

NOTE

**Effect of light/dark transition on carbon translocation
in eelgrass *Zostera marina* seedlings**Richard C. Zimmerman^{1,*}, Randall S. Alberte²¹Biology Department, University of California at Los Angeles, Los Angeles, California 90024, USA²Biological Science and Technology Program, Office of Naval Research, 800 N. Quincy St., Arlington, Virginia 22217, USA

ABSTRACT: Carbon translocation in the marine macrophyte *Zostera marina* L. (eelgrass) was investigated to elucidate the impact of light/dark transitions on sucrose partitioning between roots and shoots. After exposure of leaves to ¹⁴C-bicarbonate, the level of ¹⁴C-labelled photoassimilates increased monotonically in both leaves and fully aerobic roots of plants maintained in the light. Accumulation of ¹⁴C in roots and leaves ceased abruptly when plants were transferred to darkness that induced root anaerobiosis even though ¹⁴C levels remained high in the dark-exposed leaves. Thus, translocation of ¹⁴C photoassimilates from shoots to roots was inhibited when roots became anoxic. Anoxia induced by light limitation of photosynthesis, whether due to day/night transitions or periods of extreme light attenuation in the water column, can have an impact on carbon availability in subterranean tissues of eelgrass. As a consequence, light availability is likely to control the productivity and distribution of eelgrass in highly variable and light-limited coastal environments through its effects on carbon partitioning between shoots and roots, in addition to whole-plant carbon balance.

KEY WORDS: Seagrass · Anoxia · Translocation · Light · Photosynthesis · Carbon transport

The relationship between light availability and depth distribution/productivity of marine autotrophs is fundamental to the dynamics of primary production in the sea, and is embodied in the concept of critical depth (Sverdrup 1953). This balance of photosynthesis, respiration and growth is commonly invoked to explain the distribution, abundance and productivity of phytoplankton, macrophytic algae and seagrasses (Lüning 1981, Peckol & Ramus 1988, Duarte 1991, Platt et al. 1991).

The role of light availability in regulating the productivity and colonization depth of seagrasses is well established (reviewed by Duarte 1991), but the proxi-

mate physiological mechanisms influenced by light limitation are not always clear. Whole-plant carbon balance should limit colonization depths to a single isolume according to the critical depth concept. Different mean light requirements, however, are frequently reported for the same species of seagrasses growing in different habitats (Borum 1983, Dennison 1987, 1991, Kenworthy et al. 1991, Zimmerman et al. 1991). This suggests that light availability must regulate ecological success and colonization depths through mechanisms that are more complex than assumed by the simple carbon balance model embodied in the concept of critical depth. Unlike non-vascular marine autotrophs, seagrasses invest considerable resources and biomass in non-photosynthetic tissues (roots and rhizomes) that depend strictly upon photosynthetic shoots for the translocation of reduced carbon for maintenance and growth, as well as the transport of photosynthetically produced O₂ for aerobic respiration (Smith et al. 1984, Kraemer & Alberte 1993). Consequently, eelgrass roots become anoxic every night and during the daytime when low light levels severely limit photosynthetic O₂ production (Smith et al. 1984). While anoxic, sucrose consumption via fermentation in eelgrass roots is reduced to 65% of the aerobic rate (Smith et al. 1988), the adenylate energy charge drops to very low levels (Smith 1989) and rates of protein synthesis are reduced (Smith 1989, Kraemer & Alberte 1995). The return to full aerobic activity occurs within a few minutes after roots are re-oxygenated, even after many hours of anaerobiosis (Pregnall et al. 1987, Smith et al. 1988, Kraemer & Alberte 1995).

The ability of eelgrass roots to tolerate anoxia depends strictly on the availability of sucrose reserves imported from the shoot to maintain fermentative energy production throughout the anoxic period (Smith et al. 1988, Smith 1989). Anoxia, however, in-

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hibits phloem translocation in many vascular plants which is driven by mitochondrial respiration in phloem companion cells (Geiger & Sovonick 1975, Jackson & Drew 1984, Saglio 1985, Van Bel 1993). Acropetal transport (via xylem) of inorganic PO_4^{-3} taken up by roots of *Zostera marina* and *Z. noltii* is strongly light-dependent (McRoy & Barsdate 1970, Penhale & Thayer 1980, Brix & Lingby 1985, Pérez-Lloréns et al. 1993). The impact of light and root aerobic status on basipetal translocation of photosynthates in seagrasses that possess metabolic and structural adaptations for anoxia tolerance, however, is not well understood.

Here we sought to determine the impact of darkness-induced root anoxia on translocation of ^{14}C photoassimilates from leaves to roots of eelgrass seedlings. If translocation is O_2 -dependent in seagrasses, then prolonged daily anoxia resulting from light limitation of photosynthetic O_2 production may produce carbon limitation in roots, even though whole-plant carbon balance remains favorable (Pregnall et al. 1987, Smith et al. 1988, Zimmerman et al. 1991). Subterranean anoxia may also block the mobilization of stored sucrose from rhizomes to growing shoots and roots, particularly in the spring when growth rates increase and carbon demand is high (Zimmerman et al. 1995b, and references cited therein). Thus, the survival of eelgrass in unpredictable light environments may depend on the extent to which nighttime anoxia and periods of extreme daytime light attenuation disrupt the partitioning and flux of sucrose between shoots, roots and rhizomes (Zimmerman et al. 1991, 1994).

Materials and methods. *Zostera marina* L. seedlings were collected by hand from an intertidal population in San Francisco Bay, California, USA. Plants were gently

removed from the sediment without breaking root tips and transported to the laboratory at Hopkins Marine Station, Pacific Grove, California, where they were threaded individually through holes (3 mm diameter) punched in silicone rubber stoppers. The remaining gap around each hypocotyl was sealed with silicone grease to prevent leakage of ^{14}C -bicarbonate into the anaerobic root chamber. The shoots were sealed into 50 ml glass incubation tubes filled with filtered (0.2 μm) seawater (FSW) and 50 μCi ^{14}C -bicarbonate. The tubes were placed into an incubator filled with anoxic FSW bubbled continuously with compressed CO_2 (350 ppm) + N_2 (balance) to maintain an external anaerobic environment for the roots (Fig. 1). The O_2 concentration in the incubator was monitored by a polarographic O_2 electrode and remained below 5% of air saturation throughout the experiment. Photosynthesis-saturating irradiance (500 $\mu\text{mol quanta m}^{-2} \text{s}^{-1}$) was provided by a quartz-halogen lamp to drive photosynthesis and maintain root oxygenation (Smith et al. 1984). Incubation temperature was maintained at 15°C (ambient field temperature) using a circulating water bath.

Individual plants in tubes were removed at random every 20 min for evaluation. Root bundles were cut immediately from shoots before removing stoppers. After 3 h in the light, half the remaining tubes were transferred to an identical incubator filled with anoxic FSW bubbled with N_2 + CO_2 , but darkened to induce root anoxia. The other tubes were maintained in the light to maintain root aerobiosis. Sampling was continued at 20 min intervals for 3 h. Roots and shoots were weighed, ground separately in dH_2O [20 ml g^{-1} fresh weight (FW)], digested with an equal volume of NCS (Reg. Amersham Co.), and acidified with 1 N HCl. Radioactivity of acid-stable tissue homogenates was determined by liquid scintillation counting. Quench corrections were performed according to the sample channels ratio.

Results and discussion. Accumulation of ^{14}C -labeled photoassimilates in shoots was linear for the first 5 h of illumination (Fig. 2). Shoot ^{14}C content declined after 5 h, indicating that photosynthetic CO_2 assimilation became substrate-limited and/or O_2 -inhibited in the last 90 min of the experiment. Leaf ^{14}C concentration appeared to drop slightly after transfer to darkness, suggesting loss due to respiration or translocation (Fig 2). The decline, however, was not statistically significant (regression ANOVA, $F = 3.76$, $df = 1,8$, $p = 0.09$). Thus there was no measurable ^{14}C uptake or loss by shoots when plants were held in the dark for >3 h. In contrast, high rates of sucrose translocation to sink tissues result in rapid nighttime depletion of leaf sucrose in many anoxia-intolerant species like maize (Rufty et al. 1983, Kalt-Torres & Huber 1987).

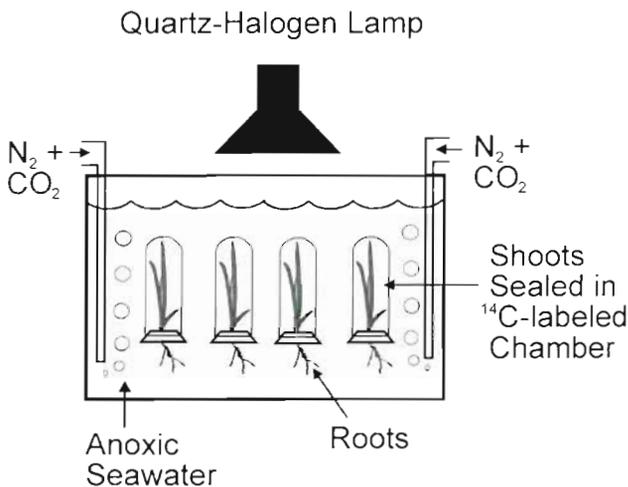


Fig. 1. Diagram of experimental apparatus for translocation experiments in *Zostera marina*

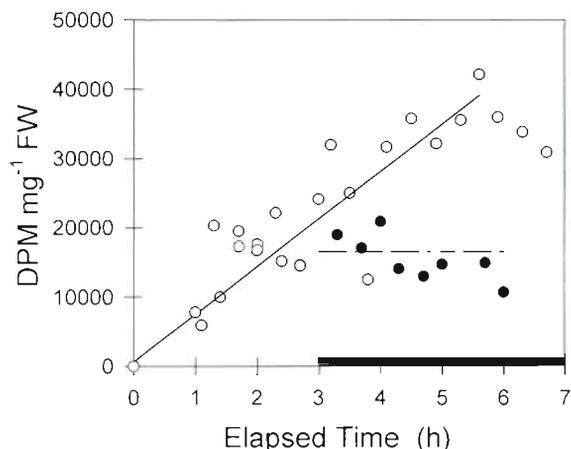


Fig. 2. *Zostera marina*. Accumulation of ^{14}C in eelgrass shoots. (O) Plants maintained in the light for 7 h. (●) Plants transferred to darkness at 3 h. The dark bar along the horizontal axis indicates the transfer of half the plants to darkness

The rate of ^{14}C accumulation in aerobic roots of illuminated eelgrass leaves followed an exponential tra-

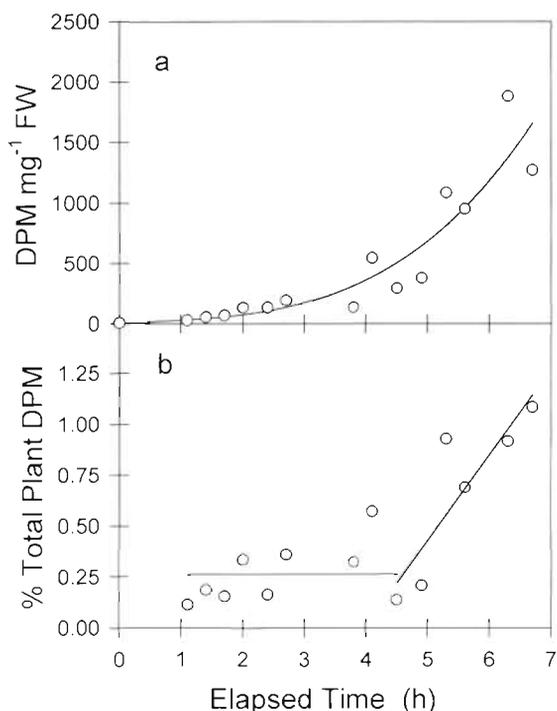


Fig. 3. *Zostera marina*. (a) Biomass-specific accumulation of ^{14}C in eelgrass roots maintained under photosynthesis-saturating irradiance throughout the duration of the experiment. The line represents a 3rd-order polynomial of DPM (disintegrations per minute) regressed against time. (b) Root ^{14}C content plotted as a percentage of total plant ^{14}C . Horizontal line between 1 and 4.5 h represents the mean percentage during that time, while the line from 4.5 to 6.6 h was determined by linear regression

jectory due to the lag between photosynthetic assimilation of ^{14}C into sucrose by shoots and the translocation of ^{14}C to the roots (Fig. 3a). The rate of ^{14}C accumulation in the roots was non-linear but monotonic as long as plants were held in continuous light. The fraction of ^{14}C found in the roots remained fairly constant at 0.26% ($\pm 0.14\%$) of total plant ^{14}C for the first 5 h. After 5 h, however, root ^{14}C content increased dramatically to 1.2% of total plant ^{14}C (Fig. 3b). At this rate of acceleration, more than 10% of the ^{14}C fixed by leaves each day would be transported to roots that represent <20% of biomass. This rapid increase in root ^{14}C after 5 h corresponded to loss of ^{14}C from the illuminated but photosynthesis-inhibited shoots in the last 90 min of the experiment. Thus, translocation in eelgrass may continue in the absence of photosynthetic carbon fixation provided roots are aerobic and translocation substrates (sucrose) remain in the leaves.

The levels of root ^{14}C continued to rise for about 1 h after plants were transferred to darkness (Fig. 4a). Unlike the roots of continuously illuminated shoots, however, root ^{14}C content did not change significantly

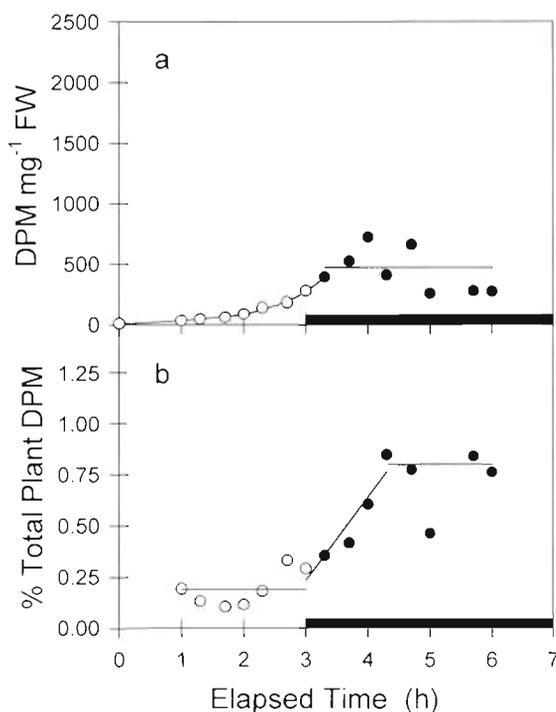


Fig. 4. *Zostera marina*. (a) Biomass-specific accumulation of ^{14}C in eelgrass roots transferred to darkness at 3 h. Black bars along the elapsed time (horizontal) axis and ● indicate the period of darkness and root anoxia. The curve before transfer to darkness represents a 3rd-order polynomial regression of DPM against time. (b) Root ^{14}C content plotted as a percentage of total plant ^{14}C . Horizontal lines from 1 to 3 h and 4.5 to 6.5 h represent the mean root percent DPM over those time intervals; the curve from 3 to 4.5 h was determined by linear regression of %DPM against time

after 1 h in the dark (Fig. 4a, regression ANOVA, $F = 4.92$, $df = 1, 5$, $p = 0.07$). The fraction of total plant DPM (disintegrations per minute) in the roots in the dark-exposed plants, however, increased significantly from 0.25 to 0.8% in the first hour after transfer to darkness (regression ANOVA, $F = 25.12$, $df = 1, 3$, $p = 0.015$) but remained constant after that (Fig. 4b). This indicates that translocation was maintained for about 1 h in the dark, during which time there was no ^{14}C fixation in the leaves. It is also consistent with the time required for fully aerobic roots to deplete lacunal O_2 reserves and become anoxic after transfer to darkness (Smith et al. 1984, 1988).

Eelgrass roots do not increase rates of glycolytic carbon consumption to maintain ATP production under anoxia (i.e. no Pasteur effect) and carbon consumption decreases to 65% of the rate under aerobic respiration (Smith et al. 1988, Smith 1989). Reduced sucrose consumption by roots under anoxia conserves sucrose resources, thereby enhancing anoxia tolerance of eelgrass roots. In contrast, the dramatic Pasteur effect in anoxia-intolerant species such as maize and rice leads to rapid depletion of root sucrose pools (Jackson & Drew 1984).

If translocation proceeded under darkness/hypoxia, levels of ^{14}C in darkened (anoxic) eelgrass roots should have been as high as in the aerobic roots of eelgrass plants maintained under photosynthesis-saturating levels of illumination. Since there was no significant accumulation of ^{14}C in eelgrass roots after 1 h in darkness, translocation in darkened plants was reduced precipitously once the roots became anoxic. Thus, phloem translocation is not maintained under root anoxia in eelgrass, despite the structural (e.g. lacunae) and metabolic (e.g. no Pasteur effect) adaptations possessed by this species for growth in anoxic marine sediments.

The inhibition of ^{14}C translocation during darkness in eelgrass seedlings probably results from cessation of respiratory activity in phloem mitochondria within the hypocotyl and root/rhizome rather than from a reduction in the source-sink gradient driving sucrose translocation. The relatively high and constant level of ^{14}C remaining in the darkened eelgrass leaves throughout the dark exposure period substantiates the lack of ^{14}C translocation from shoots to roots. It also indicates that depletion of sucrose by respiratory demand of darkened leaves is slow (Kraemer & Alberte 1995, Zimmerman et al. 1995a). Translocation rates in anoxia-intolerant species often decrease at night as the leaf sucrose pool is depleted (Ruffy et al. 1983, Kalt-Torres & Huber 1987). In contrast to anoxia-intolerant species, sucrose levels in eelgrass leaves and roots do not exhibit a measurable response to light/dark transitions on a diel basis (Zimmerman et al. 1995a). Fur-

thermore, sucrose levels in excised eelgrass roots can be sufficient to meet the requirements of anaerobic metabolism for as long as 3 d in the absence of any translocation (Smith 1989). The absence of significant carbon reserves in fast-growing species like maize means that even 1 d of low irradiance can produce carbon limitation in roots, leading to depressed rates of root respiration and nutrient uptake for 3 d or more after plants are returned to high irradiance (Massimino et al. 1981, Huber et al. 1989).

Sensitivity of ^{14}C translocation to darkness and below-ground anoxia in eelgrass has clear implications for carbon partitioning. The daily period of irradiance-saturated photosynthesis (H_{sat}) can vary between 0 and 14 h from day to day in many eelgrass habitats (Zimmerman et al. 1991, 1994). Several consecutive days of light limitation (see Zimmerman et al. 1991, 1994) can prolong the daily period of root anoxia and block translocation, thereby inducing carbon limitation in roots even though carbon reserves in the shoot or rhizome source tissues may remain high. Consequently, it may be root sucrose, rather than shoot or rhizome reserves, that determines survival of these vascular macrophytes in aquatic environments characterized by unpredictable light availability and prolonged root anoxia.

Colonization depth limits of eelgrass in the field are likely influenced by the effect of light on translocation and carbon partitioning in addition to whole-plant carbon balance normally embodied in the concept of critical depth (Dennison 1987, Duarte 1991, Kenworthy et al. 1991). Consequently, environmental impacts on resource allocation between shoots and roots also must be considered in assessment of seagrass health and in the development of management strategies to promote growth, production and distribution of these ecologically important marine macrophytes.

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