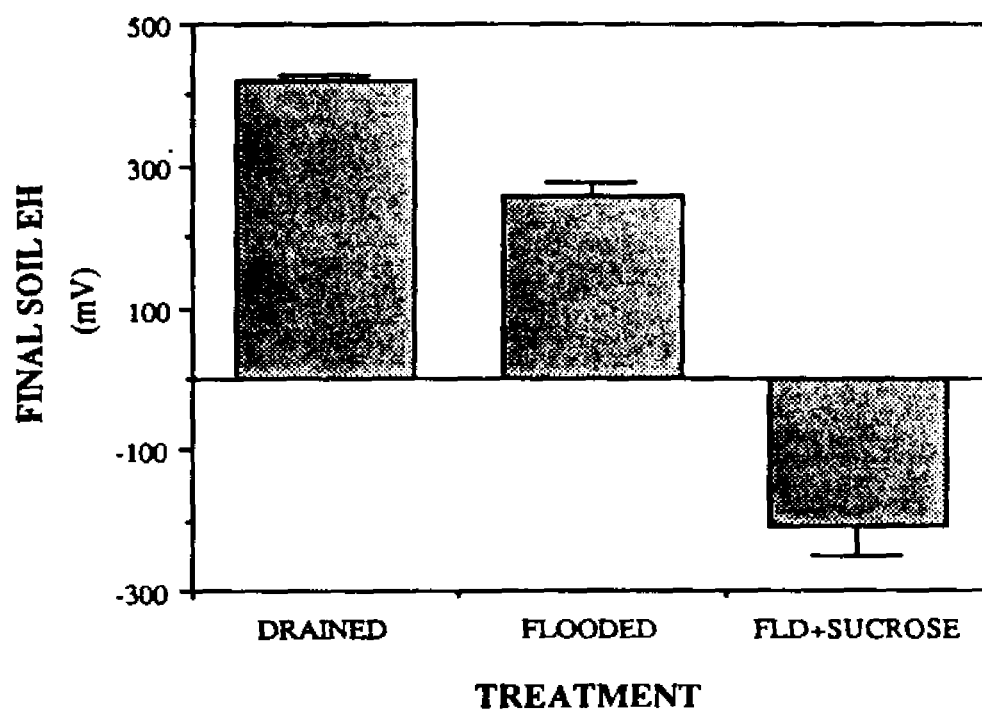


Fig. 4.1. Final soil redox potential (Eh) following drained, flooded, and flooded with 0.2% sucrose solution treatments (mean + standard error, n=12).

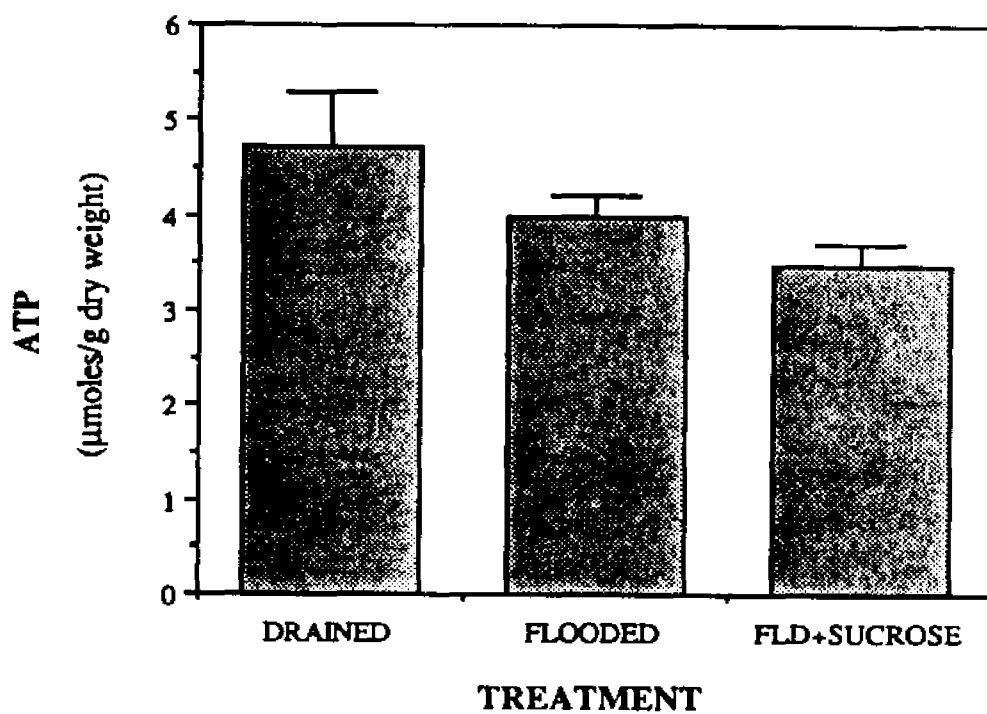


Fig. 4.2. ATP concentration in roots of *Spartina patens* following drained, flooded, and flooded with 0.2% sucrose solution treatments (mean + standard error, n=12).



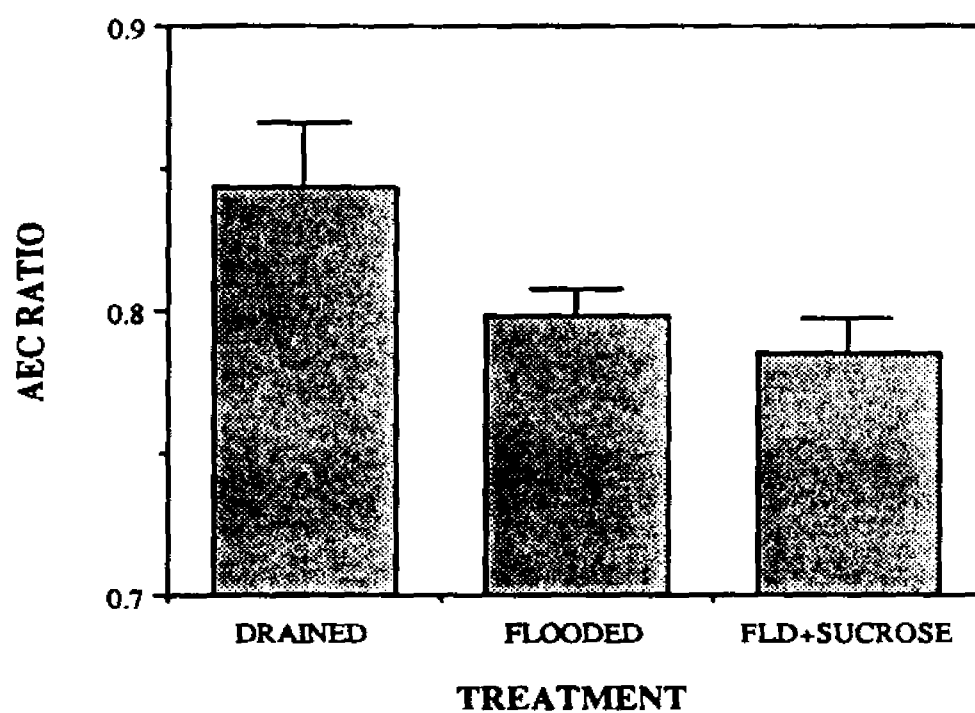


Fig. 4.3. Adenylate energy charge (AEC) ratio in roots of *Spartina patens* following drained, flooded, and flooded with 0.2% sucrose solution treatments (mean + standard error, n=12).

Table 4.3. Effects of drained, flooded, and flooded with sucrose treatments on soil redox potential (Eh), and root metabolic indicators of *Spartina patens* pretreated with flooded soil conditions(n=6). Different letter superscripts denote significantly different means within a variable row (P<0.05).

	PREFLOODED		
	Drained	Flooded	Flooded +Sucrose
Final Eh (mV)	421 <sup>a</sup>	237 <sup>b</sup>	-278 <sup>c</sup>
ATP (μmoles/g dry wt)	6.33 <sup>a</sup>	4.50 <sup>b</sup>	3.81 <sup>b</sup>
AEC Ratio	.887 <sup>a</sup>	.816 <sup>b</sup>	.777 <sup>b</sup>
ADH (μmoles/mg protein/min)	0.70 <sup>b</sup>	2.75 <sup>a</sup>	3.42 <sup>a</sup>
ADH (μmoles/g dry wt /min)	62.5 <sup>b</sup>	216.3 <sup>a</sup>	227.0 <sup>a</sup>
Protein (mg/g dry wt )	89.8 <sup>a</sup>	80.0 <sup>b</sup>	66.3 <sup>c</sup>
Malate (μmoles/g dry wt)	36.7 <sup>a</sup>	41.1 <sup>a</sup>	43.8 <sup>a</sup>

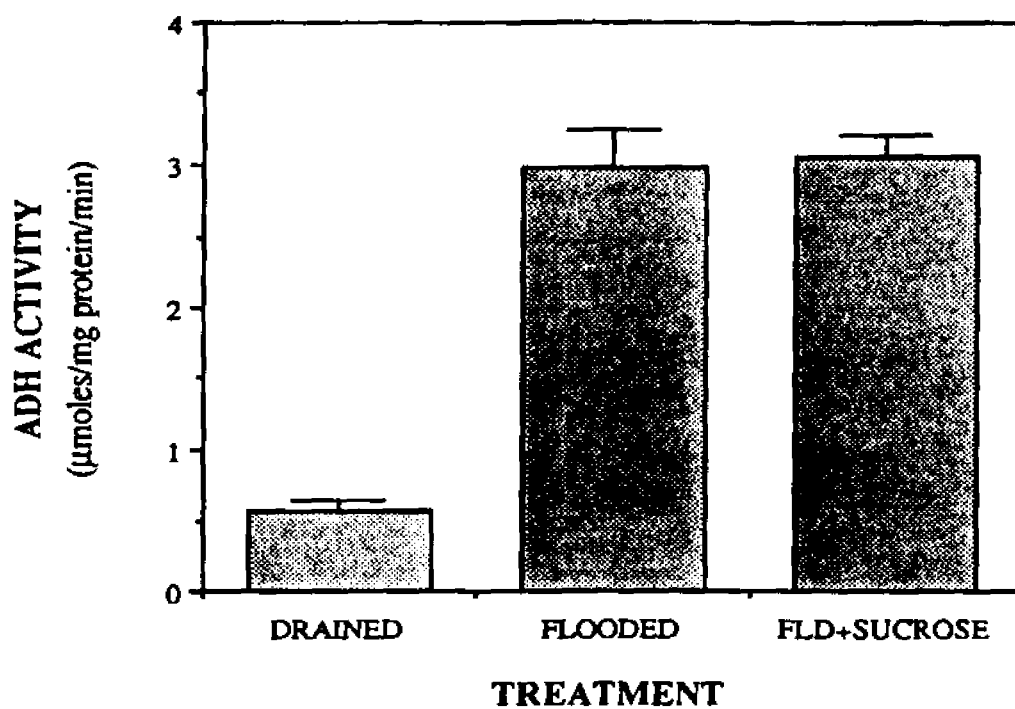


Fig. 4.4. Alcohol dehydrogenase (ADH) activity in roots of *Spartina patens* following drained, flooded, and flooded with 0.2% sucrose solution treatments (mean + standard error, n=12).

preflooded plant roots that were drained, and lowest in roots subjected to severe soil waterlogging (Table 4.3). When ADH was calculated on a dry weight basis, the trends were similar to those of ADH activity calculated on a protein basis. Root malate accumulation depended upon all effects in the model (Table 4.2). The main effect of treatment resulted in greater malate concentrations as soil waterlogging increased (Figure 4.5).

Significant correlations offer further support for the relationship between increased soil waterlogging and the metabolic responses measured: soil Eh and ADH activity:  $r = -.61$ , malate level:  $r = -.67$ , and AEC:  $r = +.34$ . In addition, ADH activity was highly positively correlated to malate concentration ( $r = +.77$ ).

## DISCUSSION

The flooding pretreatment was successful in inducing aerenchyma formation in *Spartina patens* roots, as indicated by the reduced specific gravity of the preflooded roots. In addition, the short-term treatments successfully changed soil Eh (Figure 4.1). Increases in soil waterlogging following three days of flooding treatments resulted in changes in root metabolism as shown by trends of root metabolic indicators across waterlogging treatments (Figures 4.2-5), and correlations between soil Eh and metabolic indicators.

Increases in ADH activity in response to flooding or the onset of anoxic conditions in roots of wetland plants is well established (Smith and ap Rees 1979, Jackson and Drew 1984, Chapter 1), and is thought to support increases in ethanol fermentation under anaerobiosis (Bertani et al. 1980) so that substrate level phosphorylations that produce ATP during glycolysis may continue. The ATP produced under anaerobiosis is reduced

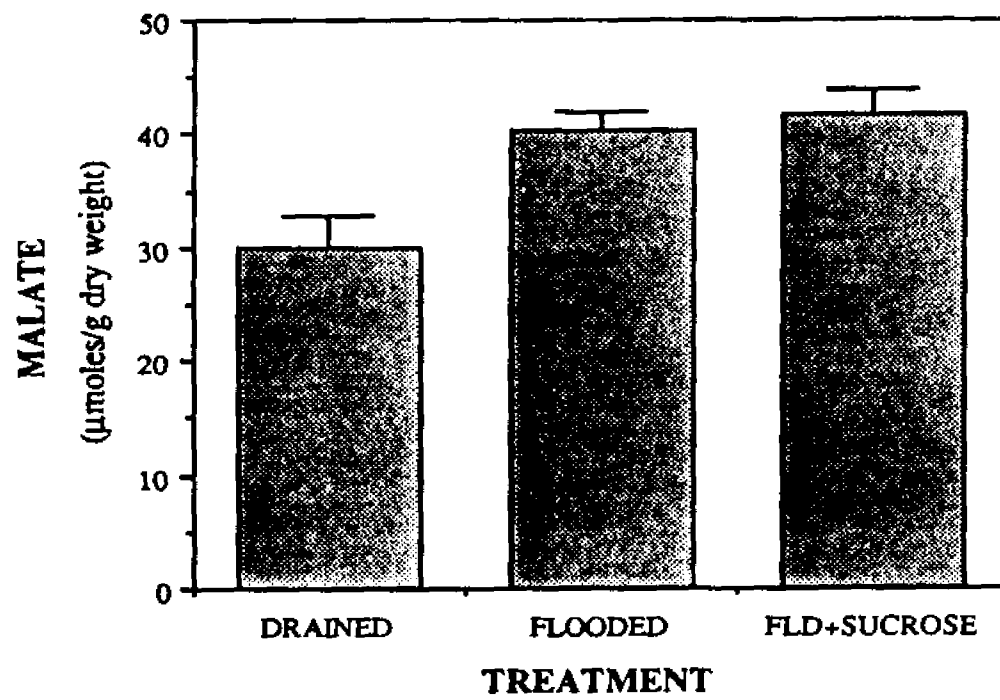


Fig. 4.5. Malate concentration in roots of *Spartina patens* following drained, flooded, and flooded with 0.2% sucrose solution treatments (mean + standard error, n=12).

(Monk and Braendle 1982, Saglio et al. 1983, Tripepi and Mitchell 1984), but is sufficient to maintain cellular integrity and promote root survival under these conditions (Roberts et al. 1984, Saglio et al. 1988).

In contrast, the accumulation of malate in response to increased soil waterlogging has not always been found. Some studies have found increases in malate concentration in wetland plant roots following flooding (Crawford and Tyler 1969, Linhart and Baker 1973), whereas others have not (ap Rees and Wilson 1984), or have found decreases in malate concentration upon transfer to anaerobic media (Smith and ap Rees 1979, Tripepi and Mitchell 1984). At present, there is no evidence that leads to an interpretation of the functional significance of malate accumulation. Nevertheless, this chapter demonstrates that increases in malate concentration in *Spartina patens* roots result from increases in soil waterlogging under controlled conditions. These results reinforce conclusions of field research that related increases in ADH activity and malate concentrations and decreases in AEC ratios in *Spartina* roots to increased soil waterlogging (Mendelssohn et al. 1981, Burdick and Mendelssohn 1987 Chapter 2).

Dramatic changes in root AEC and ADH activity occurred in preflooded roots that were drained for six days prior to harvest. Release from soil waterlogging resulted in a four-fold decrease in ADH activity and the greatest root AEC ratio of any pretreatment-treatment combination. This indicates a cessation of anaerobic fermentation and a return to aerobic respiration in these roots. In addition, the elevated AEC ratio could indicate the occurrence of aerobic metabolism of reduced metabolites which had accumulated during the flooding pretreatment. These findings contradict modeling studies that conclude aerenchyma volumes provide crosssectional areas for oxygen diffusion that are in excess of the aerobic metabolic requirements of the roots (Williams and Barber 1961, Armstrong 1972).

Continued flooding and flooding with sucrose of preflooded plants both led to high root ADH activities and moderate AEC ratios. It appears, therefore, that aerenchyma formation in preflooded roots had not increased root aeration sufficiently to significantly reduce anaerobic metabolism (as measured by ADH activity) or improve the energy status (as indicated by AEC ratios) of the roots in the flooding treatment. Benefits of aerenchyma in reducing anaerobic metabolism in *Spartina patens* (Chapter 5) and in increasing the AEC ratio in *Zea mays* (Drew et al. 1985) have been found. However, root aerenchyma volume reached 50% in the experiments with *Spartina patens* (Chapter 5), and the relatively cool temperature of the growth chamber may have slowed aerenchyma development in the flooded pretreatment which reached 39%.

Roots of predrained plants that remained drained possessed a relatively low AEC ( $0.799 \pm 0.036$ , mean  $\pm$  standard error), compared to that of the preflooded then drained roots ( $0.887 \pm 0.016$ ). This suggests that the predrained plants that remained drained may have been under some type of stress. This and other productivity studies in the greenhouse with *S. patens* (Chapter 5) and *S. alterniflora* (Mendelssohn and Seneca 1980) indicate the drained condition may not be optimal for growth of *Spartina*.

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CHAPTER 5.  
THE RELATIONSHIP BETWEEN ANATOMIC AND METABOLIC RESPONSES  
TO SOIL WATERLOGGING IN THE COASTAL GRASS *SPARTINA PATENS*

ABSTRACT

Flooding responses in *Spartina patens* propagated from plants collected in dune, swale, and marsh habitats were examined in a 63-day time course experiment. Flooding responses were significantly affected by plant source, but the differences were not interpreted to be physiologically significant. Leaf growth rates did not depend upon plant source, suggesting that there were no differences in soil waterlogging tolerance among source populations. Flooding resulted in significant declines in soil redox potential and root specific gravity (indicating increased root aeration). Root alcohol dehydrogenase activity (ADH, a measure of the capacity to ferment ethanol) increased within three days of flooding, then exhibited significant declines as root aeration increased (i.e. as root specific gravity decreased). However, maximal aerenchyma development (50% of the root volume by day 29) reduced, but did not eliminate, hypoxic stress in the roots. When plants that were flooded for 63 days were drained, ADH activity fell to levels equivalent to drained controls. These results support the following inferences: (1) Increases in soil waterlogging are accompanied by dramatic increases in ADH activity. (2) Root metabolic response to soil waterlogging diminishes once increases in root aeration decreases anaerobic metabolism. (3) Under severely reducing soil conditions, root aerenchyma formation (which adjusts to the prevalent degree of soil waterlogging) cannot provide roots with enough oxygen to support aerobic respiration completely .

## INTRODUCTION

With flooding and the onset of anoxic conditions in plant roots, survival is dependent upon establishment of an internal oxygen supply through the root system that can support aerobic respiration and detoxify reduced soil phytotoxins (Armstrong 1979). Increased root aeration through the development of aerenchyma has long been considered one of the primary adaptations of wetland plants to overcome anaerobic conditions associated with flooded soils (Sifton 1945, Iverson 1949, Armstrong 1979). Increases in growth and survival have been associated with aerenchyma that was formed in response to flooding of the rooting media (Das and Jat 1977, Yu et al. 1969, Schat 1984). Studies on aerenchyma development and oxygen transport suggest that several weeks or even months are required to complete these anatomical modifications to the roots (Das and Jat 1977, Keeley and Franz 1979, Chapter 3). In the interim, generation of root energy through anaerobic metabolism appears essential for root survival (Webb and Armstrong 1983, Roberts et al. 1984, Saglio et al. 1988).

Under flooded conditions, increases in aerobic respiration and decreases in anaerobic metabolism in flood tolerant plants have been attributed to greater aerenchyma formation in plants with greater flood tolerance, as compared to their flood-intolerant counterparts (Lambers et al. 1978, Keeley 1979). Unfortunately, previous studies did not determine if these changes were correlated with root aerenchyma development. Likewise, aerenchyma formation was invoked to explain decreases in alcohol dehydrogenase activity (ADH, an indicator of anaerobic metabolism) in the roots of rice that had been flooded over two weeks (Pedrazzini and McKee 1981). The hypothesis that increases in aerenchyma can support increased aerobic root metabolism has been suggested by Luxmore and Stolzy (1972) and confirmed in corn (Drew et al. 1985). In addition, aerobic metabolism in roots of rice was shown to decline after they were placed in anaerobic systems and the aerenchymatous paths for oxygen diffusion were flooded or sealed (Saglio et al. 1983,

ap Rees and Wilson 1984). However, no studies have been conducted that relate root aerenchyma development to changes in anaerobic metabolism. Consequently, time course experiments were conducted using *Spartina patens*, the dominant macrophyte of coastal wetlands in Louisiana, to determine if increases in aerenchyma volume were accompanied by declines in anaerobic metabolism (as indicated by ADH activity) in flooded roots. In addition, since *S. patens* is a dominant grass in a number of coastal habitats (dune, swale and marsh) which differ in ambient soil moisture, flooding responses of plants propagated in a common environment over four years were compared to determine if there were genetic differences with respect to aerenchyma formation (specific gravity), metabolic response (ADH activity), or tolerance to soil waterlogging (leaf growth) among these population sources.

## MATERIALS AND METHODS

### *Flooding experiment*

Clones of *Spartina patens* collected from dune, swale, and marsh field populations were grown in a common environment and repropagated over four years to remove any phenotypic variation between them. Ten to 12 culms from each source population were planted in 45, 2 L plastic pots with 10% (by weight) marsh sediment in a mixture of coarse to fine sand. These pots were well drained. Plants were allowed to acclimate to greenhouse conditions for two weeks prior to initiation of the experiment. Plants were fertilized with a modified Hoaglands solution (Goss 1973) which had 10 mM nitrogen supplied as  $\text{NH}_4\text{NO}_3$  on days -14, -6, 0, 16 and 32. Drained plants were watered when the surface of the soil appeared dry: on days -8, 2, 4, 7, 10, 21, 24, and 28. Thereafter they were watered every six days: day 39, 45, 51, and 57. On day 0, plants were flooded by placing the 2 L pots in 4 L buckets containing 1.7 L tap water. Water was

added to flooded plants as often as required to keep the flooding level about 2 cm over the soil surface. Soil and plants were destructively harvested on days 0 (8 July), 3, 5, 9, 16, 29, 44, and 63 (9 September, 1987). Three pots with plants from each population source were sampled for: number of culms; soil redox potential (Eh), nitrogen, and moisture; root specific gravity, and root ADH activity for each condition (drained and flooded) on each day of sampling (day 0 had no flooded treatment). Leaf length was measured every six days on all pots throughout the flooding experiment to determine leaf growth rates.

Soil Eh was measured at a 10 cm depth within 20 hours prior to harvests. Brightened platinum electrodes were checked for accuracy in pH 4 buffer with quinhydrone before use. The potential of the calomel reference electrode (-244mV) was subtracted from the readings to calculate Eh. Soil moisture was determined by gravimetric analysis and is reported on a wet weight basis. Nitrate, nitrite, and ammonia were measured with a Technicon Auto Analyzer from a 1:1 (w/v) soil to 2N KCl extraction following one hour of shaking and filtration through Whatman #2 Ashless paper (as in Burdick and Mendelssohn 1987 Chapter 2).

Roots were washed of soil and two representative samples of living roots from each pot were collected. One sample was wrapped in a damp paper towel, placed in a plastic bag and kept on ice until specific gravity measurements were made using a 25 ml pycnometer within 24 hours (as in Chapter 3). Roots in the second sample were cut into 2 cm segments, placed in plastic bags with ca. 20 ml of distilled water and frozen in liquid nitrogen. These samples were removed from the liquid nitrogen and freeze-dried. The dried roots were then ground in Wiley mill (40 mesh screen) and 0.025 g were extracted in ice-cold 0.1 M Tris buffer, pH 7.4 by vortexing 30 sec. After 30 min on ice, samples were centrifuged at 20,000 gravities for 20 min at 2-4°C. ADH activity was quantified using the method of John and Greenway (1976) by spectrophotometrically measuring the oxidation of NADH at 340 nm. The reaction was linear with time and

concentration of root extract. Recoveries of internal standards averaged  $98.7 \pm 9.2\%$  for drained and  $97.9 \pm 10.0\%$  for flooded root tissue ( $n=13$ ). Protein concentration was measured in root extracts from days 9 and 63 using a modification of the Lowery method (Total Protein, Direct Method P-5656, Sigma).

Treatments were assigned randomly and placed in a 4 by 34 matrix in a greenhouse. This completely randomized design had a factorial treatment arrangement (three Populations by two Conditions by seven sampling Days) with three replicates. In addition, a set of three replicates from each Population were harvested on Day 0 as a drained control for figure presentations, but was not included in the statistical analysis. Analysis of variance was performed with appropriate tests for fixed (Population, Condition) and random (Day) effects (SAS Institute, Inc. 1985). A log transform was performed on soil nitrogen concentration and root ADH activity to reduce non-homogeneity of the error variance. Protected least significant differences between means of treatment combinations were reported at the 0.05 level of significance if the effect was found to be significant. All correlation coefficients reported were significant at the 0.05 level.

#### *Draining experiment*

Plants from the marsh population were repropagated in a common environment over three years. Eight culms from these clones were planted in 20 pots and allowed to acclimate 40 days before half were flooded for 63 days, as in the flooding experiment. Plant roots from five flooded and drained pots were harvested on Day 63 for specific gravity and ADH activity measurements. The five remaining flooded pots were removed from the flooding buckets and allowed to drain for 21 days, whereupon roots from these and the five drained pots were harvested as on Day 63. Condition and harvest Day were factorially arranged in a completely randomized design, with differences between means reported at the 0.05 probability level.

## RESULTS

### *Soil Conditions*

Soil Eh was highly significantly affected by Condition (flooded or drained) and the interaction of Condition by Day (Table 5.1). In flooded pots, Eh fell sharply after Day 3, reaching moderately reduced levels by day 16 and highly reduced levels by Day 29 (Figure 5.1). Eh initially increased in drained pots, reaching a maximum of +632 mV on Day 9, then gradually decreased to levels similar to those at the beginning of the experiment. Soil moisture was inversely correlated with Eh ( $r=-.62$ ), with greater levels in flooded pots (16.8%) than drained pots (7.7%).

Soil nitrogen depended upon Population, Day and the Condition by Day interaction (Table 5.1). Inorganic nitrogen was greatest in the soil of dune and swale plants and least in the soil of marsh clones (Table 5.2), suggesting plants of the marsh Population took up more nitrogen from the soil than plants of dune and swale Population sources. Through Day 5, drained soil contained more nitrogen than flooded soil, probably because flooding diluted the nitrogen content of the soil (Figure 5.2). In both treatments soil nitrogen fell rapidly until Day 29 (from 2.9-4.6 g/m<sup>3</sup> on Day 3 to 0.5-1.0 g/m<sup>3</sup> on Day 29). The lowest levels were found on Day 63 because the plants had not been fertilized for 31 days.

### *Plant response*

The rate of leaf growth was dependent on Condition and Day but not their interaction (Table 5.1). This indicates flooded and drained growth rates were different, but they behaved similarly over the course of the experiment. Overall, plants in the flooded treatment exhibited slightly greater leaf growth rates than the drained plants (2.13 vs 1.80 cm/day,  $LSD_{0.05}=0.19$ ). Growth rates were relatively high through the first 9 days of



Table 5.1. Analysis of variance of the Flooding experiment for soil redox potential (Eh; sum of squares  $\times 10^{-5}$ ), log of soil nitrogen concentration, leaf growth rate, root specific gravity (sum of squares  $\times 10^2$ ), and log of root alcohol dehydrogenase (ADH) activity in *Spartina patens*.

SOURCE	Degrees of Freedom	Sum of Squares	F Value	Probability of a Greater F
Soil Eh $R^2=0.890$				
Population	2	0.456	1.53	.2558
Condition	1	40.047	28.52	.0018
Day	6	7.452	0.88	.5574
Pop X Cond	2	0.029	0.38	.6911
Pop X Day	12	1.786	3.96	.0122
Cond X Day	6	8.426	37.35	.0001
Pop X Cond X Day	12	0.451	0.43	.9453
Error	84	7.276		
Soil Nitrogen $R^2 = .710$				
Population	2	0.685	8.02	.0061
Condition	1	0.333	1.81	.2266
Day	6	15.344	13.94	.0027
Pop X Cond	2	0.013	0.12	.8847
Pop X Day	12	0.513	0.80	.6509
Cond X Day	6	1.101	3.42	.0333
Pop X Cond X Day	12	0.644	0.59	.8415
Error	84	7.597		
Leaf Growth Rate $R^2 = .781$				
Population	2	0.557	1.42	.2792
Condition	1	3.368	6.48	.0438
Day	6	71.753	23.00	.0007
Pop X Cond	2	0.069	0.06	.9401
Pop X Day	12	2.352	0.35	.9589
Cond X Day	6	3.119	0.93	.5070
Pop X Cond X Day	12	6.698	1.90	.0454
Error	84	24.643		
Root Specific Gravity $R^2 = .893$				
Population	2	2.266	4.92	.0275
Condition	1	50.085	17.94	.0055
Day	6	90.963	5.43	.0294
Pop X Cond	2	0.557	1.10	.3632
Pop X Day	12	2.763	0.91	.5622
Cond X Day	6	16.747	11.05	.0003
Pop X Cond X Day	12	3.031	1.06	.4040
Error	84	20.014		
Root ADH Activity $R^2 = .945$				
Population	2	0.180	4.76	.0301
Condition	1	13.817	156.04	.0001
Day	6	3.768	7.09	.0155
Pop X Cond	2	0.027	0.82	.4628
Pop X Day	12	0.227	1.17	.3956
Cond X Day	6	0.531	5.47	.0061
Pop X Cond X Day	12	0.194	1.10	.3731
Error	77	1.134		

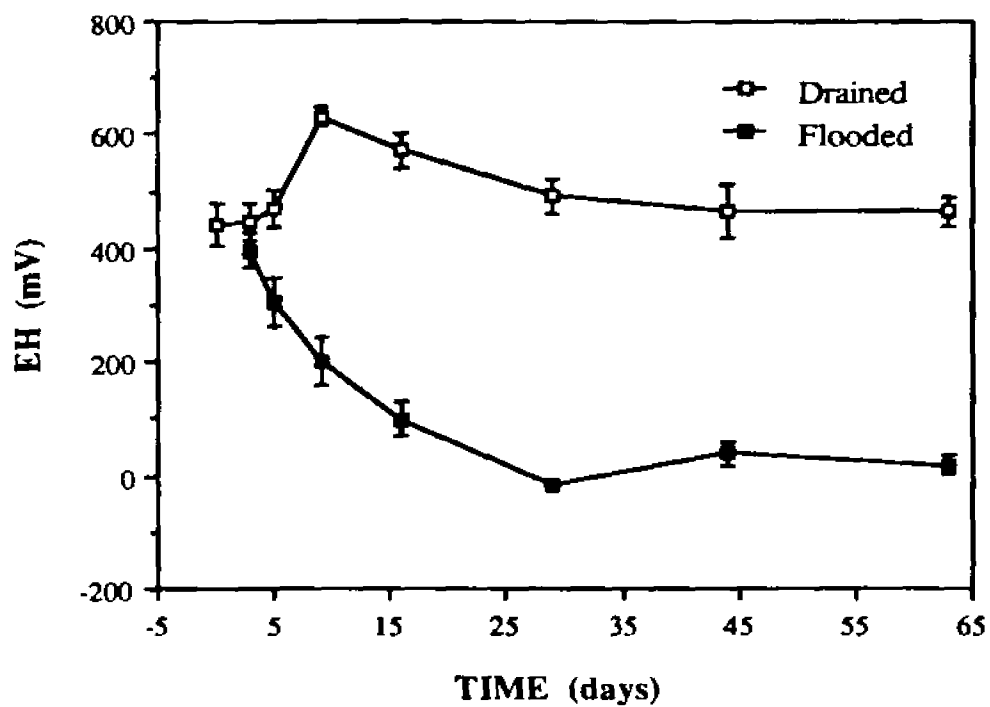


Fig. 5.1. Soil redox potential (Eh) of drained and flooded treatments over a time course of 63 days in the Flooding experiment (mean  $\pm$  standard error,  $n = 9$ ).

Table 5.2. Source population effect on soil nitrogen, leaf growth rate, root specific gravity, and root alcohol dehydrogenase (ADH) activity in *Spartina patens* for flooded plants in Flooding experiment. Different letter superscripts denote significantly different population source means (n = 21) using protected least significant differences ( $P < 0.05$ ).

	POPULATION SOURCE		
	Dune	Swale	Marsh
Nitrogen (g/m <sup>3</sup> )	1.529a	1.545a	0.839b
Growth rate (cm/day)	2.16a	2.17a	2.05a
Specific gravity	.601b	.630ab	.634a
ADH activity (μmol/g dry wt/h)	9658b	12053a	12777a

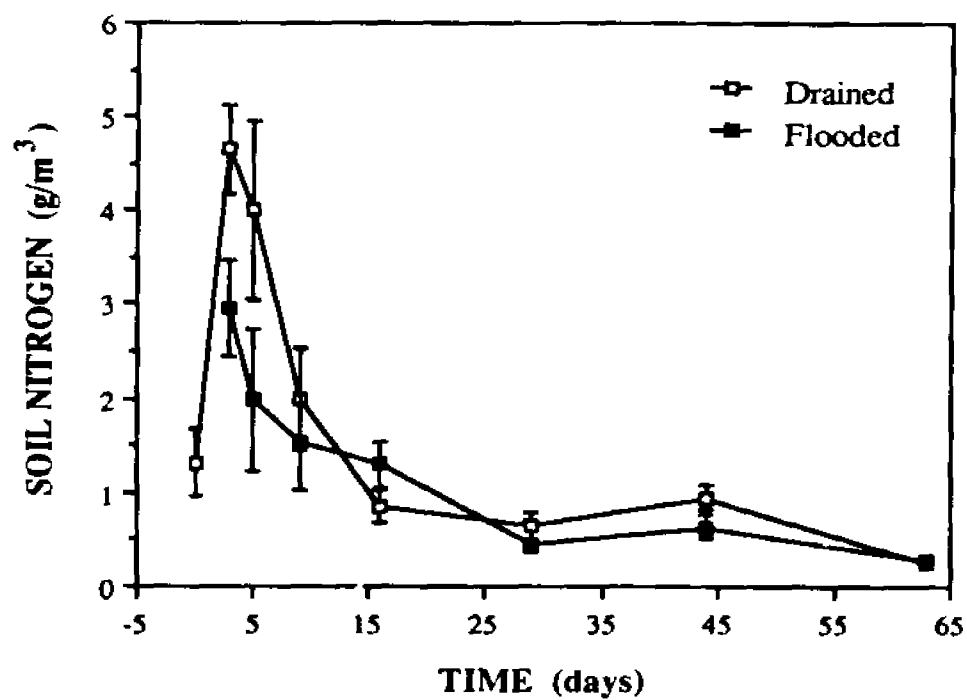


Fig. 5.2. Soil nitrogen (nitrate-nitrite plus ammonia) concentration of drained and flooded treatments over a time course of 63 days in the Flooding experiment (mean  $\pm$  standard error,  $n = 9$ ).

the experiment, then decreased to low values by the final harvest (Figure 5.3) The three-way interaction was significant, but was not interpretable.

By Day 63, the number of culms per pot had increased from 10 to 12 at the time of planting to an average of about 22. An ANOVA of culm number at harvest revealed a Condition effect that was significant at the 0.10 level ( $P=0.0739$ ), indicating there were slightly more culms under drained (17.9) than flooded (16.8) conditions ( $LSD_{0.05}=1.0$ ).

Condition, Day and their interaction were significant effects in the ANOVA for root specific gravity (Table 5.1). Flooding and the decline of soil Eh were accompanied by a rapid fall in root specific gravity through Day 29 (Figure 5.4). The fractional volume of aerenchyma in roots (porosity) is directly related to root specific gravity as detailed in Chapter 3. Thus, aerenchyma volume increased dramatically in response to the root hypoxia that accompanied flooding.

*S. patens* may have even been decreasing specific gravity in response to hypoxia in drained roots. Specific gravity in drained roots declined significantly by Day 29 (Figure 5.4). Therefore, the watering frequency of drained plants was decreased (through Day 32 the average period between watering was 3.2 days, while after Day 32 it was increased to 6.2 days) to halt further decreases in specific gravity, and by Day 63 root specific gravity had increased to earlier levels (Figure 5.4). Root specific gravity was also affected by Population source (Table 5.1). In the flooded treatments, dune clones had slightly lower root specific gravity (thus root aeration was greater) than roots of marsh clones (Table 5.2).

The analysis of variance indicated that ADH activity was significantly affected by Population, Condition, Day and Condition by Day effects (Table 5.1). Activity of ADH, which is a measure of the capacity for alcoholic fermentation, was greater in roots of marsh and swale clones than in roots of dune clones (Table 5.2). By Day 3, root ADH activity was about eight times greater in flooded than drained roots (Figure 5.5).

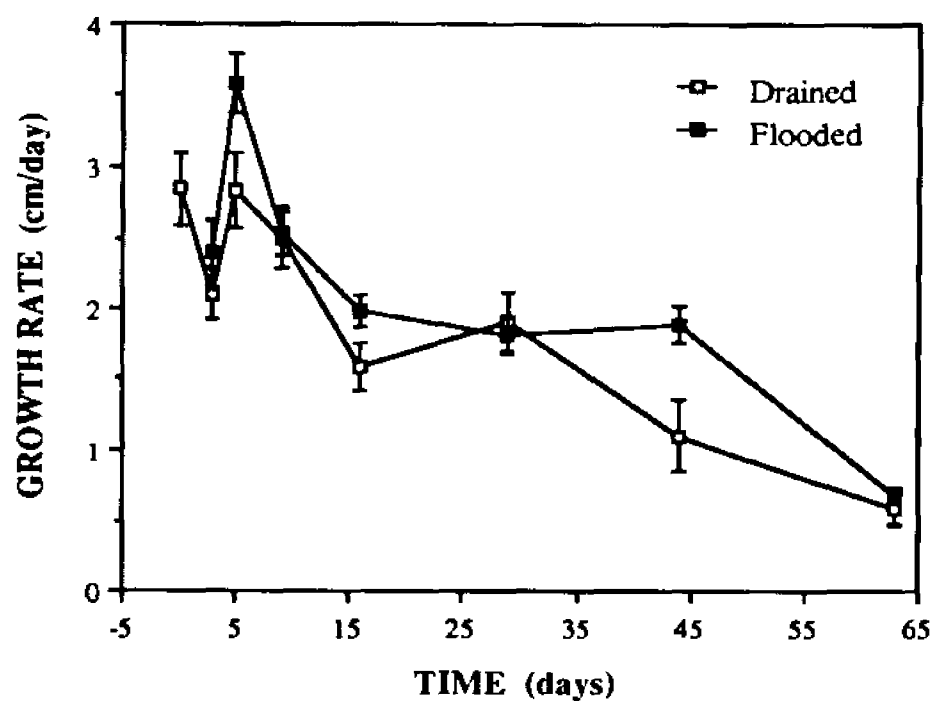


Fig. 5.3. Leaf growth rate of *Spartina patens* during the 4 to 6 days prior to harvest in drained and flooded treatments over a time course of 63 days in the Flooding experiment (mean  $\pm$  standard error,  $n = 9$ ).

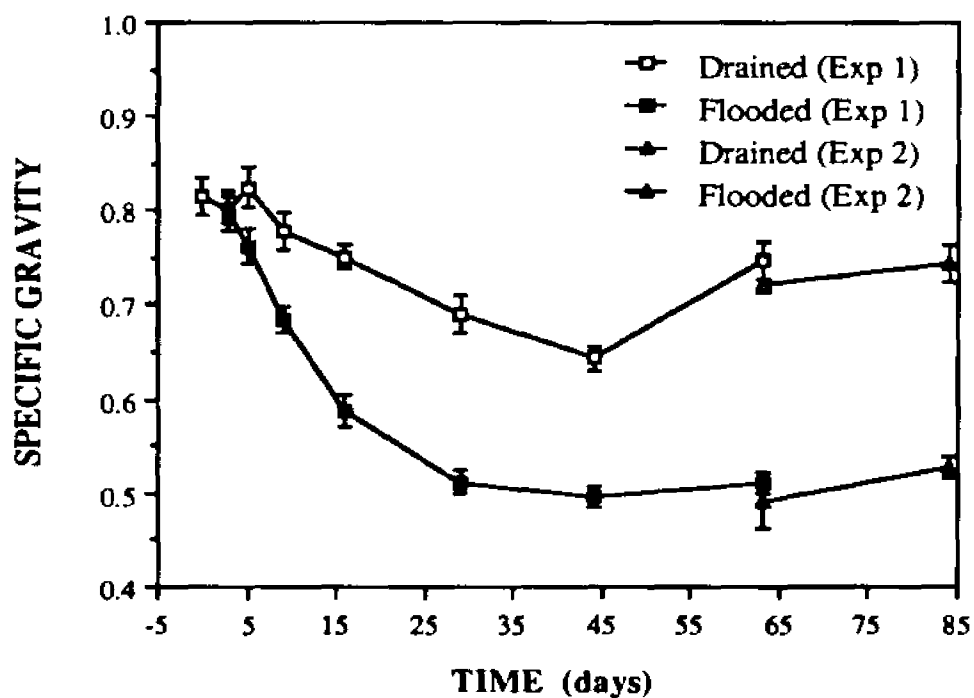


Fig. 5.4. Specific gravity of roots of *Spartina patens* during two time course experiments. In the Flooding experiment, drained and flooded conditions were maintained for 63 days (mean  $\pm$  standard error,  $n = 9$ ). The Draining experiment had flooded and drained treatments for 63 days, after which the plants assigned to the flooding treatment were allowed to drain for 21 days ( $n = 5$ ).

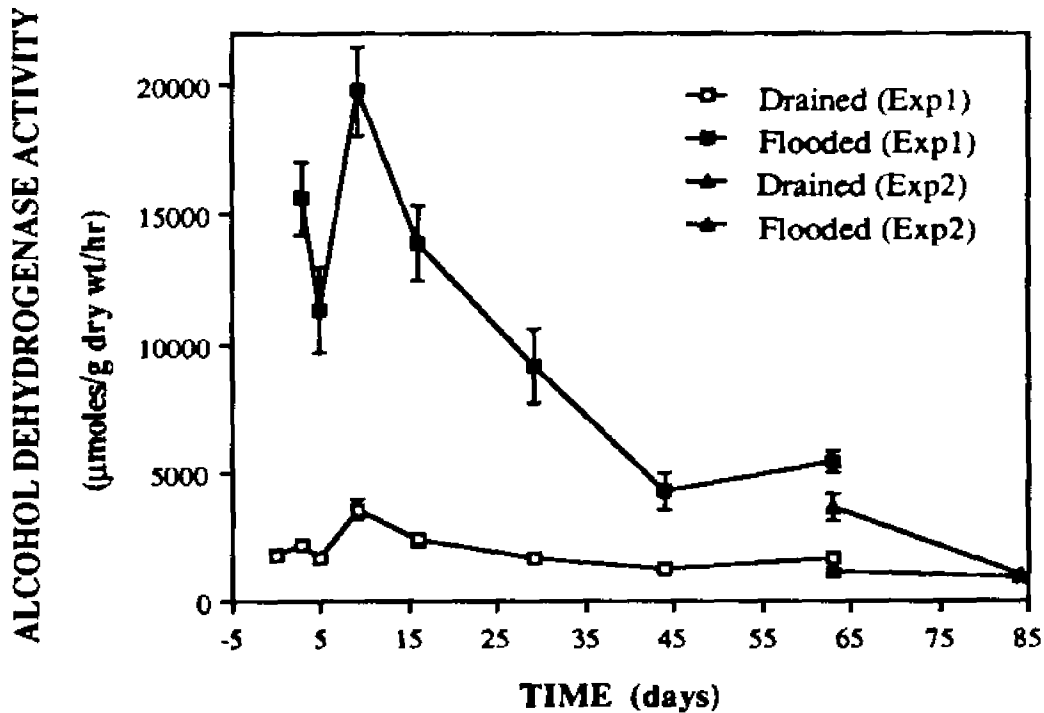


Fig. 5.5. Alcohol dehydrogenase (ADH) activity in roots of *Spartina patens* during two time course experiments. In the Flooding experiment, drained and flooded conditions were maintained for 63 days (mean  $\pm$  standard error,  $n = 9$ ). The Draining experiment had flooded and drained treatments for 63 days, after which the plants assigned to the flooding treatment were allowed to drain for 21 days ( $n = 5$ ).



It remained high and variable in flooded roots through Day 16, then decreased to levels two to three-fold greater than those of drained plants by Day 44 and showed no appreciable change at the final harvest (Figure 5.5). In the Draining experiment, roots that had been previously flooded and then were allowed to drain exhibited ADH activities equivalent to those of continuously drained roots. Root ADH activity in drained controls remained at low levels throughout both the experiments. Protein levels were only slightly (7%) greater in drained (26.8 mg/g dry wt) than flooded (25.1 mg/g dry wt) root extracts and declined between the two samplings Days (9 and 63) by only 8%. Thus, differences in root protein are not likely to have influenced the differences found in root ADH activity.

## DISCUSSION

Flooding of *Spartina patens* led to reduction of soil redox potential (Figure 5.1), decreased root specific gravity, and increased ADH activity (Figure 5.5). By Day 29, root aerenchyma had increased 35% and was accompanied by a 42% reduction in ADH activity from Day 3 of flooding. Thus, it appears that increased root aeration ameliorated root hypoxia and decreased reliance on anaerobic metabolism. ADH activity and specific gravity were positively correlated in both drained ( $r=+0.34$ ) and flooded ( $r=+0.60$ ) roots, further associating reductions in the capacity for anaerobic metabolism with increases in root aerenchyma.

Decreases in root specific gravity due to flooding has been previously demonstrated in *S. patens* (Chapter 3) and other wetland species (Schat 1984). Low nitrogen (Konings and Verschuren 1980) and low nutrients in general (Smirnoff and Crawford 1983) have also been shown to increase aerenchyma volume. In this study, it is unlikely that

decreases in root specific gravity were due to low levels of soil nutrients, since the lowest nitrogen levels and the longest interval without fertilization coincided with increases in root specific gravity in drained plants (Day 63, Figures 5.2 and 5.4).

The relationship between root anaerobiosis and stimulation of ADH activity is well established (Smith and ap Rees 1979, Davies 1980, Hook 1984, Roberts et al. 1984). Ethanol catalysis by ADH allows continued glycolysis and production of ATP (required to maintain cellular integrity) through a substrate level phosphorylation during anoxia (Roberts et al. 1984). In this study, ADH activity increased dramatically in the roots then declined gradually as root aeration increased. Since flooded *S. patens* plants continued to show growth rates equal to or greater than those of the drained controls, decreases in root ADH activity in flooded plants could not be attributed to root deterioration or exhaustion of the energy supply from the shoots as has been surmised for many species' responses to continued flooding or anaerobiosis (Keeley 1979, Saglio et al. 1980, Schat 1984, Tripeppi and Mitchel 1984).

It is likely that the maximal amount of aerenchyma (ca. 50%) had been formed in flooded roots by Day 29. However, ADH activity on Day 63 was still greater in flooded than in drained roots in both experiments. Therefore, it appears that the maximum amount of aeration in *S. patens* was not sufficient to provide the root system with enough oxygen to completely support aerobic respiration.

Some controversy exists in the literature in regard to whether anatomic or metabolic responses to flooding are of the greatest importance to plant survival and competitive ability (Armstrong 1979, Tripepi and Mitchell 1984). Of course, different life forms may rely on these two classes of adaptations to varying extents (*Oryza*: Armstrong 1971; River Birch: Tripepi and Mitchell 1984; *Salicornia*: Pearson and Havill 1988). The data presented here agree with earlier suggestions (Keeley 1979, Burdick and Mendelssohn 1987 Chapter 2) that over long-term waterlogging, anatomical adaptations prevail, but are

often incomplete. In contrast, metabolic responses are primarily associated with root hypoxia that accompanies short-term soil waterlogging. Recent evidence from a study comparing flooding responses in five freshwater species supports this view (McKee et al. 1988). In fact, McManmon and Crawford's metabolic theory of flooding tolerance (1971) is partly based on observations of flood tolerant species that exhibit reductions in ADH activity after one month of flooding in sand culture, while flood-intolerant species did not exhibit these decreases. Originally interpreted by Crawford and coworkers as under metabolic control, the reduction in ADH activity may have been due to significant aerenchyma formation in the flood tolerant species, while negligible aerenchyma may have been formed in the less flood tolerant species, as found by others (Schat 1984, McKee et al. 1988). This observation is not new (Keeley 1979), but the data presented here are the first that closely associate increased root aerenchyma with declines in ADH activity over time. In flooded *Spartina patens*, decreases in root specific gravity within one month substantially reduced root capacity for alcoholic fermentation, as indicated by ADH activity.

Soil waterlogging tolerance in *Spartina patens* that was grown from three source populations and repropagated over four years in a common environment exhibited no differences with respect to Population source since leaf growth rates did not differ among Populations. However, there were differences in flooding responses among Populations. Specifically, flooded plant roots from the marsh Population had greater specific gravity (5%) and ADH activity (32%) than roots from the dune Population. Population source differences in root specific gravity were slight, and those in root ADH activity are unlikely to be physiologically significant (Justin Roberts, personal communication). In addition, marsh clones removed more inorganic nitrogen from the soil than dune or swale clones.

Populations of the same species often exhibit ecotypic differentiation across environmental gradients. Populations of *S. patens* from the barrier islands of North Carolina show differences in response with respect to drought, nutrient deficiency, and salinity tolerance (Silander and Antonovics 1979). Ahmad and Wainwright (1974) found differences in *Agrostis* populations (dune and inland) with respect to salinity and more importantly for this discussion, waterlogging or flooding stress. Keeley (1979) found differential flood tolerance in populations of *Nyssa sylvatica* (a flood tolerant tree) from swamp, floodplain and upland habitats. The differences among population sources of *Spartina patens* found in this study suggest marsh plants may (1) be able to take up more soil nitrogen; and (2) possess greater capacity for ethanol fermentation under flooded conditions, than dune plants. These differences may be of survival value under conditions of soil waterlogging, but recall that no differences in leaf growth rates were found between flooded marsh and dune plants.

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## SYNTHESIS AND CONCLUSIONS

Soil waterlogging poses problems of oxygen deficiency for the roots of emergent wetland plants. Root oxygen deficiency in wetland plants may impair root function through its effect on metabolism, nutrient uptake, hormone balance and rhizosphere development (Chapter 1). Results from field work on *Spartina patens* (Chapter 2) showed that as soil waterlogging increases from dune to swale to marsh habitats, root aerenchyma volume increased in the respective populations of *S. patens* inhabiting these environments. In the marsh habitat where the maximum development of aerenchyma volume appears to have been reached, alcohol dehydrogenase (ADH) activity was high, indicating anaerobic ethanol fermentation was an important component of root metabolism. Furthermore, short-term temporal increases in soil waterlogging were accompanied by metabolic changes resulting in malate and lactate accumulation in roots of populations from all three habitats. Thus the following conclusions were drawn from the Chapter 2 field study: (1) Aerenchyma volume is inducible in *S. patens* and develops in response to the ambient soil waterlogging conditions. (2) In the roots of the marsh population, aerenchyma volume reached a maximum, but was still insufficient to fully support aerobic respiration. (3) Roots responded to episodes of relatively greater soil waterlogging on a metabolic level. (4) Aerenchyma development and ADH activity differed among populations, but it could not be determined whether these differences were under environmental or genetic control.

An experiment using plants that had been repropagated and grown for four years in a common environment was conducted to determine if there were genetic differences in soil waterlogging responses among population sources (Chapter 5). This study indicated



genetic differences existed, with roots of plants repropagated from the marsh population having slightly less root aerenchyma and greater ADH activity than those of the dune population. The physiological significance of these differences is doubtful, however, since there were no differences in leaf growth rates (hence no differences in flooding tolerance) among source populations.

To determine if changes in anatomy and metabolism that were observed in *Spartina patens* roots in the field were truly responses to soil waterlogging, two experiments were conducted. An experiment examining aerenchyma development demonstrated that flooding could stimulate aerenchyma formation in new as well as preexisting roots (Chapter 3). The second experiment (Chapter 4) focused on root metabolism. Results indicated that the adenylate energy charge decreased, while ADH activity and malate concentration increased with increases in soil waterlogging (Chapter 4). Thus, the anatomic and metabolic responses that were associated with soil waterlogging in the field were confirmed under experimental conditions.

The benefit and limitation of increased aerenchyma in reducing root reliance on anaerobic fermentations in *S. patens* was demonstrated in two time course experiments (Chapter 5). Initially, ADH activity increased dramatically in flooded roots, but then declined following the development of aerenchyma which increased root aeration. Aerenchyma development had reached a maximum (50% of the root volume) by Day 29. Nevertheless, after 63 days the ADH activity of flooded roots was still elevated compared to controls, suggesting that anaerobic metabolism was still important. When plants that were flooded for 63 days were drained, ADH activity fell to levels equivalent to drained controls. (Chapter 5).

I conclude that the anatomical adaptation of aerenchyma development in roots of *Spartina patens* is of great importance in maintaining aerobic respiration. However, relief of root oxygen deficiency is incomplete in environments where temporal increases in soil

waterlogging occur at a frequency and duration incompatible with maintenance of appropriate aerenchyma volume, or where the degree of soil waterlogging is severe. Moderate to severe soil waterlogging conditions resulted in root oxygen deficiencies in the presence of maximal amounts of aerenchyma (ca. 50%), as indicated by elevated ADH activities as compared to drained plants (Chapters 2 and 5).

Since the avoidance strategy of aerenchyma development cannot always supply enough oxygen to fulfill the aerobic requirements of *Spartina patens* roots, metabolic adaptations may be essential for this species in situations of severe soil waterlogging or rapid decreases in soil redox potential. Plant roots may survive anaerobiosis if: (1) a source of reduced carbon (i.e. glucose) is available for glycolysis, (2) the metabolic machinery exists (or can be made rapidly) to generate ATP via substrate level phosphorylations, (3) the redox potential of the cell is maintained, and (4) NADH generated in glycolysis is reoxidized in order to recycle  $\text{NAD}^+$  for continued glycolysis (Hochachka 1980). Biochemical modifications in root metabolism classified as either compensatory or exploitative strategies may fulfill the last three conditions if the first is met. *Spartina patens* appears to favor ethanol fermentation as the final glycolytic step under anaerobiosis, representing the compensatory strategy. This is indicated by increased ADH activity under flooded conditions (Chapters 4 and 5). The substrate level phosphorylation produced in this pathway results in a moderate adenylate energy charge ratio, which appears to support root survival under conditions of soil waterlogging (Burdick and Mendelssohn 1987 Chapter 2, Chapter 4). Recent studies cite the beneficial effects associated with alcoholic fermentation in roots stressed by anoxia (Roberts et al. 1984, Saglio et al. 1988).

Use of adaptations that are characteristic of the compensatory strategy by *S. patens* does not necessarily exclude the occurrence of other metabolic changes that may represent an exploitative strategy. Davies (1980) proposed that alcohol fermentation was stimulated

by decreases in pH brought about by lactate fermentation with the onset of root anaerobiosis. This metabolic control was subsequently confirmed (Roberts et al. 1984, Hoffman et al. 1987), and a pattern of metabolic indicators that supports this hypothesis was observed in *S. patens* in the field research (Burdick and Mendelssohn 1987 Chapter 2). Elevated lactate levels in marsh roots were positively correlated with ADH activity and ethanol accumulation.

In addition to lactate, malate was also found to accumulate in roots under soil waterlogging stress (Burdick and Mendelssohn 1987 Chapter 2, Chapters 4 and 5). McManmon and Crawford (1971) cited this metabolite as an alternate endproduct of glycolysis favored by flood tolerant plants (exploitative strategy), since it is relatively non-toxic as compared to ethanol. If the preferred end product of glycolysis under anoxia was malate in roots of *S. patens*, two problems arise: (1) the accumulation of malate was one to two orders of magnitude smaller than would be expected, and (2) malate fermentation results in no net adenylate phosphorylation (Jackson and Drew 1984). The adaptive significance of malate accumulation in response to soil waterlogging is unclear, and malate accumulation may not represent an exploitative strategy. However, the formation of malate does recycle the  $\text{NAD}^+$  required for further glycolysis (Lance and Rustin 1984, Jackson and Drew 1984). In addition, malate may accumulate in roots experiencing hypoxia in order to recycle  $\text{H}^+$  between cellular compartments (Hochachka 1980), provide a pool of carbon skeletons for amino acid metabolism, or perform a regulatory function (Lance and Rustin 1984) as found for lactate accumulation (Roberts et al. 1984, Hoffman et al. 1987).

In conclusion, *Spartina patens* exhibits responses to soil waterlogging that are characteristic of all three strategic approaches to survival in anaerobic environments. This species dominates the vegetation in several coastal habitats where the severity of soil

waterlogging varies both spatially and temporally. The exceptional ability of *Spartina patens* to be ecologically successful in dune as well as marsh environments may be due to the variety and effectiveness of the soil waterlogging responses that it possesses.

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## VITAE

David M. Burdick was born on December 30, 1954, in Flushing, Queens, New York. Through age 11, I was able to appreciate animals, plants and natural phenomena both at home and on summer vacations to the seashore. A move to Cape Cod, Massachusetts strengthened my affinity with marine life. In 1977, I graduated cum laude from Hobart College in Geneva, New York with a Bachelor of Science degree in Chemistry. I returned to Cape Cod to work on variety of marine investigations at the Provincetown Center for Coastal Studies. To broaden my research experience, I accepted a position in the Environmental Chemistry Laboratory of the National Marine Fisheries Service at Sandy Hook, New Jersey. I came to Louisiana State University supported by an Alumni Federation Fellowship and studied physiological ecology of marsh grasses under the direction of Dr. Irving A. Mendelssohn. Specifically, I investigated waterlogging responses in *Spartina patens*, a dominant grass of coastal Louisiana. In 1987, I was awarded the Joe Lipsey, Sr. Memorial Scholarship. At present, I have accepted a postdoctoral position in the Biology Department at the Woods Hole Oceanographic Institute, Woods Hole, Massachusetts.


# DOCTORAL EXAMINATION AND DISSERTATION REPORT

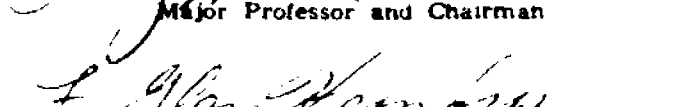
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Major Field: Marine Sciences

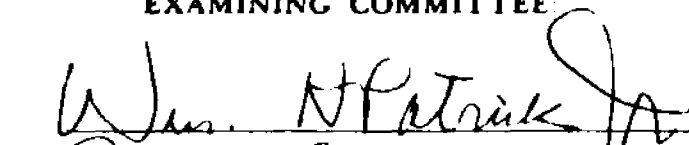
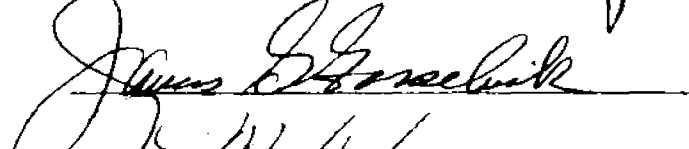
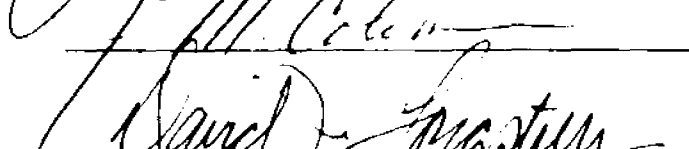
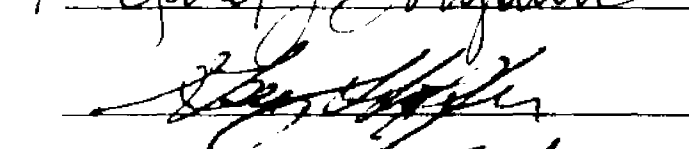


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Approved:

  
Major Professor and Chairman

  
Dean of the Graduate School

## EXAMINING COMMITTEE

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