NOTE

Is light involved in the vertical growth response of seagrasses when buried by sand?

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ABSTRACT: Involvement of light in the vertical growth response of Cymodocea nodosa (Ucria) Ascherson seedlings to sand burial was tested by comparing the growth response of seedlings that were buried and seedlings that were buried but had the shoot meristem illuminated using an optic fiber. Mortality of shoots was higher in the buried and illuminated shoots than in those only buried. The number of new leaves and the length of the vertical rhizome internodes produced during the experiment tended to decrease when the shoots were buried and illuminated, while the length of the leaf sheaths was not affected. Results indicate that light is one of the environmental signals that control the vertical growth response of C. nodosa when buried by sand, and that the shoot meristem is the place where the changes in the light environment of the shoot are detected.

KEY WORDS: Sand burial · Mortality · Vertical growth · Seedlings · Cymodocea nodosa

Most seagrasses grow on shallow (<20 m; Duarte 1991) unconsolidated substrata (sand, mud) where physical energy often causes disturbance to the meadows (Fonseca & Kenworthy 1987), such as burial by sand. Burial may be caused by sand mobilised by physical processes (Williams 1988, Marbá et al. 1994a, b, Marbá & Duarte 1995) or the activity of benthic organisms (Suchanek 1983, Ziebis et al. 1996). Burial by moving sand dunes can greatly influence the spatial structure and life-history of seagrasses (e.g. Cymodocea nodosa, Marbá & Duarte 1995).

Most seagrass species have differentiated short rhizomes that grow vertically (Tomlinson 1974, cf. Duarte et al. 1994). The presence of these short rhizomes with vertical growth allows seagrasses to react to sediment burial by growing vertically, which has been reported for Cymodocea nodosa (Marbá & Duarte 1994, 1995,

Marbá et al. 1994a) and also for other species (Patriquin 1973, Boudouresque et al. 1984, Gallegos et al. 1993, Marbá et al. 1994b, Duarte et al. 1997). The ability of seagrasses to react to sand burial by growing vertically is an essential process to assure the long-term survival of the seagrass meadows during disturbance caused by either natural or human processes.

The only experimental evaluations of the vertical growth response of seagrasses to sand burial were in aquaria for Cymodocea nodosa (Marbá & Duarte 1994) and in field experiments for Thalassia hemprichii, Cymodocea serrulata, Cymodocea rotundata, Halodule uninervis and Syringodium isoetifolium growing in a mixed SE Asian seagrass meadow (Duarte et al. 1997). Burial of seagrasses causes mortality of shoots (Marbá & Duarte 1994, Duarte et al. 1997), but those which survive grow faster as reflected by an increase in the number of leaves produced, the length of the vertical rhizome internodes, the length of the leaf sheaths, and the growth rates of the vertical rhizomes (Marbá & Duarte 1994, Duarte et al. 1997). T. hemprichii, S. isoetifolium and H. uninervis also show an increase in vertical rhizome branching when buried by sand (Duarte et al. 1997). The combined results of these responses are a relocation of the shoot meristem closer to the sediment surface and a reduction in the proportion of photosynthetic tissue that remains buried. These results are similar to those found in plants growing in coastal sand dunes (Disraeli 1984, Maun & Lapierre 1984, Zhang & Maun 1990, 1992, Maun 1994), where burial events are common, or in salt marshes (Pezeshki et al. 1992). Although the vertical growth response of seagrasses to sediment burial is relatively well described, the mechanism that triggers the vertical growth response to sediment burial remains unknown. Possible mechanisms involve a response triggered by the shading of the meristems and/or growth stimulation from the nutrients associ-

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ated with the sediments deposited over the plants. However, a possible increase in the availability of nutrients associated with the sediment load does not seem to play a role in the vertical growth response (Zhang & Maun 1992, Marbá & Duarte 1994).

The goal of this study was to test if a light switch is involved in the control of the vertical growth response of seagrasses upon sediment burial. I tested this hypothesis on *Cymodocea nodosa*, a seagrass species whose vertical growth response to sand burial is well known (Marbá & Duarte 1994, 1995, Marbá et al. 1994a). If the increased growth rate of the vertical rhizomes of *C. nodosa* when buried by sand was driven by the lack of light imposed by burial, the supply of light to a buried plant would inhibit the vertical growth response. I further hypothesised that the place where the changes of the light environment would be detected is the meristem situated at the base of the shoot because it is there that new rhizome and leaf tissue is produced. I used an optic fiber to illuminate the meristematic region of shoots buried by sand and compared the vertical growth response of these shoots with that of shoots that were equally buried but received no irradiance on the buried meristem.

Material and methods. Seedlings of *Cymodocea nodosa* (Ucria) Ascherson were obtained from the edge of a shallow (–0.4 m) patchy meadow located on the bay side of the sand bar that delimits Alfacs Bay, NE Spain (40° 36.15' N, 0° 43.08' W). On 28 June 1996 the seedlings were carefully collected by hand so that damage to the root system was minimal, placed in plastic containers with ambient seawater, and kept in the shade and cool while transported to the laboratory (within 4 h), where they were transferred to an aquarium with running seawater. The aquaria used in the experiment were connected to an open system that continuously pumped seawater, resulting in a water residence time of about 1 h. Each aquarium was illuminated with 1 Sylvania F36W Gro-Lux fluorescent tube and 4 Osram Concentra R63 60 W lights that provided a photon flux density [PFD; photosynthetically active radiation (PAR) was measured] of 80 to 120 µmol m^{-2} s^{-1} at the level of the seedlings in a light:dark cycle of 14 h·10 h. The bottom of the 2 aquaria used in the experiment was covered with a 5 to 6 cm thick layer of sand collected at the same site of seedling collection. Following a 10 h acclimation period, 10 sticks of a slow-release NPK fertilizer ('Le Clou Miracle', A.M.O.S.L., Granollers, Spain) were broken into pieces and inserted into the sediment of each experimental aquarium. This addition represented a nutrient load of 7.29 g N m^{-2} and 3.18 g P m^{-2} per month, which has been shown to prevent nutrient limitation of the growth of this species in the area where the seedlings were collected (Pérez et al. 1991, Marbá & Duarte 1994). Thirty seedlings were planted in each of the 2 experimental aquaria in such a way that the meristematic region of each shoot was just below the sediment surface, and left to acclimate to environmental conditions in the aquaria for 5 d.

On 4 July 1996 all the shoots of the seedlings were marked by punching a hole just below the top of the leaf sheath of the oldest leaf in the shoot. Each seedling was then haphazardly assigned to one of the following 3 treatments: (1) burial under a 4 cm sand layer; (2) burial under a 4 cm sand layer with the meristematic region illuminated with an optic fiber; and (3) no burial (control plants). Burial under 4 cm of sand has been shown to promote the greatest vertical growth response in *Cymodocea nodosa* seedlings (Marbá & Duarte 1994). Burial was achieved by enclosing each seedling within an opaque PVC cylinder 5 cm in diameter and 6 cm in height. The PVC cylinder penetrated 2 cm into the sediment so that a 4 cm burial was obtained when it was filled with sand (Fig. 1). Control seedlings were enclosed in a similar PVC cylinder but only 2 cm in height, which did not protrude from the sediment. Illumination of the shoot meristematic zone was achieved using a 1 m long, 1.5 mm diameter plastic optic fiber. The fiber conducted the irradiance captured from a fluorescent tube used to illuminate the aquaria to the meristematic region where it delivered a PFD (PAR) of 2.9 µmol m^{-2} s^{-1}. The end delivering the captured irradiance was held in position using a 1 cm long, 5 mm diameter clear plastic tube (Fig 1). The plastic tube used to position the optic fiber to the shoot meristematic region was also placed in the shoots of the other 2 treatments to control for any possible effects. Ten seedlings were assigned to each experimental treatment in each aquarium. Each of the seedlings represents, however, an independent experimental unit because the treatment was applied to each of the seedlings independently (cf. Marbá & Duarte 1994).

The seedlings were harvested 35 d (8 August 1996) after the application of the treatment, placed in plastic bags, and kept moistened and cool until the morphometric measurements were done (within the following 5 h). These morphometric measurements included counting the number of living and dead shoots on each seedling, the number of leaves produced during the experiment, the length of the leaf sheath, and the sequence of internodal length of the rhizome of each living shoot. The significance of the effect of the treatments on the survival of the seedlings was tested by constructing Model II 2 × 2 contingency tables (Sokal & Rohlf 1981) with the number of living and dead seedlings at the end of the experiment of each burial treatment and the control, and performing a *G*-test of independence (Sokal & Rohlf 1981). The null hypothe-
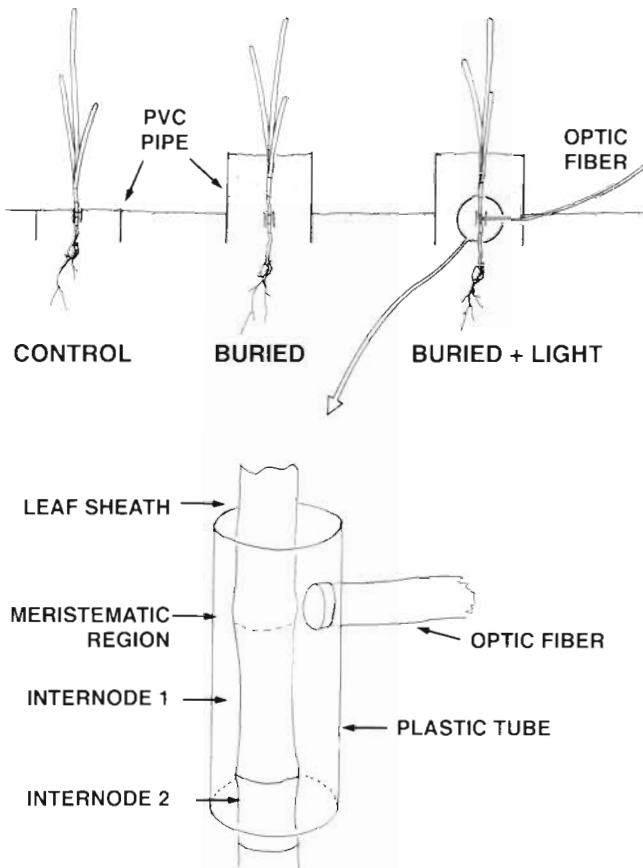
Fig. 1 Illustration of how the experimental treatments were applied to each *Cymodocea nodosa* seedling, and detail of how the tip of the optic fiber was held at the level of the shoot meristematic region (see 'Materials and methods' for dimensions)

sis of this test is that the proportion of living and dead seedlings is independent of the experimental treatment and, therefore, the expected proportion of dead seedlings in each treatment will be the product of the proportion of seedlings assigned to each treatment times the overall proportion of dead seedlings in the experiment. The G-value obtained is tested against the critical values of a χ^2 distribution for 1 degree of freedom (Rohlf & Sokal 1981, Sokal & Rohlf 1981). Differences in the rate of appearance of new leaves, the length of the leaf sheath, and the lengths of the 2 last rhizome internodes produced between treatments were tested using ANOVA (Sokal & Rohlf 1981). Whenever ANOVA results were significant, a post-hoc Tukey HSD multiple comparisons test was used to detect which of the treatments differed. Prior to the statistical analysis the data were tested for normality and homoscedasticity and transformed if necessary. Whenever transformation did not meet the parametric assumptions, the Kruskal-Wallis non-parametric

ANOVA and the Mann-Whitney U-test (Sokal & Rohlf 1981) were used.

Results and discussion. The proportion of shoots that died following burial of the seedlings (51.4%) was not statistically different from that of the control treatment (44.1%, $G = 0.362 \ll \chi^2_{0.05,1} = 3.841$), but increased (to 67.7%) when the meristem of the buried seedlings was illuminated ($\chi^2_{0.10,1} = 2.706 < G = 3.627 < \chi^2_{0.05,1} = 3.841$; Fig. 2).

The rate of appearance of new leaves tended to be slower in the buried shoots that had the meristem illuminated than in the other treatments (Fig 3a), but this difference was not significant (ANOVA with square-root-transformed data, $F = 1.7831$, p = 0.18). The length of the leaf sheath was smaller in the control treatment than for buried seedlings (Fig. 3b; ANOVA, $F = 43.5301$, p < 0.0001), independently of whether buried meristems were illuminated or not (post-hoc Tukey's HSD, p = 0.66). The length of the last, youngest internode (internode 1) was smaller in the control plants than in buried plants [Fig. 3c; Kruskal-Wallis, H (2 df, n = 46) = 28.6949, p < 0.0001]. There was, however, no significant difference in internodal length between buried and buried and illuminated seedlings (Mann-Whitney U-test, $U = 80.00$, p = 0.80). The length of the second youngest internode (internode 2) did not differ among treatments [Kruskal-Wallis, H (2 df, n = 46) = 2.4271, p < 0.30], although buried plants with illuminated meristems tended to produce internodes somewhat smaller that those produced by the seedlings that were only buried (Fig. 3c).

The effects of sand burial on the mortality and vertical growth of *Cymodocea nodosa* seedlings were consistent with those described previously (Marbá & Duarte 1994). The burial of *C. nodosa* seedlings by
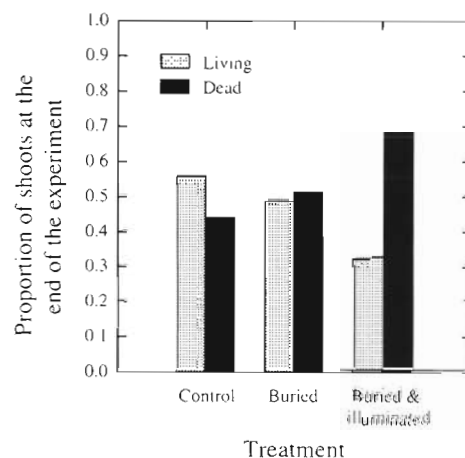

Fig. 2. *Cymodocea nodosa*. Proportion of shoots living or dead at the end of the experiment (after 35 d) in the different experimental treatments. Number of shoots: control, n = 34; buried, n = 35; buried with the meristematic region illuminated, n = 31
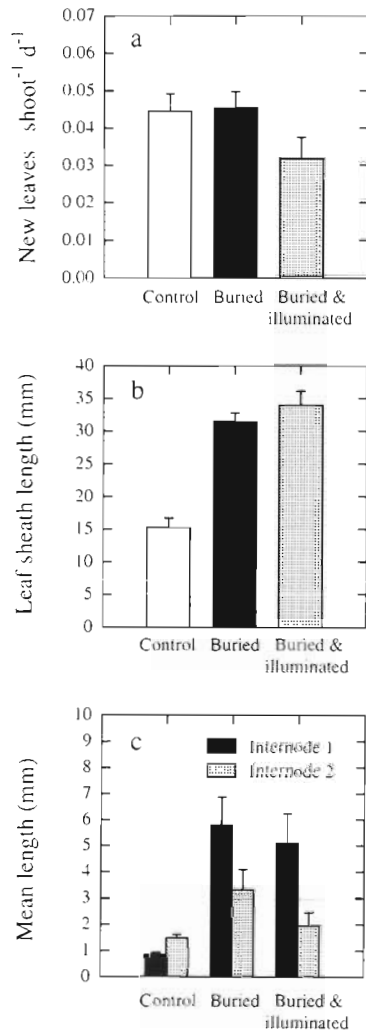
Fig. 3. *Cymodocea nodosa.* (a) Leaf appearance rate, (b) length of the leaf sheath and (c) length of the 2 last, youngest, internodes of the rhizome of each shoot in the different experimental treatments. Error bars indicate +1 SE. Number of shoots: control, n = 19 [18 in (a)]; buried, n = 17; buried with the meristematic region illuminated, n = 10 [9 in (c)]

sand produced an increase in the mortality of shoots when the meristem was illuminated, but not when the meristem remained in darkness (Fig. 2). The presence of light at the shoot meristem also had effects on the vertical growth response. The production of new leaves and, therefore, of vertical internodes showed a tendency to decrease when the meristems of buried shoots were illuminated (Fig. 3a), while the length of the vertical rhizome internodes elongating during the experiment also showed a tendency to decrease when the shoot meristem was illuminated (Fig 3c). The illumination of the meristem did not seem to affect the length of the leaf sheath (Fig 3b). These results indi-

cate that the supply of light to the meristems of the shoots buried by sand precludes the growth response of *C. nodosa* seedlings to burial. This causes an increase in the mortality of the shoots, and a decline in the production of new leaves and the length of the vertical rhizome internodes.

Light is not only an energy source for plants but also one of the main factors controlling plant morphogenesis. Phytochrome, cryptochrome, UV-B photoreceptors and protochlorophyllide *a* are the photoreceptors known to be involved in the control of plant morphology (Salisbury & Ross 1992). The shading imposed by a leaf canopy promotes stem elongation and inhibits stem branching of shade-intolerant plants both through a decrease in the total amount of light and a relative increase in the amount of far-red radiation received by the plants (Ballaré et al. 1990, 1991, Salisbury & Ross 1992). These photomorphogenic responses have an adaptive value to alleviate plant competition for light. Furthermore, far-red radiation reflected by neighbouring plants promotes stem elongation even before the plant experiences shading which represents a mechanism to detect the presence of neighbouring plants and avoid competition (Ballaré et al. 1990, Ballaré et al. 1992, Aphalo & Ballaré 1995). Reports of photomorphogenic processes in seagrasses are scarce. When *Halodule wrightii* grows under a dense canopy of *Thalassia testudinum* the length of rhizome internodes and the number of branches of the plants are greater and smaller, respectively, than when it grows alone (Tomasko 1992). Experimental manipulation of the ratio between red and far-red light (R:FR) received by the plants shows that the length of rhizome internodes increases by 18% when the R:FR ratio decreases from 0.96 to 0.55 and suggests that phytochrome might be involved in the response (Tomasko 1992). The results of the present study provide strong evidence of a light-triggered switch in the control of the vertical growth response of *Cymodocea nodosa* when buried by sand and, therefore, the coupling between vertical growth and sediment accretion. The results also indicate that the shoot meristem is the place where the photoreceptor responsible for the detection of burial through changes in the light environment of the shoot is located. The nature and specific functioning of this photoreceptor should be further investigated to understand the control of the vertical growth of seagrasses as a mechanism to survive disturbance.

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