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UV-B induces leaf reddening and supports photosynthesis in the seagrass *Thalassia testudinum*

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ABSTRACT

Numerous seagrass species growing in intertidal and shallow subtidal areas around the world produce red leaves, but the factors responsible for the induction of leaf reddening in seagrasses are poorly understood. We investigated the responses of transplanted green-leafed and *in situ* red-leafed *Thalassia testudinum* shoots growing in high light areas in the lower Florida Keys, USA, to four light treatments: 1) full solar radiation with UV excluded (PAR); 2) full solar radiation with UV–B excluded (PAR + UV–A); 3) full solar radiation reduced by 50% (50% Ambient); and 4) full solar radiation (Ambient). In our first experiment, green-leafed shoots were transplanted from a 1 m depth (MLW) to the four light treatments in 0.2 m depth (MLW). In our second experiment, *in situ* red-leafed shoots growing at depths between 0.2 m and 0.5 m were exposed to the four light treatments. Within one week, new leaf tissue from green-leafed shoots transplanted into shallow water accumulated anthocyanins and began to turn red in treatments receiving full spectrum solar radiation (Ambient; 50% Ambient) while transplanted green-leafed shoots in the two treatments that excluded UV–B (PAR and PAR + UV–A) had low anthocyanin content and remained green. Although we quickly induced red coloration in leaves of green-leafed shoots, reducing light levels (including UV–B) for seven weeks did not cause leaves of *in situ* red leafed shoots to decrease anthocyanin content or turn green. Instead, red leaves increased photosynthetic pigments in all treatments except Ambient. In addition, the PAR+UV -A treatment had lower effective quantum yields at midday compared to the PAR, 50% Ambient, and Ambient treatments, as well as lower relative electron transport rates compared to the PAR and Ambient treatments. We conclude that exposure to UV–B induces anthocyanin accumulation and red coloration in green-leafed shoots and contributes to the maintenance of high levels of photosynthesis in red-leafed shoots of *T. testudinum*. We also propose that *T. testudinum* in the clear, shallow waters of the lower Florida Keys produces a red-leafed variant, a genetically differentiated form of this species, with permanently red leaves since anthocyanin accumulation and red coloration in leaves of red-leafed shoots was not reversible in this and a longer-term study.

1. Introduction

Numerous seagrass species with red leaves have been found growing in intertidal and clear shallow subtidal waters of the Tropical Atlantic, Tropical Indo-Pacific, and Temperate Southern Oceans bioregions (Novak and Short, 2010; Short et al., 2007). Similar to terrestrial plants, red coloration in seagrass leaves is caused by the accumulation of anthocyanins, water-soluble pigments produced via the flavonoid biosynthetic pathway (Fyfe, 2003, 2004; McMillan, 1983; Novak and Short, 2011). In a previous study, we showed that anthocyanins can act as a sunscreen in seagrasses, enabling red leaves to maintain higher effective quantum yields at midday compared to green leaves (Novak and Short, 2011). Research with terrestrial plants has demonstrated that anthocyanins can serve as a sunscreen and antioxidant in leaves during periods of high light stress by absorbing both ultraviolet (280–400 nm) and visible (400–750 nm; also referred to as PAR) regions of the solar spectrum (see review Gould et al., 2002).

In terrestrial plants, leaves may be red throughout a plant's life or they may turn red while growing, during senescence, or in response to environmental stress. Stressors shown to induce leaf reddening in terrestrial plants include enhanced ultraviolet (UV)/visible radiation, cold temperatures, nutrient limitation, herbivory and pathogen attack (Chalker-Scott, 1999; Gould et al., 2002). The permanent and/or transient nature of red coloration in seagrass leaves is not fully understood although there is evidence that reddening is photoinduced in some seagrasses. Trocine et al. (1981) observed reddish methanol extracts after exposing the seagrass *Halophila engelmanni* to enhanced ultraviolet–B (UV–B; 280–320 nm) radiation. More recently, we found that anthocyanin content in green-leafed *Thalassia testudinum* shoots was positively related to UV and visible irradiance although no relationship was observed between anthocyanin content in red-leafed *T. testudinum* shoots and those same parameters (Novak and Short, 2011).

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Ultraviolet irradiance reaching the Earth's surface has increased over the last thirty years (Herman, 2010; McKenzie et al., 2011) and climate models predict global warming will cause further increases in the tropics and high southern latitudes even as the stratosphere recovers from ozone depletion (Hegglin and Sheperd, 2009). While UV radiation can be beneficial to photosynthesis in some seagrasses growing in high light environments (Figueroa et al., 2002; Hanelt et al., 2006; Hanelt and Roleda, 2009; Hegglin and Sheperd, 2009) excess UV radiation has been shown to negatively affect photosynthetic capacity (Dawson and Dennison, 1996; Detres et al., 2001) and photosynthetic efficiency (Dawson and Dennison, 1996; Figueroa et al., 2002; Larkum and Wood, 1993; Ralph and Burchett, 1995; Trocine et al., 1981), with factors such as morphology, secondary metabolite production, and leaf epiphytes influencing the magnitude of the seagrass response (Abal et al., 1994; Brandt and Koch, 2003; Dawson and Dennison, 1996; Detres et al., 2001; Kunzelman et al., 2005; Larkum and Wood, 1993; Trocine et al., 1981).

The present field study was conducted in the shallow subtidal waters of the lower Florida Keys to determine whether 1) various components of the light spectrum induce anthocyanin accumulation and reddening in green-leafed *T. testudinum* shoots; and 2) reduction of various components of the light spectrum affects anthocyanin levels, redness, and/or other physiological characteristics of red-leafed *T. testudinum* shoots. Our work is part of an ongoing effort to develop a comprehensive understanding of the cause and adaptive significance of the expression of red coloration in seagrass leaves (Novak and Short, 2010; Novak and Short, 2011).

2. Methods

2.1. Site description and experimental design

The lower Florida Keys comprise 30 carbonate islands that separate the Atlantic on the east from the Gulf of Mexico on the west (Schomer and Drew, 1982). Nearshore waters are generally shallow and seagrass meadows, dominated by *T. testudinum*, are the primary benthic vegetation (Fourqurean et al., 2001; Zieman et al., 1989). *T. testudinum* shoots with one or more leaves expressing red coloration have been observed in shallow subtidal waters on both the Atlantic and Gulf of Mexico sides of the lower Florida Keys (Novak and Short, 2010). Red pigmentation in leaves varies from cross or vertical striations to leaves that are entirely red. Patches of *T. testudinum* consisting of shoots with entirely red leaves (red-leafed shoots) have been observed at a number of locations growing adjacent to patches of *T. testudinum* with entirely green leaves (green-leafed shoots; Fig. 1). Red-leafed shoots have higher concentrations of photo-protective pigments (anthocyanins and UV-absorbing compounds), higher effective quantum yields ($\Delta F/F_{m'}$) at high ambient irradiance, as well as shorter, narrower, and lighter-weight leaves than leaves from green-leafed shoots (Novak and Short, 2011).

Two field experiments were performed in the lower Florida Keys between June 1 and August 17, 2007 each using four light treatments which included: 1) full solar radiation with UV excluded (PAR); 2) full solar radiation with UV–B excluded (PAR + UV–A); 3) solar radiation reduced by 50% (50% Ambient); and 4) full solar radiation (Ambient). The exclusion of UV was achieved using Acrylite OP3 polycarbonate sheets, which are opaque to wavelengths below 400 nm, but allow full transmittance underwater in the PAR region. The exclusion of UV–B was achieved using Mylar 92D sheets, which are opaque to wavelengths below 320 nm, but allow full transmittance underwater in the PAR region. To reduce ambient light by 50% we used two sheets of neutral density screen. Transmittance in the UV and visible region was verified with a UV dosimeter (Apogee, UT, USA) and a LI-COR meter (LI-COR, NE, USA). To ensure stability of the light filters, a PVC frame was placed around the Acrylite, Mylar, and neutral density screens. Each 50 cm × 50 cm apparatus was placed 15 cm above the tips of the seagrass shoots and anchored into the sediment with stainless steel threaded rods in each corner. All filters remained submerged throughout the experimental period. Filters were cleaned daily to prevent fouling and transmittance of light through filters was checked weekly to ensure that the filters maintained their spectral properties. Water temperature was recorded under each light treatment at 30-min intervals using iButton temperature loggers (Maxim Corporation, MA, USA) to determine if light filters were affecting the temperature of the water column. The temperature loggers were encased in silicon and attached to stakes at the center of each light treatment. No difference in temperature was observed among the treatments in both experiments.

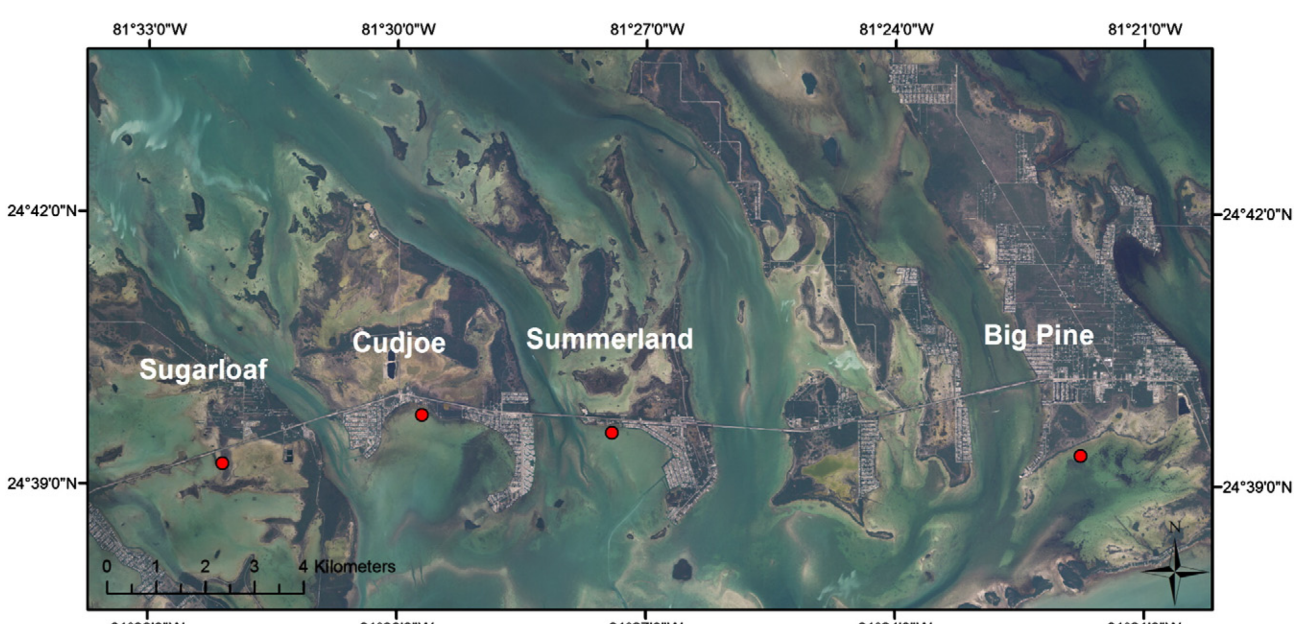

Fig. 1. Map of the lower Florida Keys, USA with the location of leaf reddening study sites (dots).