Anaerobic Substrate Tolerance in Sporobolus virginicus (L.) Kunth

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ANAEROBIC SUBSTRATE TOLERANCE IN SPOROBOLUS VIRGINICUS (L.) KUNTH.[1]

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ABSTRACT

The purpose of this study was to determine if and how the two genetically distinct forms, marsh and dune, of *Sporobolus virginicus* (L.) Kunth. tolerate anaerobic substrates. The treatments in the hydroponic study, conducted in the greenhouse for approximately 6 months, involved growing the marsh and dune forms in aerobic, anaerobic, and alternating aeration treatments. Plants were examined for morphological and physiological responses to the aeration treatments. In response to the continuous anaerobic treatment, the dune form of *S. virginicus* exhibited increased stolon biomass, but no difference of total biomass or rhizome aerenchyma when compared with the aerobic treatment. In response to alternating aeration, rhizome aerenchyma increased, total biomass decreased, and stolon biomass remained constant. Belowground transport of oxygen enabled the root tissue in all of the aeration treatments to maintain aerobic respiration. The marsh form grown in the alternating aeration treatment had the same total biomass but more rhizome aerenchyma when compared to the aerobic treatment. Growth in the continuous anaerobic treatment resulted in a reduction of total biomass and increased rhizome arenchyma. Marsh form roots did not appear to be respiring anaerobically or producing ethanol or additional malate at the time of harvest; however, root respiration was higher in the anaerobic and alternating treatments. The marsh and dune forms of *S. virginicus* were able to adjust morphologically or physiologically or to use existing morphological features to tolerate anaerobic substrates. Thus, it appears that the distribution of the two forms of *S. virginicus* found in coastal sand dunes and in salt marshes is not limited by differences in ability to tolerate waterlogged soils.

SINCE *Sporobolus virginicus* (L.) Kunth., a grass of worldwide distribution in the tropics and sub-tropics, is found in both wetland and nonwetland habitats, its anaerobic substrate tolerance capacity is of interest. A perennial with numerous branching and creeping rhizomes, it typically forms extensive colonies on sandy or muddy seashores and in saline marshes (Hitchcock, 1971). *Sporobolus virginicus* has two genetically distinct growth forms which because of their distributions are designated here as marsh and dune forms. The marsh form is listed as the type specimen of *S. virginicus* by Hitchcock (1971) and is described as var. *minor* Bail. by Smith-White (1979) in Australia. It has blades approximately 5 cm in length, culms 10–40 cm in height, and grows at the fringes of saltpans, in the low and high marsh, and along river banks. The dune form is listed as a robust form of *S. virginicus* by Hitchcock (1971) and is referred to as var. *virginicus* by Smith-White (1979). It has culms up to 1 m in height, longer and broader leaves, and generally occurs on sandy beaches and coastal dunes. Sapelo Island, Georgia (approx. 31°25′N latitude and 81°15′W longitude) is the northern most reported collection site where both forms grow in adjacent habitats in North America (Gallagher, 1979). When raised from seed under identical conditions the two forms retain their divergent morphological characteristics.

The occurrence of both the marsh and dune forms on Sapelo Island provided an opportunity to look for intraspecific differences in the anaerobic substrate tolerance characteristics of *S. virginicus*. Breen, Everson and Rodgers (1977) studied the effects of salinity and inundation on various ages of young marsh form *S. virginicus* plants growing in pots. They found that the young plants are sensitive to inun-

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dation (flooding of pots), but once established they grow well under waterlogged conditions associated with freshwater environments. Increased salinity caused an inhibition of growth as was reported earlier by Gallagher (1974). Thus, *S. virginicus* is not an obligate halophyte, but both forms can tolerate high salinities.

The ability of marsh plants to grow in waterlogged habitats, even though the availability of oxygen is low (Williams and Barber, 1961), has been attributed to a number of morphological and physiological characteristics.

The development of an extensive internal ventilation system is a morphological adjustment which can allow a plant to avoid an anoxic substrate (Greenwood, 1967; Armstrong, 1981). Small continuous intracellular spaces in cortical tissues are characteristic of most roots of terrestrial plants (Armstrong, 1978), whereas much larger gas spaces typically develop in wetland roots in response to soil anaerobiosis. Tissue with large amounts of gas space is often loosely referred to as aerenchyma (Armstrong, 1980). Kawase and Whitmoyer (1980) showed evidence of the initiation of increased aerenchyma development in the cortex of sunflower stems after only 24 hr of waterlogging. The arenchyma system may lower resistance to gas flow (which is chiefly diffusional) and reduce the respiratory demand per unit volume of tissue (Armstrong, 1980). Roots may be able to maintain oxygen concentrations adequate for normal growth since oxygen can diffuse from aerial parts to underground organs (Conway, 1940; Vartapetian, Andrews and Nuridinov, 1978). However, increased oxygen transport capacity of flood tolerant species is not always induced by anaerobiosis (Greenwood 1967; Lambers, Steingrover and Smakman, 1978).

Plants possessing physiological resistance to anoxia are able temporarily to tolerate the absence of molecular oxygen in the environment by means of metabolic adjustments (Vartapetian, 1978). The most studied features of root metabolism in response to the lack of molecular oxygen have been the control of the metabolic rate and the diversification of end products of glycolysis. One reported combination of these responses involves stimulated glycolysis, ethanol production, and ATP synthesis (Boulter, Coult and Henshaw, 1963; Hook, Brown and Kormanik, 1971; Keeley, 1979; Mendelssohn, McKee and Patrick, 1981). Ethanol, a potential phytotoxin, could be lost from the roots by diffusion or remetabolism (Cossins and Beevers, 1963; Barclay and Crawford, 1981; Armstrong, 1981). An alternate response involves the accumulation of malate (via oxaloacetate) as the major end product of anaerobic respiration and simultaneous limitation of anaerobic respiration rates (Crawford and McManmon, 1968; Crawford and Tyler, 1969; Crawford, 1978; Mendelssohn et al., 1981). Thus ethanol production is avoided but there is no net ATP formation. These variations in metabolic responses to anoxia have also been shown to occur intraspecifically in *Senecio vulgaris* L. (Crawford, 1966), *Veronica peregrina* L. (Linhart and Baker, 1973) and *Spartina alterniflora* Loisel. (Mendelssohn et al., 1981).

We studied *Sporobolus virginicus* to determine if both forms could tolerate anaerobic substrates, what tolerance mechanisms are used, and whether or not intraspecific differences exist. Tolerance was defined as the ability to maintain or increase total plant biomass when grown in the anaerobic treatment. In Georgia the dune form is found primarily on well-drained sites on sea island fore dune ridges, while the marsh form is most frequently associated with waterlogged soils in the upper elevation zones of salt marshes. These observations lead us to hypothesize that one of the factors separating the distribution of the forms is a differential response to the aeration conditions in the two habitats. The anaerobic substrate tolerance mechanisms of the two forms were examined by determining the morphological and physiological responses in a hydroponic study. Morphological data included biomass distribution, rhizome aerenchyma development, and physical characteristics of the plants. Physiological data included excised root respiration rates, alcohol dehydrogenase and malate dehydrogenase activities, and ethanol and malate concentrations.

MATERIALS AND METHODS—The study design consisted of two forms of *Sporobolus virginicus,* marsh and dune, and three aeration treatments: aerobic (AE), anaerobic (AN) and alternating (AE/AN). Each aeration treatment contained 17 marsh form plants and 13–15 dune form plants.

Plant material for the study was originally collected from Sapelo Island, by Gallagher, and maintained as potted stock plants in a greenhouse. Several weeks prior to the study, apical portions of rhizomes were removed from the potted plants and placed in small hydroponic chambers. When these cuttings had adequate root and leaf growth for study initiation, they were transferred to greenhouse trays.

The hydroponic study was conducted in 1.8-m × 0.6-m × 15-cm greenhouse trays lined with plastic. The hydroponic solution was half-strength Hoagland's solution (Hoagland and Arnon, 1950). Plants were held in styrofoam sheets floating on the nutrient solution. The AE treatment, representing a habitat where oxygen is not limited as in the dunes, was saturated with dissolved oxygen by continuous bubbling. The AN treatment, representing a continuously anaerobic substrate such as the low marsh, was maintained anaerobic by intermittent bubbling with nitrogen. The AE/AN treatment was switched between the AE and AN condition every 2 wk to create short-term anaerobiosis as could be found in the high marsh and dune slacks. Dissolved oxygen levels were determined with a modified Winkler procedure (Strickland and Parsons, 1972).

Aboveground (above-styrofoam) material was divided into stems, leaves, reproductive tissue (inflorescences), and stolons (above-styrofoam horizontal stems often continuous with rhizomes). The belowground (below-styrofoam) material was divided into roots and rhizomes (underground stems). Weights were taken for all plant portions after constant weight was reached in a 60-C drying oven. Maximum culm height, number of aboveground culms and five random stem diameters were determined for each plant. The mean of the areas of 30 random leaves (including leaf sheaths) per treatment was used to calculate aboveground plant surface area for each plant.

Just prior to harvest, six whole fresh rhizomes were removed from each form in a given treatment. Freehand sections were made from the portion of the rhizome between the third and fourth nodes back from the apex. The cross-sections were photographed through a microscope at 4× magnification. A compensating polar planimeter was used to determine the area of the aerenchyma (central air space) and the whole cross-section (Harris, Lowry and Chapus, 1981) from photographic prints at ×14.5 total magnification. The area of aerenchyma was expressed as a percentage of the cross-sectional area of the rhizome. The percent aerenchyma was determined for 42 cross-sections for each form in each treatment.

Root respiration measurements were made on apical portions of roots from six plants of each form in a given treatment. Roots were excised at dawn just prior to whole-plant harvest. Roots for each plant were washed, patted dry with tissue paper, weighed, and divided between two 50-cc incubation syringes. One syringe from each plant was flushed with ambient air, and a second was flushed with dry nitrogen to determine CO_2 evolution during aerobic and anaerobic incubations. Replicate gas aliquots from the incubation syringes were injected into a Beckman Infra-red Gas Analyzer Model 865 to determine the CO_2 concentrations which were used to calculate root CO_2 evolution. A standard curve was constructed using varying quantities of 1% CO_2 and the balance air. Respiration rates are expressed as μl CO_2 evolved/gfw/hr.

Just prior to whole-plant harvest, apical portions of roots were removed for determination of alcohol dehydrogenase and malate dehydrogenase activity, and of ethanol and malate concentrations. Determinations were made for three plants of each form in a given treatment. Plant roots were washed, blotted dry with tissue paper, weighed, and frozen until the analyses were performed.

Alcohol dehydrogenase (ADH) and malate dehydrogenase (MDH) activities were determined on extracts from approximately 0.8 g of root tissue per plant. The frozen root material was homogenized in cold 0.1-M Trizma buffer at pH 8.0 (Crawford, 1966; Crawford and McManmon, 1968). The slurry was cold centrifuged and the supernatant was frozen quickly for later analysis. The enzyme activities were determined spectrophotometrically (Bergemeyer, 1963). The activities for ADH and MDH are expressed as units per gfw root tissue; these are international units (IU) where a unit of enzyme will convert 1 μm of substrate per min under the specified conditions.

Malate and ethanol were extracted from frozen root material in cold 6% perchloric acid, and heavier material separated out by centrifugation. Following neutralization of the supernatant with potassium carbonate and methyl orange indicator, the malate and ethanol concentrations were determined spectrophotometrically according to the methods of Bergmeyer (1963) and of Crawford and Tyler (1969). Reagents for the analyses were obtained from Sigma Chemical Co. and Boehringer Mannheim Co.

All data were analyzed statistically using analysis of variance which compensated for unequal numbers of replicates when necessary. Posteriori contrasts by Duncan's Multiple Range were performed at alpha level 0.05 (unless otherwise indicated).

RESULTS AND DISCUSSION—*Biomass*—The marsh and dune forms of *Sporobolus virginicus* grown in the aerated treatment had the same total dry weight biomass (Fig. 1) and aboveground plant surface area (Table 1). However, the two forms did distribute their biomass differently with the marsh form having less reproductive tissue, more stolons, much more underground biomass (Fig. 1, 2), and more

TABLE 1. *Mean aboveground plant surface area (mm²) of* Sporobolus virginicus *(L.) Kunth. marsh and dune forms grown in aerobic (AE), anaerobic (AN), and alternating (AE/AN) hydroponic solutions. Different lower case letters indicate statistical differences with Duncan's Multiple Range Analysis (alpha level 0.05)*

	Plant form	
Aeration	Normal	Robust
AE	27,275 c,d	21,470 b,c
AE/AN	29,540 d	15,385 a,b
AN	11,925 a	14,158 a,b

culms than the dune form (Fig. 3). The marsh form culms were shorter and approximately half the diameter in comparison (Fig. 3).

The marsh form had significantly less total dry weight in the AN treatment than in the AE treatment (Fig. 1). The biomass of leaf, stem, and rhizome tissue was also less in the anaerobic treatment. The number and the diameter of the culms remained the same (Fig. 3), but their height was reduced in the anaerobic substrate. Aboveground plant surface area was also reduced (Table 1), but the aboveground : belowground biomass ratio remained the same (Fig. 2). Essentially, the marsh form was dwarfed when grown in the anaerobic treatment. Marsh form stolon biomass was the same in both the treatments, but it increased as a percentage of the total biomass in the AN treatment (Fig. 1). The proportional increase of stolons, often as aboveground extensions of rhizomes, may increase the capacity for internal oxygen transport to belowground parts. The marsh form grown in the alternating treatment had the same total biomass as that grown in the AE treatment. However, the AE/AN plants produced less underground biomass as roots and rhizomes in response to the alternating aeration treatment (Fig. 2).

The dune form growing in the AN treatment had the same total dry weight and aboveground surface area as that grown in the AE treatment

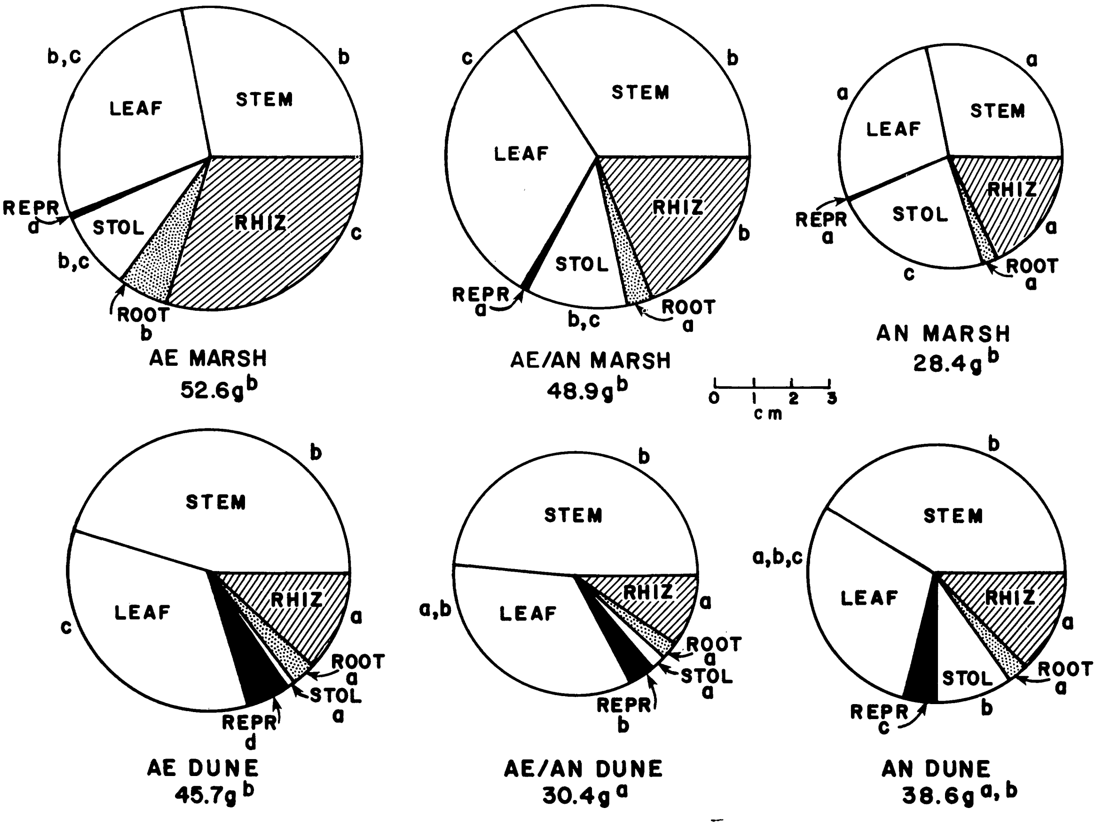

Fig. 1. Mean biomass data for *Sporobolus virginicus* (L.) Kunth. marsh and dune forms grown in aerobic (AE), anaerobic (AN), and alternating (AE/AN) hydroponic solutions. One-cm² circle area equals 1-g dry weight of tissue. Different lower case letters for a given plant part indicate statistical differences with Duncan's Multiple Range Analysis (alpha level 0.05). REPR (inflorescences), STOL (stolons), RHIZ (rhizomes).

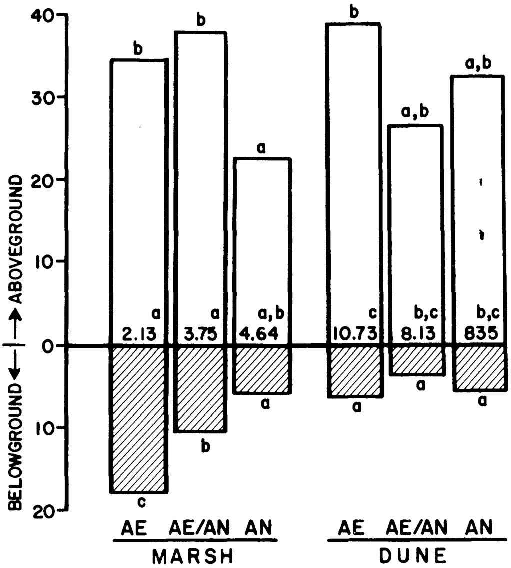

Fig. 2. Bars represent mean aboveground and belowground biomass (gdw) of *Sporobolus virginicus* (L.) Kunth. marsh and dune forms grown in aerobic (AE), anaerobic (AN), and alternating (AE/AN) hydroponic solutions. Number inside the bar is the mean aboveground: belowground biomass ratios. Different lower case letters for a given variable indicate statistical differences with Duncan's Multiple Range Analysis (alpha level 0.05).

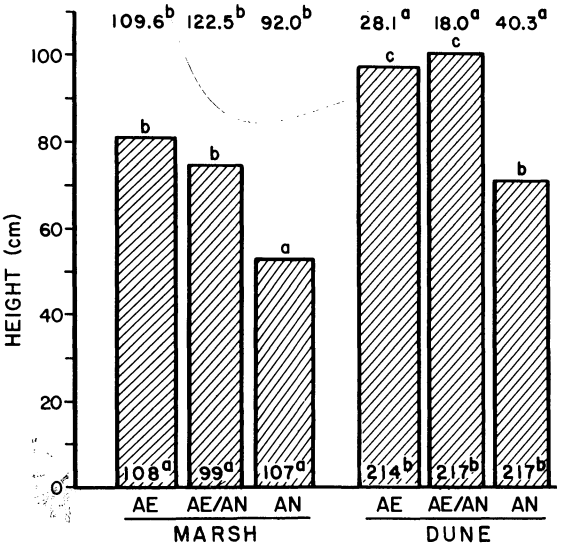

Fig. 3. Bars represent mean maximum culm height (cm) of *Sporobolus virginicus* (L.) Kunth. marsh and dune forms grown in aerobic (AE), anaerobic (AN), and alternating (AE/AN) hydroponic solutions. Number inside bar is the mean stem diameter (mm × 10^2). Number over the bar is the mean number of culms. Different lower case letters for a given variable indicate statistical differences with Duncan's Multiple Range Analysis (alpha level 0.05).

(Table 1, Fig. 1). The anaerobic substrate also did not affect the amount of underground biomass (Fig. 2), number of culms, stem diameter, or aboveground : belowground biomass ratio (Fig. 3). Growth in the AN treatment did result in increased stolon biomass, decreased reproductive tissue, and shorter culms (Fig. 1, 3). As stated before, stolons may increase capacity for internal oxygen transport to roots and rhizomes.

A comparison of the responses of the forms in regard to biomass production and resource allocation indicates that the marsh form was altered more than the dune form when subjected to constant anaerobic substrate conditions. However, the AE/AN treatment reduced the biomass of the dune form and not the marsh form. The increased dune form stolon production observed in the AN treatment was not found in the AE/AN treatment. The lack of stolon production in the AE/AN treatment probably contributed to the decreased total biomass.

Rhizome aerenchyma—The marsh and dune forms had 23% and 36% aerenchyma, respectively, when grown in the AE environment (Fig. 4). The marsh form showed an increase in the percent rhizome aerenchyma when grown in both the continuous (AN) and short-term (AE/AN) anaerobic treatments. This increased the potential for transport of oxygen from aerial to below-styrofoam portions of the plant and

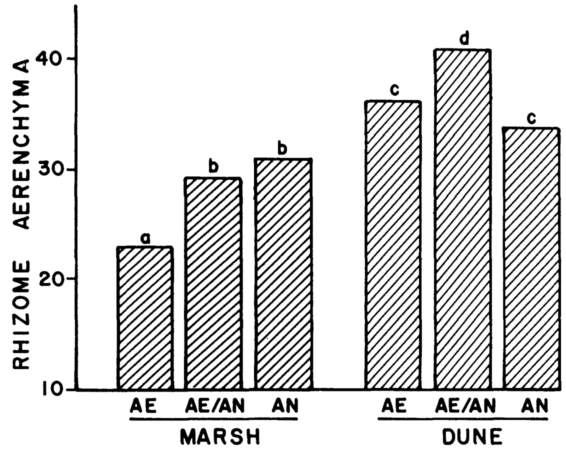

Fig. 4. Mean rhizome aerenchyma (expressed as a percentage of the cross-sectional area of the rhizome) of *Sporobolus virginicus* (L.) Kunth. marsh and dune forms grown in aerobic (AE), anaerobic (AN), and alternating (AE/AN) solutions. Different lower case letters indicate statistical differences with Duncan's Multiple Range Analysis (alpha level 0.05).

may have reduced the amount of respiratory demand per unit volume of below-styrofoam tissue (Williams and Barber, 1961; Armstrong, 1980). However, there was a concurrent reduction of aerial biomass in the AN treatment whereas no reduction in total biomass occurred in the AE/AN treatment. The increased rhizome aerenchyma and the reduction of rhizome biomass seemed to enable the plant to avoid any major reduction of the aerial biomass when the anaerobiosis was only temporary.

The dune form did not differ in percent rhizome aerenchyma when grown in the AE and AN treatment (Fig. 4). The quantity of stolon tissue increased under continuous anaerobiosis, while the total biomass remained the same. The AE/AN treatment did have a higher percent rhizome aerenchyma, but still had less total biomass than in the AE or AN treatments. This difference in response between the constant anaerobic and the alternating aerobic anaerobic environments may be related to the increased stolon biomass which was evident in the AN treatment but lacking in the AE/AN treatment. Increased stolon production in *S. virginicus* dune form appeared to be a morphological adjustment to continuous (long-term) anaerobic substrates, whereas increased rhizome aerenchyma appeared to be an adjustment to alternating (short-term) anaerobic substrates.

Respiration rates—Plant tissue intolerant of the lack of molecular oxygen will usually respond to incubation in nitrogen with a burst of anaerobic respiration which produces elevated levels of CO_2 and ethanol. Tolerant plant tissue often responds to the lack of molecular oxygen by depressing the respiration rate, resulting in lower levels of CO_2 and ethanol production (Crawford, 1978). The marsh and dune forms both exhibited significantly lower levels of CO_2 evolution when the root tissue was incubated in nitrogen as compared to incubation in ambient air. This indicates that the root tissue of both forms may tolerate a lack of molecular oxygen by reducing the anaerobic respiration rate.

Since treatment respiration rate ratios (aerobic incubation rate/anaerobic incubation rate) were not significantly different, the excised root respiration rates for incubations in air and nitrogen were averaged to get a mean respiration rate for each form in each treatment (Fig. 5). The dune form showed the same mean rate in all three aeration treatments. The dune root tissue in the AE treatment was growing in an aerated solution and should have been carrying on aerobic respiration. Since the mean rates for the AE/AN and AN treatments were the same as the AE treatment, the dune root tissues in the AE/AN and AN substrates were also respiring aerobically while growing in the hydroponic solutions. During the study, the plants appeared to be avoiding the anaerobic substrate with the morphological adjustments, rhizome aerenchyma and stolons, allowing transport of sufficient oxygen belowground to the roots.

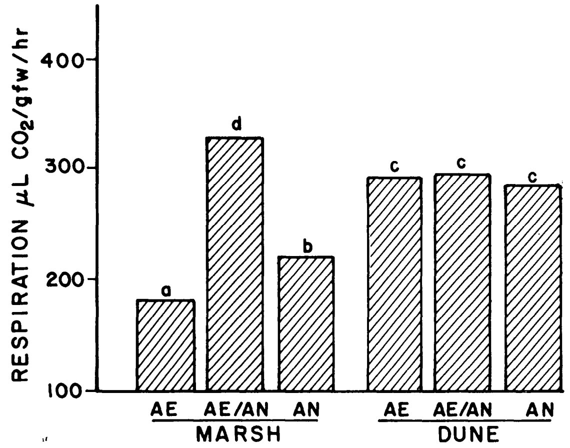

Fig. 5. Mean respiration rates (μl CO_2 $gfw^{-1}hr^{-1}$) of excised root tissue. *Sporobolus virginicus* (L.) Kunth. marsh and dune forms were grown in aerobic (AE), anaerobic (AN), and alternating (AE/AN) hydroponic solutions. Different lower case letters indicate statistical differences with Duncan's Multiple Range Analysis (alpha level 0.05).

The marsh form plants grown in the AE/AN and AN treatments showed higher mean respiration rates than those grown in the AE treatment (Fig. 5). This suggested that the root tissues from the AE/AN and AN environments were in different physiological states than that from the aerated environment. One factor could have been periods of partial or total anoxia in the roots during the course of the study. The resulting anaerobic respiration may have affected the physiological state of the root tissue by altering the amount of substrate, enzyme levels, and available energy.

Enzyme and metabolite analysis—The dune and marsh forms showed no detectable ADH activity in any of the treatments. The analysis for ethanol showed just a trace of ethanol in the roots of both forms in all the treatments but did not vary with form or aeration treatment. Therefore, the roots were not carrying on detectable levels of anaerobic respiration leading to ethanol production, in any of the treatments at the time of harvest.

The marsh and dune forms generally showed the same amount of MDH activity in their roots, with the mean being approximately 24,000 ± 154 (SD) IU/gfw root tissue. The marsh and dune forms had a mean of approximately 0.44 ± 0.21 (SD) mg malic/gfw root tissue (3.3 μM/gfw root tissue). Some of the variability evident in the MDH and malate may have been introduced in the extraction procedure. Crawford and Tyler (1969) report malate concentrations ranging from 0 to 4.0 μm/gfw root tissue for *Juncus effusus* L., *Carex aerencia* L., and various species of *Senecio*. Mendelssohn et al. (1981) found from 1 to 4 μm malate/gfw root tissue for *Spartina alterniflora* Loisel, and they suggested that malate was accumulating during temporary anaerobiosis in streamside *S. alterniflora*. In *S. virginicus* there was no difference in either form in response to the aeration treatment. Perhaps neither form of *S. virginicus* experienced anaerobiosis in its roots under the conditions of the experiment, or perhaps they did not produce malate as an alternative to ethanol in response to temporary or prolonged anaerobiosis as has been suggested for other plants (Crawford, 1978).

CONCLUSIONS—The marsh and dune forms of *Sporobolus virginicus* were both able to tolerate anaerobic hydroponic substrates. However, there were differences in their responses to the anaerobic treatment.

The marsh form of *S. virginicus*, generally found in wetter habitats, responded to the anaerobic substrate by reduction of the total biomass, increased development of rhizome aerenchyma, and redistribution of the biomass. The result was a dwarfed plant with the same number of culms and reduced underground biomass. Root tissues from the anaerobic or alternating treatments showed no indication of anaerobic respiration, production of ethanol, or enhanced malate accumulation at the time of harvest. However, root respiration was higher in the AN and AE/AN treatments.

The dune form, which came from a dry, sandy habitat, responded to the continuous anaerobic substrate by altered distribution of biomass with increased production of stolons, but no change in total biomass. Since the dune form had less underground biomass and more rhizome aerenchyma than the marsh form, the additional stolons seemed to enable it to transport sufficient oxygen belowground to maintain aerobic root respiration and avoid any affect on overall biomass. The dune form grown in the alternating treatment had less total biomass than that grown in either the aerobic or anaerobic treatments. Increased rhizome aerenchyma, without increased stolon production, seemed to be the response to the alternating (short-term) anaerobiosis. The aboveground : belowground biomass ratio was unaffected by either continuous or alternating anaerobiosis in the hydroponic substrates. The root tissues showed no indications of anaerobic respiration or production of ethanol or additional malate.

Both forms of *S. virginicus* were able to adjust either morphologically or physiologically or to use existing morphological features to tolerate anaerobic substrates. It thus appears that the distribution of the marsh and dune forms is not limited by differences in their ability to tolerate the anaerobic conditions found in the marsh habitat.

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