### 1. REPORT DATE (DD-MM-YYYY)
- 14

### 2. REPORT TYPE
- New Reprint

### 3. DATES COVERED (From - To)
- -

### 4. TITLE AND SUBTITLE
- Linking leaf chlorophyll fluorescence properties to physiological responses for stress detection in coastal plant species.

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- U.S. Army Research Office  
  P.O. Box 12211  
  Research Triangle Park, NC 27709-2211

### 8. SPONSOR/MONITOR'S ACRONYM(S)
- ARO

### 9. PERFORMING ORGANIZATION REPORT NUMBER
- 49364-EV.1

### 10. SPONSOR/MONITOR'S REPORT NUMBER(S)
- 49364-EV.1

### 11. DISTRIBUTION AVAILABILITY STATEMENT
- Approved for public release; distribution is unlimited.

### 12. SUPPLEMENTARY NOTES
- The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision, unless so designated by other documentation.

### 13. ABSTRACT
- Effects of salinity and drought on physiology and chlorophyll fluorescence were used to evaluate stress in two coastal plants, Myrica cerifera (L.) and Phragmites australis (Cav.) Trin. ex Steud. Drought and salinity stress were induced and measurements of stomatal conductance, photosynthesis, xylem pressure potential (c) and fluorescence were conducted following treatment. The onset of stress began at 2 g l−1 for M. cerifera, and 5 g l−1 for P. australis, as seen by significant decreases in physiological measurements. Despite the physiological effects of drought, salinity, barrier island, shrub, thicket.

### 14. SECURITY CLASSIFICATION OF:
- a. REPORT
  - UU
- b. ABSTRACT
  - UU
- c. THIS PAGE
  - UU

### 15. LIMITATION OF ABSTRACT
- UU

### 16. NUMBER OF PAGES
- 19

### 17. NAME OF RESPONSIBLE PERSON
- Donald Young

### 18. TELEPHONE NUMBER
- 804-828-1562
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Effects of salinity and drought on physiology and chlorophyll fluorescence were used to evaluate stress in two coastal plants, Myrica cerifera (L.) and Phragmites australis (Cav.) Trin. ex Steud. Drought and salinity stress were induced and measurements of stomatal conductance, photosynthesis, xylem pressure potential (\(c\)) and fluorescence were conducted following treatment. The onset of stress began at 2 g l\(^{-1}\) for M. cerifera, and 5 g l\(^{-1}\) for P. australis, as seen by significant decreases in physiological measurements. Despite the physiological effects of salinity, there was no significant difference in darkadapted fluorescence (F\(_{v}/\)F\(_{m}\), where F\(_{m}\) is the maximal fluorescence in darkadapted leaves) for either species at any salinity level. Significant decreases in the light-adapted measurement DF/F\(_{\#m}\) (F\(_{\#m}\) is maximal fluorescence in lightadapted leaves) occurred at 10 g l\(^{-1}\) in M. cerifera and P. australis, days before visible stress was evident. The quantum yield of xanthophyll-regulated thermal energy dissipation (FNPQ, where NPQ is non-photochemical quenching of chlorophyll fluorescence) increased with decreasing DF/F\(_{\#m}\). Drought studies showed similar results, with significant decreases in physiological measurements occurring by day 2 in M. cerifera and day 4 in P. australis. Differences in DF/F\(_{\#m}\) were seen by day 5 for both species, whereas F\(_{v}/\)F\(_{m}\) showed no indication of stress, despite apparent visible signs. Xanthophyll-cycle-dependent energy dissipation may be the underlying mechanism in protecting photosystem II from excess energy in salinity- and droughttreated plants.
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ARO Report Number 49364.1-EV
Linking leaf chlorophyll fluorescence properties t ...
Linking leaf chlorophyll fluorescence properties to physiological responses for detection of salt and drought stress in coastal plant species

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Effects of salinity and drought on physiology and chlorophyll fluorescence were used to evaluate stress in two coastal plants, Myrica cerifera (L.) and Phragmites australis (Cav.) Trin. ex Steud. Drought and salinity stress were induced and measurements of stomatal conductance, photosynthesis, xylem pressure potential ($\psi$) and fluorescence were conducted following treatment. The onset of stress began at 2 g l\textsuperscript{-1} for M. cerifera, and 5 g l\textsuperscript{-1} for P. australis, as seen by significant decreases in physiological measurements. Despite the physiological effects of salinity, there was no significant difference in dark-adapted fluorescence ($F_v/F_m$, where $F_m$ is the maximal fluorescence in dark-adapted leaves) for either species at any salinity level. Significant decreases in the light-adapted measurement $\Delta F/F_m$ ($F_m$ is maximal fluorescence in light-adapted leaves) occurred at 10 g l\textsuperscript{-1} in M. cerifera and P. australis, days before visible stress was evident. The quantum yield of xanthophyll-regulated thermal energy dissipation ($\Phi_{NPQ}$, where NPQ is non-photochemical quenching of chlorophyll fluorescence) increased with decreasing $\Delta F/F_m$. Drought studies showed similar results, with significant decreases in physiological measurements occurring by day 2 in M. cerifera and day 4 in P. australis. Differences in $\Delta F/F_m$ were seen by day 5 for both species, whereas $F_v/F_m$ showed no indication of stress, despite apparent visible signs. Xanthophyll-cycle-dependent energy dissipation may be the underlying mechanism in protecting photosystem II from excess energy in salinity- and drought-treated plants.

Introduction

Plants are usually exposed to more radiant energy than is needed for photosynthesis and have evolved numerous mechanisms that safely dissipate excess light to avoid photoinhibition and photooxidation (Flexas and Medrano 2002). Under conditions of stress, these mechanisms for disposing of excess energy do not work efficiently, thus causing changes in the competing reactions of photochemistry, heat loss and fluorescence. Any process that affects the function of PSII and associated de-excitation

Abbreviations – $\Delta F/F_m$, fraction of absorbed photons that are used for photochemistry in a light-adapted leaf; $\Phi_{NPQ}$, quantum yield of xanthophyll-regulated thermal energy dissipation; $\psi$, xylem pressure potential; $A_{net}$, net photosynthetic rate; $F_m$, maximal fluorescence in dark-adapted leaves; $F_{m}'$, maximal fluorescence in light-adapted leaves; $F_o$, minimal fluorescence in dark-adapted leaves; $F_o'$, minimal fluorescence in light-adapted leaves; $F_s$, steady-state fluorescence; $F_v/F_m$, maximum quantum use efficiency of PSII in the dark-adapted state; $F_v'/F_m'$, effective quantum use efficiency of PSII in the light-adapted state; H–F, Huynh–Feldt; NPQ, non-photochemical quenching of chlorophyll fluorescence; PRI, physiological reflectance index.
pathways will have an effect on chlorophyll fluorescence because the fluorescence signal is assumed to originate primarily from PSII (Krause and Weis 1991). Changes in chlorophyll function take place before changes in chlorophyll content, and therefore alterations in the fluorescence signal occur before any visible signs are apparent (Krause and Weis 1991). The percentage of absorbed light used in photosynthesis or dissipated as heat can be estimated by chlorophyll fluorescence parameters and is directly related to plant physiological processes (Flexas and Medrano 2002). Understanding the physiological mechanisms of various environmental stressors is critical in predicting how disturbances will influence future plant community patterns and distributions.

The physiological condition of plants is indicative of plant productivity, adaptability to stress and a general indication of the environment in which they grow (Zarco-Tejada et al. 2002). Plant growth depends on photosynthesis, which is affected by environmental factors such as salinity, drought, temperature and light. Stress may be apparent in morphological and physiological characteristics, which represent integrated responses to multiple environmental factors. Early detection of stress could identify plant physiological condition at larger spatial and temporal scales before visible effects are apparent (Zarco-Tejada et al. 2002). The dark-adapted parameter for maximum quantum use efficiency ($F_v/F_m$) has been widely used to detect changes in the photosynthetic apparatus as a result of stress (Baker and Rosenqvist 2004). This parameter is not efficient for large-scale use in the field because it is problematic to dark adapt an entire canopy. Research into light-adapted measurements of fluorescence is imperative as dark adaptation is not currently practical at scales beyond the leaf level. Steady-state fluorescence and other light-adapted parameters are easily applied to field situations, as dark adaptation is not required, and light-adapted measurements are possible to use at the canopy scale (Evain et al. 2004, Gamon et al. 1990, Zarco-Tejada et al. 2003).

Application of leaf level measurements to the landscape becomes increasingly difficult as species and structural diversity are increased. Not surprisingly, research has focused on fluorescence and canopy level remote sensing for agricultural species, which usually form monospecific canopies (Baker and Rosenqvist 2004, Flexas et al. 2000, Gamon et al. 1990, Zarco-Tejada et al. 2003). Fluorescence appears promising at detecting specific stressors, but there are many challenges for use in natural environments with multiple stressors. Experiments conducted in controlled environments at the leaf level can relate physiological responses to fluorescence for known stressors, which can then be applied to field situations to identify specific types of stress. Scaling up to the landscape will be easiest if using species that form monospecific canopies naturally in the ecosystem.

The objective of our study was to evaluate the effects of drought and salinity on plant physiological responses and functionality of the photosynthetic apparatus, measured by chlorophyll fluorescence. We used two focal species that form monospecific canopies (Myrica cerifera and Phragmites australis) to link leaf fluorescence patterns and physiological responses with different kinds and degrees of environmental stress. Comparisons of light- and dark-adapted measurements of fluorescence were made to determine the ability to detect stress before any visible signs were apparent.

**Materials and methods**

**Plant materials**

*M. cerifera* L. (wax myrtle), Myricaceae, is a relatively salt intolerant, evergreen shrub that forms extensive, dense thickets and is the dominant woody species on most barrier islands of the southern United States (Ehrenfeld 1990, Young et al. 1994). *P. australis* (Cav.) Trin. ex Steud. (common reed), Poaceae, is an invasive perennial grass that has formed numerous large colonies, fringing freshwater and saltwater marshes of the North American Atlantic Coast (Chambers et al. 1999). Because of limited numbers and variations in size, seedlings of *M. cerifera* were obtained from a nursery using local seed stock. Seedlings were transplanted into 2-l plastic pots and acclimated for at least 4 weeks prior to experimentation. Rhizomes of *P. australis* were collected from a brackish water population at Oyster, Virginia (37°17'N; 75°56'W), which is located in the Eastern Shore of Virginia. Rhizomes were transplanted into 2-l plastic pots and grown for 3 months in the environmental chamber before experimentation.

**Induction of stress**

Plants were grown in a Conviron environmental chamber (CMP 3244, Controlled Environments Limited, Asheville, NC) under a photosynthetic photon flux density of approximately 700 μmol m$^{-2}$ s$^{-1}$, 48% relative humidity, a photoperiod of 14 h and a day/night temperature of 30/25°C. Exposure to soil salinity was increased weekly with concentrations of 0, 2, 5, 10 and 15 g l$^{-1}$ for *M. cerifera* and up to 20 g l$^{-1}$ for *P. australis* using dilutions of a commercial mixture that approximates total ocean salts (Instant Ocean, Aquarium Systems, Mentor, OH). Major cations present in the mixture are Na$^+$, K$^+$, Ca$^{2+}$ and Mg$^{2+}$ (5.1, 0.18, 0.19 and 0.62 g l$^{-1}$, respectively) and the major anion is Cl$^-$ (8.9 g l$^{-1}$) (Atkinson and Bingman 1997). Salinity concentrations
were monitored with a conductivity meter (model 33, YSI, Yellow Springs, OH; Tolliver et al. 1997). Drought stress was induced by not watering plants, and responses were compared with well-watered control plants.

**Measurements of gas exchange and fluorescence**

Plant responses to drought and salinity treatments were quantified by measuring stomatal conductance to water vapor, leaf net photosynthesis ($A_{\text{Net}}$), midday leaf xylem pressure potentials ($\psi$) and leaf fluorescence ($n = 10$ per treatment). Measurements were conducted midday (10:00–14:00 h) on days 1, 3 and 5 following salinity treatments and days 1, 2, 4, 5 and 7 for drought experiments. Rates of stomatal conductance and leaf net photosynthesis were measured using a portable infrared gas analyzer at a light intensity of 700 μmol m$^{-2}$ s$^{-1}$, 48% relative humidity and 28°C (LI-6400, LI-COR Biosciences, Lincoln, NE). Midday leaf $\psi$ were quantified with a Scholander pressure chamber (Model 650, PMS, Corvallis, OR).

Light-adapted and dark-adapted measurements of chlorophyll fluorescence were conducted on the 4th or 5th fully expanded leaf of each plant using a pulse amplitude modulated leaf chamber fluorometer (LI-6400, LI-COR Biosciences, Lincoln, NE). Minimal fluorescence values in the dark-adapted state ($F_o$) were obtained by application of a low-intensity red measuring light source (630 nm), whereas maximal fluorescence values ($F_m$) were measured after applying a saturating light pulse of 8000 μmol m$^{-2}$ s$^{-1}$. Minimum ($F_o$) and maximum ($F_m$) values of fluorescence in the light-adapted state at 700 μmol m$^{-2}$ s$^{-1}$ were also obtained in this manner. Using these parameters, the following ratios were calculated: effective quantum use efficiency of PSII in the light-adapted state, $F_v/F_m = (F_m-F_o)/F_m$, fraction of absorbed photons used for photochemistry, $\Delta F/F_m = (F_m-F_o)/F_m$, quantum yield of xanthophyll-regulated thermal energy dissipation, $\Phi_{NPQ} = (F_v/F_m) - (F_o/F_m)$, where $NPQ$ is non-photochemical quenching of chlorophyll fluorescence and $F_s$, the steady-state fluorescence (Hendrickson et al. 2004), and maximum quantum use efficiency of PSII in the dark-adapted state, $F_v/F_m = (F_m - F_o)/F_m$. Leaves were dark adapted for 30 min using dark-adapting leaf clips (LI-COR Biosciences, Lincoln, NE) for $F_v/F_m$ measurements.

**Statistical analysis**

Variations in photosynthetic characteristics, stomatal conductance, leaf $\psi$ and fluorescence relative to control plants over time were analyzed with repeated measures analysis of variance for each stress experiment (Zar 1999). Day was specified as the repeated factor (within subjects) and treatment as fixed effect (between subjects). The validity of a within-subject test depends on sphericity of the data (Von Ende 1993). A measure of deviation that addresses this assumption, the Huynh–Feldt (H–F) correction (Huynh and Feldt 1976), was calculated and adjusted $P$ values (H–F $P$) were reported. Significant differences in treatment means for individual days were identified with Tukey’s tests ($\alpha = 0.05$). In addition, differences in fluorescence were identified and evaluated in response to the specific stressor.

**Results**

**Physiological responses to salinity**

Both species showed physiological responses to salinity treatments at low salinity levels. There was a significant day × treatment interaction for stomatal conductance ($F = 10.80, P < 0.0001$), photosynthesis ($F = 10.37, P < 0.0001$) and leaf $\psi$ ($F = 5.83, P < 0.0001$) in *M. cerifera*. Tukey’s multiple comparisons for individual days revealed that control and treatment plants differed significantly by day 3 of 2 g l$^{-1}$ salinity for both stomatal conductance and photosynthesis (Fig. 1) and remained significantly different throughout the experiment. This was followed by a significant decrease in leaf $\psi$ from $-0.46 \pm 0.04$ on day 0 of salt-stressed plants to $-0.67 \pm 0.07$ on day 5 of 2 g l$^{-1}$ salinity ($F = 9.30, P < 0.0001$; Fig. 1). Visible signs of stress (i.e. browning and fallen leaves) were not observed in treatment plants until day 5 of 10 g l$^{-1}$.

*P. australis* also exhibited significant day × treatment interactions for stomatal conductance ($F = 17.96, P < 0.0001$), photosynthesis ($F = 18.35, P < 0.0001$) and $\psi$ ($F = 2.56, P = 0.0199$). Significant differences in stomatal conductance occurred on day 1 of 5 g l$^{-1}$ salinity for *P. australis* between control and treatment plants, followed by a significant decline in leaf $\psi$ by day 3 of 5 g l$^{-1}$ (Fig. 1). Photosynthesis in salinity treated plants remained significantly lower than control plants by day 3 of 10 g l$^{-1}$ salinity (Fig. 1) and continued to decrease throughout the experiment. Visible signs of stress were apparent by day 3 of 15 g l$^{-1}$ in salinity-treated plants of *P. australis*.

**Chlorophyll fluorescence responses to salinity**

A significant day × treatment interaction occurred in the light-adapted measurements $\Delta F/F_m$ ($F = 3.14, P = 0.0005$) and $F_v/F_m$ ($F = 14.49, P = 0.0019$) in *M. cerifera*. $\Phi_{NPQ}$ ($F = 3.46, P = 0.0005$) increased in salinity-treated plants. Significant differences in $\Delta F/F_m$, $F_v/F_m$ and $\Phi_{NPQ}$ occurred by day 1 of 10 g l$^{-1}$ salinity.
Control plants had an average $D_{Fm}$ value of $0.55 \pm 0.01$ and $F_{v}/F_{m}$ value of $0.60 \pm 0.00$. By day 1 of 10 g l$^{-1}$, $D_{Fm}$ of salinity-treated plants was $0.37 \pm 0.04$, which continued to decline to $0.29 \pm 0.02$ at the end of the experiment. Average $NPQ$ for control plants was $0.26 \pm 0.01$. $NPQ$ increased to $0.50 \pm 0.01$ by the last day in salinity-treated plants. There were no significant differences in $F_{v}/F_{m}$ between control and treatment plants over the course of the experiment. On the last day of measurements, the average value of salinity-treated plants decreased to $0.79 \pm 0.01$ but was not significant.

A significant day × treatment interaction was also seen in $D_{Fm}$ (F = 5.15, P < 0.0001), $F_{v}/F_{m}$ (F = 5.08, P < 0.0001) and $NPQ$ (F = 9.33, P < 0.0001) in $P. australis$. These significant reductions in PSII efficiency occurred by day 5 of 10 g l$^{-1}$ salinity, 3 days prior to apparent visible stress (Fig. 2). $P. australis$ control plants had a higher $D_{Fm}$ of $0.61 \pm 0.01$ and $F_{v}/F_{m}$ value of $0.67 \pm 0.00$ compared with control plants of $M. cerifera$. By day 5 of 10 g l$^{-1}$, $F_{v}/F_{m}$ of salinity-treated plants had decreased to $0.57 \pm 0.02$ and to $0.54 \pm 0.03$ by day 5 of 15 g l$^{-1}$. $D_{Fm}$ declined to $0.32 \pm 0.03$ by the end of the experiment.

**Fig. 1.** Effect of salinity on stomatal conductance to water vapor (gwv), $A_{net}$ and $\Psi$ for $M. cerifera$ and $P. australis$ over time. Closed symbols and open symbols represent means with s.e.s for control and treatment plants, respectively.
experiment, whereas $\Phi_{NPQ}$ increased from 0.16 ± 0.01 to 0.44 ± 0.02. As in *M. cerifera*, there were no significant changes in $F'/F_m$ for *P. australis* between control and salinity-treated plants throughout the experiment ($F = 0.63$, $P = 0.6902$) despite declines in physiological responses (Fig. 2). By the end of the experiment, $F'/F_m$ values of salinity-treated *P. australis* plants had dropped slightly to 0.78 ± 0.01 compared with control plants (0.80 ± 0.00), but this change was not significant.

Significant positive linear relationships (i.e. slope significantly different from 0) occurred between $F'/F_m$ and stomatal conductance ($r^2 = 0.40, F = 139.85, P < 0.0001$),

![Graph showing changes in $F'/F_m$, $\Delta F'/F_m$, and $\Phi_{NPQ}$ for *M. cerifera* and *P. australis* with incremental increases in salinity. Closed symbols and open symbols represent means with SEs for control and treatment plants, respectively.]
photosynthesis \( (r^2 = 0.40, F = 135.75, P < 0.0001) \) and leaf \( \psi \) \( (r^2 = 0.49, F = 135.75, P < 0.0001) \) for \( M. cerifera \) (Fig. 3). Significant positive linear relationships were also seen between \( F'_v / F'_m \) and stomatal conductance \( (r^2 = 0.25, F = 73.98, P < 0.0001) \), photosynthesis \( (r^2 = 0.32, F = 106.25, P < 0.0001) \) and leaf \( \psi \) \( (r^2 = 0.25, F = 71.98, P < 0.0001) \) for \( P. australis \) but with much less predictive power (Fig. 3). Linear relationships were also observed between \( \Delta F/F'_m \) and measurements of gas exchange for both species (data not shown).

**Physiological responses to drought**

Significant reductions in physiological parameters of drought-treated plants were observed early in both species. \( M. cerifera \) showed significant day \( \times \) treatment interactions in stomatal conductance \( (F = 42.55, P < 0.0001) \), photosynthesis \( (F = 28.42, P < 0.0001) \) and leaf \( \psi \) \( (F = 12.37, P < 0.0001) \) in response to drought. Control and treatment plants differed significantly by day 2 for all three physiological parameters (Fig. 4). Visible signs of stress were observed on day 6 of drought treatment in some \( M. cerifera \) plants. By day 7, all plants showed signs of drought stress as evidenced by wilted leaves. \( P. australis \) exhibited similar physiological responses to drought, with significant day \( \times \) treatment interactions in stomatal conductance \( (F = 23.60, P < 0.0001) \), photosynthesis \( (F = 28.06, P < 0.0001) \) and leaf \( \psi \) \( (F = 17.90, P < 0.0001) \). Leaf \( \psi \) was significantly lower in drought plants by day 2 of the experiment, followed by a significant reduction in both stomatal conductance and photosynthesis by day 4 (Fig. 4). Drought-stressed plants had rolled and wilted leaves by day 5 of the experiment.

**Chlorophyll fluorescence responses to drought**

A significant day \( \times \) treatment interaction for \( \Delta F/F'_m \) \( (F = 13.39, P < 0.0001) \), \( F'_v / F'_m \) \( (F = 14.87, P < 0.0001) \)
and $\Phi_{NPQ}$ ($F = 11.35$, $P = 0.0001$) was seen in $M. cerifera$. Drought-stressed plants had significantly lower $\Delta F/F_m$ values by day 2 of the experiment ($0.44 \pm 0.01$) compared with control plants ($0.58 \pm 0.01$), which occurred 4 days prior to visible signs of stress (Fig. 5). $\Delta F/F_m$ values continued to decrease until the end of the experiment, when values were down to $0.17 \pm 0.01$. Decreasing $\Delta F/F_m$ values were accompanied by increasing $\Phi_{NPQ}$ values. By day 7, $\Phi_{NPQ}$ increased to $0.56 \pm 0.01$, compared with control plants, which had an average $\Phi_{NPQ}$ of $0.25 \pm 0.01$. There were no significant changes in $F_v/F_m$, throughout the experiment between control and treatment plants ($F = 2.42$, $P = 0.1211$), even when visible signs of stress were obvious. $P. australis$ also exhibited significant interactions between day and treatment for $\Delta F/F_m$ ($F = 12.66$, $P < 0.0001$), $F_v/F_m$ ($F = 15.02$, $P < 0.0001$) and $\Phi_{NPQ}$ ($F = 13.22$, $P < 0.0001$). Significant decreases in $\Delta F/F_m$, $F_v/F_m$ and $\Phi_{NPQ}$ of drought-stressed plants were also observed by day 4 of treatment (Fig. 5). Again, despite physiological responses and visible signs of stress, there was no significant difference in $F_v/F_m$ between well-watered and drought-stressed plants ($F = 1.61$, $P = 0.2072$).

$F_v/F_m$ exhibited a significant positive linear relationship with stomatal conductance ($r^2 = 0.37$, $F = 68.34$, $P < 0.0001$), photosynthesis ($r^2 = 0.36$, $F = 66.42$, $P < 0.0001$) and leaf $\psi$ ($r^2 = 0.50$, $F = 119.90$, $P < 0.0001$) for $M. cerifera$ (Fig. 6). Significant positive linear relationships were also seen between $F_v/F_m$ and stomatal conductance ($r^2 = 0.45$, $F = 94.93$, $P < 0.0001$), photosynthesis ($r^2 = 0.51$, $F = 122.18$, $P < 0.0001$) and leaf $\psi$ ($r^2 = 0.43$, $F = 92.38$, $P < 0.0001$) for $P. australis$ (Fig. 6) under drought stress. Again, significant linear relationships were observed between $\Delta F/F_m$ and gas exchange measurements for both species (data not shown).

**Discussion**

$M. cerifera$ is salt sensitive (Young 1992) whereas $P. australis$ is able to grow in brackish and saltwater...
marshes (Amesberry et al. 2000). However, both species occur in coastal environments and must withstand periods of drought and episodic flooding with brackish water. This was evident in the physiological responses to both salinity and drought treatments. As seen in the rapid decreases of stomatal conductance, photosynthesis and leaf xylem water potential compared with P. australis (5 g l\(^{-1}\)), M. cerifera was stressed at a lower concentration of salinity (2 g l\(^{-1}\)). Stomatal closure appears to be responsible for the reductions in photosynthesis as a result of salinity stress in both species. This rapid physiological response by M. cerifera enables survival of short-term episodes of salinity by keeping tissue chloride concentrations low within the leaves (Tolliver et al. 1997). The response in P. australis was much slower compared with M. cerifera, with maintained significant photosynthetic stress not apparent until 10 g l\(^{-1}\). Visible signs of stress were obvious earlier in M. cerifera, with browning and wilting leaves occurring at 10 g l\(^{-1}\), whereas visible effects of salinity were not seen in P. australis until 15 g l\(^{-1}\).

There is no change in the maximum quantum use efficiency of open PSII centers (F\(_{v}/F_{m}\)) in the early stages of salinity stress, indicating that salinity does not induce

![Fig. 5. Changes in the fluorescence measurements F\(_{v}/F_{m}\), F\(_{v}/F_{m}\), ΔF/F\(_{m}\) and Φ\(_{NPQ}\) for M. cerifera and P. australis as drought increases over time. Closed symbols and open symbols represent means with s.e. for control and treatment plants, respectively.](image-url)
sustained photodamage (Baker and Rosenqvist 2004, Morales et al. 2005). However, some studies have shown effects on $F_v/F_m$ after 30 min of dark adaptation as a result of salinity stress (Belkhodja et al. 1994, Castillo et al. 2005, Lee et al. 2004), and perennial plants may be more sensitive to salinity compared with annuals (Morales et al. 2005). Other studies have shown little to no effect of salinity on $F_v/F_m$ at low to mid-salinity levels (Morant-Manceau et al. 2004, Netondo et al. 2004, Redondo-Gomez et al. 2006) even when leaf growth and gas exchange were reduced. This is because salinity initially decreases stomatal conductance, which reduces photosynthesis, leaving PSII unaffected in the early stages of stress (Baker and Rosenqvist 2004).

In our study, $F_v/F_m$ was not affected by salinity in *M. cerifera* and *P. australis* for the duration of the experiment, suggesting that there was no damage to PSII, even at mid-range salinity levels. Conversely, significant changes in the efficiency of PSII ($\Delta F/F_m$ and $F'_v/F'_m$) were observed at 10 g l$^{-1}$ in both species, prior to visible signs of salt stress. Decreases in these light-adapted fluorescence parameters were coupled with increases in $\Phi_{NPQ}$ in both species. Values of $\Phi_{NPQ}$ were higher by the end of the experiment in *M. cerifera*. Changes in fluorescence were detectable sooner in *M. cerifera* than in *P. australis*, but significant decreases in $\Delta F/F'_m$ occurred within 8–9 days of physiological stress for both species. Reductions in fluorescence measurements in *M. cerifera* were likely because of chloride toxicities. Tolliver et al. (1997) found significantly higher tissue chlorides in *M. cerifera* plants over time at 10 g l$^{-1}$ salinity, the concentration at which changes in chlorophyll fluorescence were observed. In comparison, *P. australis* is considered a salt excluder by its ability to maintain a relatively constant Na$^+$ concentration within the shoot tissues at different salinity levels (Vasquez et al. 2006). Therefore, osmotic stress and not salt toxicities was most likely the cause of reductions in fluorescence measurements in *P. australis*.

Decreases in $\Delta F/F'_m$ and increases in $\Phi_{NPQ}$ with no subsequent decline in $F_v/F_m$ for both species suggest an
enhancement in thermal dissipation in PSII in such a way as to match the decrease in photosynthesis in order to avoid photodamage (Qiu et al. 2003). Xanthophyll-cycle-dependent energy dissipation may be the underlying mechanism in protecting the photosynthetic apparatus from excess energy in the salinity-treated plants (Demmig-Adams and Adams 1996). In addition to enhanced thermal dissipation, increased photorespiration may be a potential mechanism for avoiding photodamage in salt-stressed plants (Downton et al. 1990). Stępień and Klbus (2006) also reported that \( \Phi_{PS2} (\Delta F/F_m) \) was sensitive to salt stress in Cucumis sativus leaves, whereas there was no change in \( F_v/F_m \) at any salinity level. \( F_v/F_m \) began to decline in both species by the end of the salinity experiments, but this decline occurred well after visible signs of stress were apparent. Declining \( F_v/F_m \) values could indicate the possibility of photoinhibition had the experiment continued (Krause and Weis 1991). Alternatively, lower \( F_v/F_m \) could be because of sustained thermal energy dissipation after dark adaptation associated with the xanthophyll cycle (Adams and Demmig-Adams 1995), as evidenced by high values of \( \Phi_{NPQ} \) in stressed plants. Variations in \( \Delta F/F_m \) are more sensitive to salinity stress compared with changes in \( F_v/F_m \). Thus, light-adapted fluorescence measurements are better indicators for detecting salinity stress before severe damage occurs in these species.

M. cerifera and P. australis had similar responses to drought. All three physiological parameters were significantly lower in drought-treated plants of M. cerifera by day 2 of the experiment. Leaf \( \psi \) dropped by day 2 of drought for P. australis, but stomatal conductance and photosynthesis were not affected until day 4. Visible signs of stress were evident earlier in P. australis, with rolling leaves seen by day 5 of drought as opposed to day 6 in M. cerifera. Again, stomatal closure was likely the mechanism responsible for reductions in photosynthesis in both species (Cornic 1994, Medrano et al. 2002). Stomatal effects on photosynthesis as a result of drought do not initially affect the efficiency of PSII, as measured by \( F_v/F_m \) (Baker and Rosenqvist 2004, Flexas and Medrano 2002). In our study, drought did not affect \( F_v/F_m \) for either M. cerifera or P. australis. de Mattos et al. (1997) found that coastal plants maintained high \( F_v/F_m \) values throughout the day, even at high leaf-to-air vapor deficit values. Saltmarsh et al. (2006) found no significant change in afternoon \( F_v/F_m \) values for P. australis subjected to drought over the first 7 days. \( F_v/F_m \) values in our experiment did slightly decrease by day 7, but this was not significant, and visible signs of stress were apparent in all plants by the end of the experiment. Some studies have reported significant declines in afternoon \( F_v/F_m \) as water stress increases (Liberato et al. 2006, Souza et al. 2004), whereas other authors have reported no significant change in \( F_v/F_m \) during drought stress or only after severe water stress (Marques da Silva and Arrabaca 2004, Miyashita et al. 2005, Subrahmanyam et al. 2006). However, these experiments did not address whether or not visible damage was evident.

Our results indicate that water stress did not cause damage to PSII by the end of the experiment, or before any visible damage to the leaves occurred, as expected. Significant decreases in \( \Delta F/F_m \) and \( F_v/F_m \) were evident by day 4 in both species, which occurred 1–2 days before visible signs of stress. Subrahmanyam et al. (2006) also found significant changes in \( F_v/F_m \) in water-stressed plants relative to control plants. A reduction in \( \Delta F/F_m \) with increasing \( \Phi_{NPQ} \) in the absence of change in \( F_v/F_m \) suggests an increase in thermal energy dissipation, likely because of the xanthophyll cycle (Demmig-Adams and Adams 1996). In addition, increased photorespiration may also be a way of protecting PSII as this is a common response in water-stressed plants (Flexas and Medrano 2002).

The possibility of xanthophyll-cycle-dependent energy dissipation in both species under drought and salinity treatments may enable rapid stress detection at the canopy level. The physiological reflectance index (PRI) is sensitive to changes in de-epoxidation state of the xanthophyll cycle and subsequent accumulation of zeaxanthin (Gamon et al. 1990). \( \Delta F/F_m \) and \( \Phi_{NPQ} \) have been correlated to PRI under drought conditions (Evain et al. 2004, Peñuelas et al. 1997). Because \( \Delta F/F_m \) and \( \Phi_{NPQ} \) changed progressively as stress was increased in our experiment, investigation into the links between PRI and fluorescence may prove powerful for linking leaf level measurements to the landscape. Changes in fluorescence were observed prior to signs of visible stress, which may be an important consideration, especially for drought, when linking fluorescence to PRI at the canopy level (Evain et al. 2004, Gamon et al. 1992). Further research into identifying the fluorescence signal in reflectance data is key to using fluorescence remote sensing on a large scale.

Acknowledgements – D’Arcy Mays provided suggestions for the statistical analyses. Pablo Zarco-Tejeda commented on an earlier draft of this manuscript. This research was support by a grant to D. R. Y. from the United States Army Research Office. The comments of Wah Soon Chow and two anonymous reviewers are greatly appreciated.

References

Tolliver KS, Martin DW, Young DR (1997) Freshwater and saltwater flooding response for woody species common to barrier island swales. Wetlands 17: 10–18

Edited by W. S. Chow