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EFFECTS OF *LABYRINTHULA* INFECTION ON THE PHOTOSYNTHETIC CAPACITY OF *THALASSIA TESTUDINUM*

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ABSTRACT

Blackened, necrotic lesions on *Thalassia testudinum* leaves are frequently associated with seagrass die-off in Florida Bay. A previously undescribed species of the marine slime mold, genus *Labyrinthula,* is the primary causal agent of these lesions. When *Labyrinthula* infection was present, variations in lesion coverage resulted in significant differences in dry-weight based photosynthesis versus irradiance (P/I) responses of *Thalassia* leaf tissue, reducing photosynthetic capacity and oxygen output. Maximum photosynthetic rate, P_{max}, decreased to below zero when lesions covered 25% or more of the leaf tissue. In addition, respiration rates in infected leaves were up to three times greater than in adjacent, uninfected tissue. Alpha (α), the initial slope of the P/I relationship, exhibited little change with low lesion coverage but was usually reduced with higher lesion coverage. These results show that the presence of *Labyrinthula* lesions impair photosynthesis of *Thalassia* leaf tissues and might reduce oxygen available for transport to belowground tissues, possibly making *Thalassia* more susceptible to hypoxia and sulfide toxicity.

Rapid, widespread, and recurring mortality of the seagrass *Thalassia testudinum* Banks ex König (turtle grass) in Florida Bay, at the southern tip of the Florida Peninsula, is continuing. Since the summer of 1987, more than 4,000 ha of seagrass beds have been lost, and an additional 23,000 ha have been affected (Robblee et al., 1991). Die-off of *Thalassia* appears to be density-dependent and, thus far, has been observed only in areas that previously supported very dense *Thalassia*-dominated populations. Low-density, chronically stressed populations, such as bank-top *Thalassia* and the stunted *Thalassia*-dominated populations of the lower salinity basins in northeast Florida Bay appear unaffected by this phenomenon.

Die-off patches vary in size from small (<1 to 2 m diam) isolated patches within dense beds to a single, large elliptical area almost 2 km long in Rabbit Key Basin (Robblee et al., 1991). The patches spread and coalesce resulting in a contagious distribution pattern which seems to be dependent on short-shoot density and, possibly, shoot-to-shoot contact. Between November 1988 and January 1989, an isolated, dense *Thalassia* bed in Sunset Cove experienced significant die-off. Sunset Cove is over 30 km east of the main area of die-off in western Florida Bay, but it is adjacent to the Everglades National Park boat dock where the research vessels that sample die-off areas are moored. Die-off at this location and the density-dependent distribution patterns of die-off patches observed elsewhere both indicated that a transmissible agent might be involved in this phenomenon.

An endemic species of *Labyrinthula* has been suggested as the possible cause of the Florida Bay *Thalassia* die-off (Porter and Muehlstein, 1989). *Labyrinthula* is the most common eucaryotic organism isolated from affected *Thalassia* during die-off episodes (Porter and Muehlstein, 1989). This marine slime mold is related to the pathogenic species *L. zosterae* Porter and Muehlstein, which is the causative agent of the catastrophic wasting disease of the temperate eelgrass, *Zostera marina* L. (Muehlstein et al., 1988). The endemic *Labyrinthula* sp. from Florida Bay also produces blackened, necrotic lesions similar to those symptomatic of wasting disease (Porter and Muehlstein, 1989). In this study, we investigated the effect of *Labyrinthula* infection and the resulting lesions on the photosynthesis versus ir-

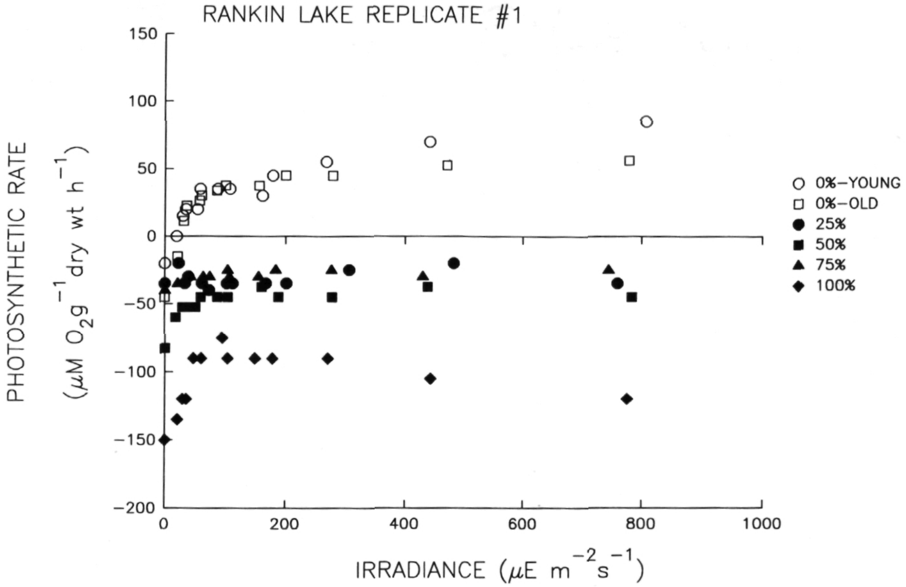

Figure 1. Photosynthesis versus irradiance responses of *Thalassia testudinum* leaf segments from Rankin Lake as a function of leaf age and percentage of lesion cover.

radiance (P/I) responses of field-collected *Thalassia* leaf tissue. Our hypothesis was that infection by *Labyrinthula* decreases the photosynthetic capacity of *Thalassia* leaves, thereby reducing the amount of oxygen available for transport to belowground tissues. Reduced oxygen transport to roots and rhizomes increases their susceptibility to hypoxic stress and sulfide toxicity, both of which might play important roles in the die-off process (Carlson et al., 1990).

MATERIALS AND METHODS

Short-shoots of *Thalassia testudinum* were collected from four locations in Florida Bay that were experiencing seagrass die-off: Rankin Lake, Johnson Key Basin, Rabbit Key Basin, and Sunset Cove (Robblee et al., 1991, Fig. 1). Short-shoots having leaf lesions characteristic of *Labyrinthula* infection were gently excavated from the sediments to minimize damage to the roots and rhizomes and to ensure that the shoots were undamaged. The plant material was transported back to the laboratory within 6 h of collection and stored in aerated, seawater-filled buckets in low light until required for the photosynthesis versus irradiance (P/I) measurements. All experiments were conducted within 4 days of plant collections.

The effect of necrotic lesions on the photosynthetic capacity of *Thalassia* leaves was assessed by comparing the P/I characteristics of individual 1-cm-long leaf segments without visible lesions to those of segments with about 25%, 50%, 75%, and 100% lesion coverage. Lesion-free segments were obtained from the mid-to-basal section of both the youngest and the oldest leaves of a short-shoot (0%-young and 0%-old treatments, respectively). Where possible, the remaining lesion-containing leaf segments were also obtained from the same short-shoot. Verification that the leaf lesions on leaf segment samples were due to infection by *Labyrinthula* sp. was obtained by plating random samples of surface sterilized (0.5% sodium hypochlorite for 2 min, followed by three rinses in sterile seawater) leaf segments on SSA isolation media amended with antibiotics (0.25 g·liter^{-1} each of streptomycin and penicillin) and 3 mg·liter^{-1} geranium dioxide (Muehlstein et al., 1988). After 2 to 5 days in culture, fusiform cells interconnected by filamentous "slimeways" (Porter, 1968) began to move out on the agar surface only at the cut ends of the plated *Thalassia* leaf segments that had lesions. These cell colonies were identical to those in cultures we previously isolated from Florida Bay *Thalassia* leaf segments that were identified as *Labyrinthula* (David Porter, University of Georgia, pers. comm.).

Leaf segments used in the P/I measurements were cut while submerged in a petri dish filled with

Leaf segments used in the P/I measurements were cut while submerged in a petri dish filled with ambient seawater from the collection site and were incubated overnight in individual wells of a 6-well multiwell culture plate containing filtered (0.45 μm) N_2-sparged seawater from the collection site. The following day, leaf segments were removed from the multiwells in random order and placed in 2 ml of N_2-sparged filtered seawater from the collection site in a well-stirred, temperature-controlled glass reaction chamber of a Hansatec DW/1 oxygen-electrode system. The Clark-type polarographic oxygen electrode was calibrated using $N_{2(gas)}$ and air-saturated filtered seawater.

Photosynthesis versus irradiance (P/I) relationships were determined for each randomly chosen leaf segment at ambient-site temperatures (which ranged from 26–32°C) using neutral-density filters and a Kodak projector lamp. Rates of photosynthesis and respiration were measured as the change in dissolved oxygen concentration over a standardized measurement interval (2 min) in the closed (no gaseous head space) reaction chamber. For each P/I run, leaf tissues were first incubated in the dark (10-min equilibration, 2-min respiration measurement interval). The tissues were then subjected to 12 light levels ($\approx$10 to $\approx$600 $\mu E \cdot m^{-2} \cdot s^{-1}$ photosynthetically active radiation [PAR = 400 to 700 nm] as measured by a Li-Cor 2π quantum sensor) in increasing order (1-min equilibration, 2-min photosynthetic measurement interval). At the end of each P/I run, leaf tissues were removed from the chamber, rinsed three times in deionized water, and dried at 60°C for dry-weight determinations. One complete P/I treatment series was run per day and four replicate P/I series were run per site. Photosynthetic and respiratory rates are expressed as $\mu M\ O_2 \cdot g^{-1}$ dry $wt \cdot h^{-1}$.

Respiration rates and the P/I characteristics α and P_{max} were determined for each P/I run (Fourqurean and Zieman, 1991). The initial slope (α) of the P/I curve, which indicates photosynthetic efficiency, was calculated by linear regression of the dark respiration rate plus the photosynthetic rates at the first four to six light levels (whichever yielded the highest coefficient of determination [r^2]). Light-saturated photosynthetic rate (P_{max}) was calculated by averaging the net photosynthetic rates at light levels >300 $\mu E \cdot m^{-2} \cdot s^{-1}$ PAR. Light-saturation (I_k) and light-compensation (I_c) points could not be calculated for most of the P/I runs because of the frequent, negative P_{max} values.

Data were evaluated for normality and homogeneity of variance and were log transformed, if necessary, to satisfy the assumptions of parametric statistics. Analysis of variance (ANOVA) was utilized to assess sources of variability in P/I characteristics attributable to lesion cover and collection site. Where significant treatment effects were determined, Duncan's Multiple Range tests were utilized to compare treatment means. All calculations were performed using Statistical Analysis System (SAS) programs (SAS Institute, Inc., 1985).

RESULTS

Thalassia leaf segments without lesions exhibited typical light-saturated photosynthesis; segments with lesions all exhibited reduced photosynthetic rates. Figure 1, which illustrates the photosynthesis versus irradiance responses of one treatment series replicate from Rankin Lake, is representative of the P/I response patterns of all the sites. In this example, the most obvious treatment effect was a decrease in P_{max} with increasing leaf age and lesion coverage; there was no net oxygen production for leaf segments with lesions over the entire range of light intensities. Age and lesion effects on P_{max} were significant ($F_{5,23}$ = 6.04, 10.05, 2.79, and 3.12 for Rankin Lake, Johnson Key, Rabbit Key and Sunset Cove, respectively) and relatively consistent for leaf samples from all the collection sites (Fig. 2), with the treatment threshold for net oxygen production at saturating irradiances being between 25–75% lesion coverage (Fig. 2).

Lesion coverage generally had a significant effect on respiration rates ($F_{5,23}$ = 2.39, 4.94, 4.94, and 5.32 for Rankin Lake, Johnson Key, Rabbit Key, and Sunset Cove, respectively) and respiration rates were higher for leaf segments with lesions than for young or old leaf segments with no lesions at all sites (Fig. 2). The apparent lack of respiration in the lesion-free leaf segments from Rabbit Key Basin and Sunset Cove is an artifact of our equilibration procedure. The 10-min equilibration time we adopted had been standardized before we obtained the samples from these locations. Leaf segments from both Rabbit Key Basin and Sunset Cove generally had much lower respiration rates than comparable segments from Rankin Lake and Johnson Key Basin (Fig. 2). Rabbit Key samples also had the lowest α and P_{max} of the four sites. The response of α to leaf age or lesion coverage

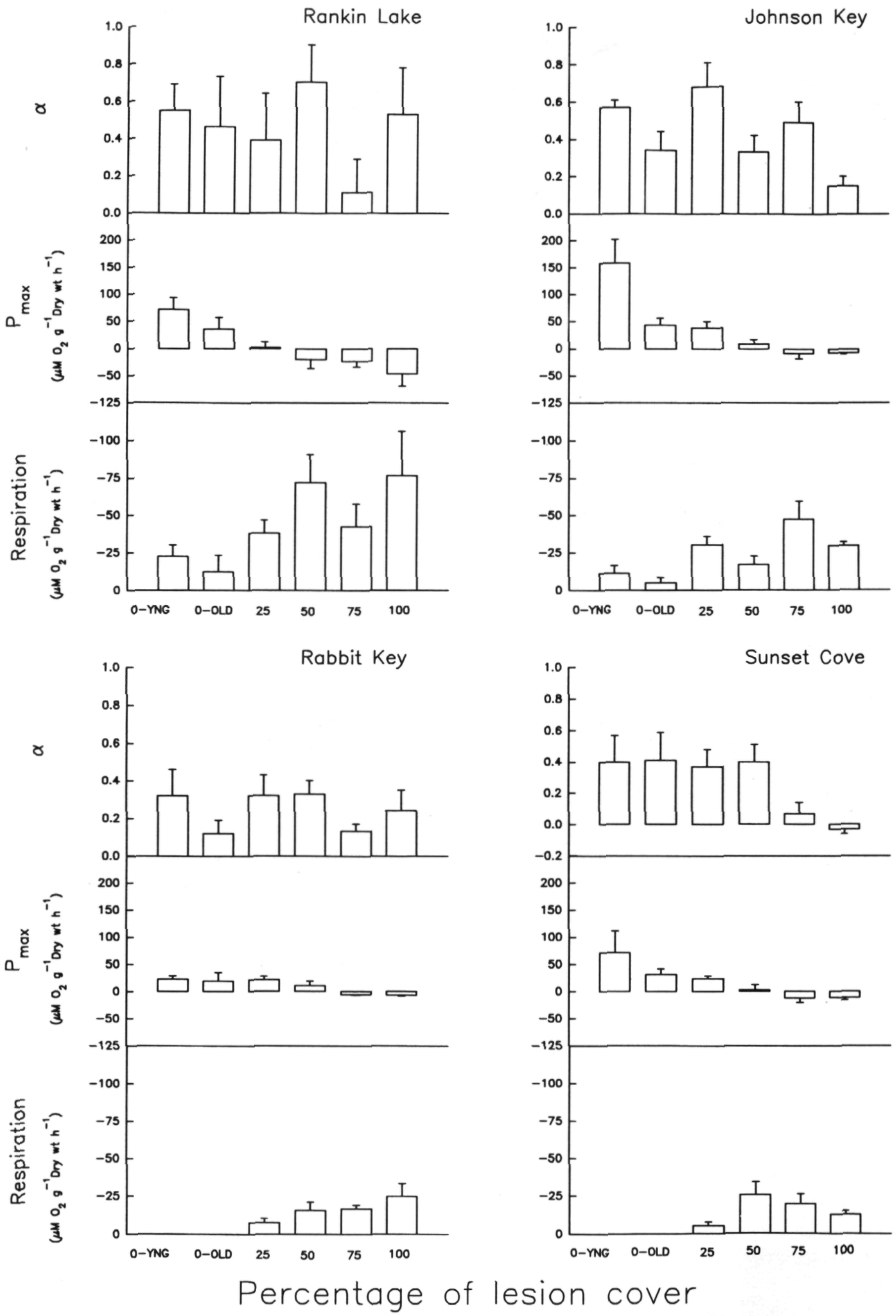

Figure 2. Photosynthesis versus irradiance characteristics and respiration rates (mean ± standard error, N = 4) for *Thalassia testudinum* leaf segments from four sites in Florida Bay as a function of leaf age and percentage of lesion cover.

was inconsistent and was only significant for the Johnson Key population ($F_{5,23}$ = 4.52). However, α was always lowest for segments with the highest lesion coverage (75% or 100%, Fig. 2).

DISCUSSION

A growing body of evidence suggests that environmental stresses weaken seagrasses, making them vulnerable to disease (Den Hartog, 1987; Short et al., 1988; Muehlstein, 1989). Reduced freshwater inflow to Florida Bay due to drought and diversion of upland runoff has caused significant increases in salinity, changing the bay from an estuary to a persistently hypersaline, marine lagoon (Schomer and Drew, 1982; Smith et al., 1989; Fourqurean et al., 1992). The recent anomalously long, time interval without hurricanes in the Florida Bay region has also reduced the frequency of major low-salinity pulses through the system and has resulted in an increased accumulation of sediment in many basins (Zieman et al., 1989). These changes allowed *Thalassia* to develop to very high densities and biomass in basins adjacent to the Everglades shoreline during the time period preceding the initial outbreak of seagrass die-off (Zieman et al., 1989).

The contagious distribution patterns, density dependence, spread rates, and frequent presence of leaf necroses associated with die-off all suggest the involvement of a pathogenic organism. *Thalassia*-seedling bioassays indicated that no acute toxicity was associated with water, sediment, or plant material from Florida Bay die-off sites (Carlson et al., 1990). However, all seedlings inoculated with *Labyrinthula* isolated from Florida Bay developed necrotic lesions within one week. The spread of the lesions was quite slow, and none of the seedlings died during the course of the experiment, possibly because of the mitigating influence of the stored seed reserves (Durako and Moffler, 1987).

We found that leaf lesions, which are symptomatic of infection by *Labyrinthula* sp., reduce the photosynthetic capacity and generally increase the respiratory oxygen demand of *Thalassia* leaves. Consequently, the amount of oxygen available for transport to belowground tissues in infected short-shoots is decreased or eliminated. Severely infected leaf tissues actually exhibit net respiration even when exposed to high light levels. The reduced photosynthetic capacity of leaves infected with *Labyrinthula* is likely a direct result of reduced pigment levels in the necrotic, blackened tissues. We were interested in the effect of *Labyrinthula* infection on the oxygen balance and, secondarily, the carbon budget in *Thalassia,* which is a function of above- and below-ground biomass and respiratory demands (Fourqurean and Zieman, 1991). Thus, photosynthetic rates were determined on a tissue dry weight basis. Because chlorophyll levels in the leaves were not measured, we cannot speculate on whether *Labyrinthula* infection effects photosynthetic efficiency.

In addition to reduced photosynthetic capacity and increased respiration, diseased *Thalassia* short-shoots also exhibit reduced conductance of oxygen (Durako et al., 1992). Combined with the elevated sediment sulfide levels characteristic of Florida Bay's highly organic, carbonate sediments (Carlson et al., 1990), the responses of the leaf tissues to infection by *Labyrinthula* make *Thalassia* more susceptible to hypoxia and sulfide toxicity, which may be the proximal causes of death. If high sediment sulfide concentrations and rhizome hypoxia are the proximal causes of death in the seagrass decline in Florida Bay, then the die-off phenomenon may be limited to systems dominated by carbonate sediments, and its effects may be attenuated in areas where terriginous, iron-rich sediments limit dissolved sulfide concentrations.

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