Transaction of the Kansas Academy of Science
vol. 115, no. 1/2
p. 5-18 (2012)

Effects of flooding on photosynthesis, chlorophyll fluorescence, and oxygen stress in plants of varying flooding tolerance

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Physiological effects of flooding might be interpretable through measures of photosynthesis and respiration in plants. Photosynthesis and respiration processes were measured in *Phalaris arundinacea* L. (reed canarygrass), *Phaseolus vulgaris* L. (common bean), *Pisum sativum* L. (pea), *Vicia faba* L. (broad bean), and *Zea mays* L. (maize) following three days of flooding. From this, we propose a model of physiological flooding tolerance regarding photosynthetic and respiratory metabolism. Tolerance to flooding results from an ability to maintain photosynthesis and high stomatal conductance when flooded, and to avoid oxygen shortage in roots. The most flooding-tolerant attributes were observed in the wetland grass *Phalaris arundinacea*. Oxygen was not limiting for root respiration when flooded, and photosynthetic rates and stomatal conductance increased in the flooding treatment. The flooding-tolerant *Zea mays* and the broad bean *Vicia faba* were the next most flooding tolerant, as indicated by increased photosynthesis rates and stomatal conductance with flooding, but both displayed signs of oxygen stress from three days of flooding. The flooding-sensitive *Phaseolus vulgaris* and *Pisum sativum* were the most impacted by flooding, particularly *P. vulgaris*. Roots of *P. vulgaris* and *P. sativum* experienced oxygen stress in flooded treatments. Photosynthesis and stomatal conductance decreased in flooding treatments, and there was evidence of increased photoprotection with flooding in *P. vulgaris*. An ability to allocate light energy to photochemistry during flooding also appeared to be related to flooding tolerance. Plant flooding tolerance is mainly determined by an ability to maintain respiratory processes in affected tissues.

Keywords: Alcohol dehydrogenase, Anaerobic, Phalaris arundinacea, Phaseolus vulgaris, Photosynthesis, Pisum sativum, Vicia faba, Waterlogging, Zea mays

Introduction

All higher plants are aerobes and thus depend on oxygen for mitochondrial respiration (Brix and Sorrell 1996). Most plants can survive short-term absence of oxygen (≤ 1 hr) without cell death. Carbohydrate reserves can help the cells respire anaerobically while stored carbon supplies last. In these cases, ATP stores are quickly depleted in active cells, and mitochondrial swelling is usually observed within minutes (Drew 1997). Longer absences of oxygen can result in irreversible damage to mitochondria and cell viability, which generally occurs following 15 hours of anaerobiosis (Perata and Alpi 1993).

Under the low oxygen caused by flooding, ATP synthesis from oxidative phosphorylation is decreased (Drew 1997). Accordingly, respiration pathways shift from oxidative to fermentative (Kato-Noguchi and Morokuma 2007). In plants, the majority of the fermentative metabolic flux proceeds via decarboxylation of pyruvate by pyruvate decarboxylase (PDC) and subsequent reduction to ethanol via alcohol dehydrogenase (ADH) (Roberts et al. 1984).

The rate-limiting step of fermentation is the reduction of acetaldehyde to ethanol via ADH (Zabalza et al. 2009), making ADH activities especially meaningful in tissues subjected to low oxygen. Increased activities of PDC and ADH are commonly measured when plants are flooded (e.g., Maricle et al. 2006; Yin et al. 2009). Furthermore, increased ADH or PDC activities are sometimes correlated with the degree of flooding susceptibility of a plant (Yin et al. 2009). By contrast, increased ADH activities can also be directly related to the degree of flooding tolerance of the plant (Mugnai, Marras, and Mancuso 2011). Pairing ADH activities with other measures of flooding tolerance can provide a more clear interpretation of physiological responses to flooding.

There are several ways plants adapt to substrates with low oxygen. Plant roots are especially prone to the effects of hypoxia since they are non-photosynthetic tissues submerged in the waterlogged sediment (Drew et al. 1994). To counter this oxygen shortage, wetland plants form aerenchyma tissue, with abundant air spaces to ventilate submerged portions of the plant (Jackson and Armstrong 1999). Many flooding tolerant plants can direct a bulk flow of air through aerenchyma to support respiration in submerged tissues (e.g., Armstrong 1979; Dacey 1980; Armstrong and Armstrong 1990). Additionally, other common adaptations to help plants with low oxygen include forming adventitious roots above water (Song et al. 2011) and fast growth to keep portions of the plant in contact with atmospheric oxygen (Luo et al. 2009).

In addition to decreased aerobic respiration, flooding of roots commonly causes stomatal closure in leaves (Rodríguez-Gamir et al. 2011), which can reduce CO_2 uptake (Pezeshki 2001). Stomatal limitations on photosynthesis are manifested through a decrease in the internal CO_2 concentration (C_i) in a leaf (Farquhar and Sharkey 1982), easily measured with gas exchange equipment (Field, Ball, and Berry 1989). A reduction in CO_2 availability results in a decreased electron sink in chloroplasts, which can result in excess light energy available (Allen 1995). This has the potential to generate reactive oxygen species that can damage membranes and proteins (Allen 1995). Efficiency of photosynthesis and light harvesting can be assessed by following the fate of absorbed light energy in a plant. This is particularly meaningful in conditions where CO_2 fixation is reduced relative to light harvesting (e.g., flooding-induced stomatal closure) (Demmig-Adams and Adams 1992). It has been suggested that flooding tolerant plants are better able to maintain energy flow through PSII compared to flooding sensitive species (Sarkar and Panda 2009). Use of chlorophyll fluorescence measures allow calculations of the routing of radiant energy toward photosynthesis or lost as heat (Demmig-Adams et al. 1996), providing additional indications of how photosynthesis is affected during stress.

From the preceding, a general model of plant flooding tolerance can be developed (Bailey-Serres and Voesenek 2008). Flooding tolerance mechanisms include the ability to transport oxygen internally to submerged tissues (Maricle and Lee 2002; Maricle and Lee 2007), a high capacity to respire anaerobically (Maricle et al. 2006) and maintain glycolytic metabolism during flooding (Drew 1997), an ability to keep stomata open and maintain photosynthesis rates during flooding (Jones et al. 2006; Parent et al. 2011), and the capacity to avoid oxidative damage from excess light energy not

used in CO_2 fixation (Arbona et al. 2008). Tolerant species perform some or all of these, whereas more sensitive species do not. It can be hypothesized the degree of physiological flooding tolerance is related to the extent these mechanisms are employed by plants.

Plant species are often categorized by relative flooding tolerance. For example, the system of Bray, Bailey-Serres, and Weretilnyk (2000) recognizes: (1) The most tolerant plants are "wetland plants," capable of sustained growth in flooded soils, e.g., *Oryza sativa* L. (rice) or *Echinochloa crus-galli* (L.) Beauv. (barnyard grass). (2) The next most tolerant plants are called "flooding tolerant plants," determined as those that can survive flooding for short duration (a few days), e.g., *Zea mays* L. (maize) or *Triticum aestivum* L. (wheat). (3) The most sensitive plants are designated "flooding sensitive plants," those that cannot survive even short-term flooding, e.g., *Pisum sativum* L. (pea) or *Glycine max* (L.) Merr. (soybean) (Bray, Bailey-Serres, and Weretilnyk 2000).

The objective of this study was to verify the preceding model of flooding tolerance, and propose how flooding affects root respiration, leaf gas exchange, and light harvesting in leaves. In this study, effects of flooding were investigated on several common plant species ranging in flooding tolerance. It was hypothesized the wetland grass *Phalaris arundinacea* L. (reed canarygrass) would be more tolerant of flooding compared to the crop species *Phaseolus vulgaris* L. (common bean), *Pisum sativum* (pea), *Vicia faba* L. (broad bean), and *Zea mays* (maize). Furthermore, the flooding-tolerant *Z. mays* was hypothesized to be more tolerant of flooding compared to the flooding-sensitive *P. vulgaris*, *P. sativum*, and *V. faba*. Tolerance to flooding was investigated by comparing root ADH activity, leaf gas exchange, and chlorophyll fluorescence between flooded and drained plants in greenhouse experiments.

Materials and Methods

Plant Material and Growing Conditions

Seeds of *Phaseolus vulgaris*, *Pisum sativum*, *Vicia faba*, and *Zea mays* were purchased commercially, planted in potting soil, and germinated in greenhouse conditions. Pots were 11 cm x 11 cm and contained 5 to 8 seeds each. *Phalaris arundinacea* seedlings were collected from sites of growth near Hays, KS (38° 57′ N, 99° 23′ W), and were repotted in potting soil and grown in the greenhouse. All plants were of similar developmental stage. Greenhouse conditions were natural lighting with an average PPFD of 1100 µmol m^{-2} s^{-1} at midday and average radiant energy was 600 W m^{-2} at midday. Greenhouse temperatures were variable, but were normally near 33°C during afternoons and 21°C at night during the study. Plants were watered daily with tap water.

Plants of uniform size (15 to 30 cm in height) were divided into treatment and control groups containing four to ten individuals per species. For flooding treatments, plants were placed in Rubbermaid 61 cm x 40 cm x 22 cm plastic tubs. Tubs were filled with water to soil level (ca. 10 cm). Plants in control treatments were kept on the greenhouse bench; soil remained moist and water was allowed to drain freely. Flooding treatments lasted three days.

Anaerobic Enzyme Activity

Following three days of flooding, plant roots were harvested, frozen in liquid

nitrogen, and kept at -80°C (Maricle et al. 2006). Root alcohol dehydrogenase (ADH) enzyme assays were conducted after John and Greenway (1976). Tissue samples were ground in liquid nitrogen with a mortar and pestle. Cold extraction buffer was added at 5 mL g^{-1}, consisting of 50 mM HEPES with 5 mM $MgCl_2$, 2 mM cysteine hydrochloride, and 2% PVP-40 at pH 8.0. The mixture was homogenized and then centrifuged at 10,000 g for 10 min at 4°C. Assays were conducted at 25°C in a spectrophotometer. Enzyme activity was determined as the rate of NADH oxidation, measured as a decrease in absorbance at 340 nm (Maricle et al. 2006) . Background rates of substrate conversion with enzyme extract were measured with a buffer containing 40 mM bicine and 5 mM $MgCl_2$ at pH 8.0 in the presence of 80 µM NADH. Reaction rates were calculated following addition of 10 mM acetaldehyde (Maricle et al. 2006).

Photosynthesis Measures

Prior to root harvest, photosynthesis measures were taken with a Li-Cor LI-6400XT portable photosynthesis system (Li-Cor Biosciences, Inc., Lincoln, NE) following three days of flooding treatment. Leaf chamber conditions were set at a flow rate of 400 µmol s^{-1} with 385 ppm CO_2, and irradiance was set at 1500 µmol quanta m^{-2} s^{-1}. Measurements included photosynthesis rate (A_{1500}), stomatal conductance to water vapor (g_s), intercellular CO_2 concentration (C_i), transpiration (E), steady-state fluorescence in the light (F_s), minimum fluorescence from a light-exposed plant (F_0'), maximum fluorescence from a light-exposed plant (F_M'), and the quantum yield of photosystem II (Φ_{PSII}). The proportion of absorbed light energy that was allocated to photochemistry (P) or thermal dissipation (D) was calculated after Hichem, Naceur, and Mounir (2009):

$$P = \frac{F_M' - F_0'}{F_M'} \times q_p \qquad (1)$$

$$D = 1 - \frac{F_M' - F_0'}{F_M'} \qquad (2)$$

$$X = \frac{F_M' - F_0'}{F_M'} \times (1 - q_p) \qquad (3)$$

where X is the proportion of absorbed radiant energy in excess of P and D (Hichem, Naceur, and Mounir 2009), and P + D + X = 1.00.

Data Analysis

Data were analyzed with analysis of variance (ANOVA), with species and treatment as factors (StatView 5.0, 1998 SAS Institute, Inc., Cary, NC). All analyses were performed at α = 0.05.

Results

Root Enzyme Assays

Root alcohol dehydrogenase (ADH) activities ranged from 0.21 to 5.19 µmol g^{-1} min^{-1} across species and treatments

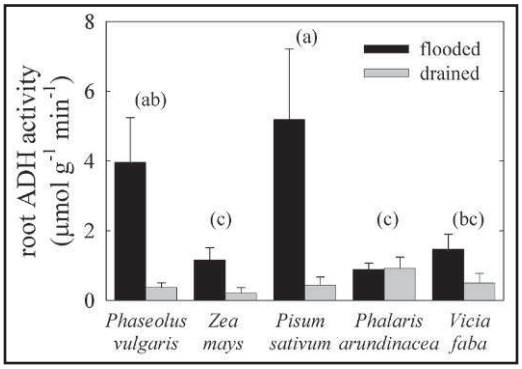

Figure 1. The effect of flooding on root alcohol dehydrogenase (ADH) activities (µmol g^{-1} min^{-1}) for plants in the study. Bars are means of 4 to 10 replicate plants ± SE. Letters over the bars indicate significant differences between species at α = 0.05. Bars that share a letter are not significantly different.

(Fig. 1). Root ADH activities increased with flooding, as ADH activities were significantly higher in plants from flooded treatments compared to drained treatments (ANOVA, $p < 0.001$). ADH activities were significantly higher in *P. sativum* and *P. vulgaris* compared to *Z. mays*, *P. arundinacea*, and *V. faba* (ANOVA, $p = 0.009$; Fig. 1). A significant species x treatment interaction (ANOVA, $p = 0.003$) indicates root ADH activities in *P. arundinacea* did not increase in the flooded treatment.

Gas Exhange

Leaf-level photosynthesis rates at 1500 µmol quanta m^{-2} s^{-1} (A_{1500}) ranged from 2.1 to 14.4 µmol CO_2 m^{-2} s^{-1} across species and treatments (Fig. 2*a*). A_{1500} was significantly higher in *Z. mays* compared to all other species (ANOVA, $p < 0.001$; Fig. 2a). A significant species x treatment interaction (ANOVA, $p = 0.016$) indicates differential responses of species to flooding. Whereas A_{1500} increased with flooding in *Z. mays*, *P. arundinacea*, and *V. faba*, A_{1500} decreased with flooding in *P. sativum* and *P. vulgaris*.

Stomatal conductance (g_s) followed a similar pattern as A_{1500} and ranged from 0.031 to 0.153 mol H_2O m^{-2} s^{-1} across species and treatments (Fig. 2*b*). Mean g_s was significantly higher in *P. arundinacea* and *V. faba* compared to *P. sativum* and *P. vulgaris* (ANOVA, $p = 0.016$). A significant species x treatment interaction (ANOVA, $p = 0.011$) indicates differential responses of species to flooding. Species-specific patterns of g_s in response to flooding were similar to changes in A_{1500}; g_s increased with flooding in *Z. mays*, *P. arundinacea*, and *V. faba*, but decreased with flooding in *P. sativum* and *P. vulgaris*.

Internal (intercellular) CO_2 (C_i) ranged

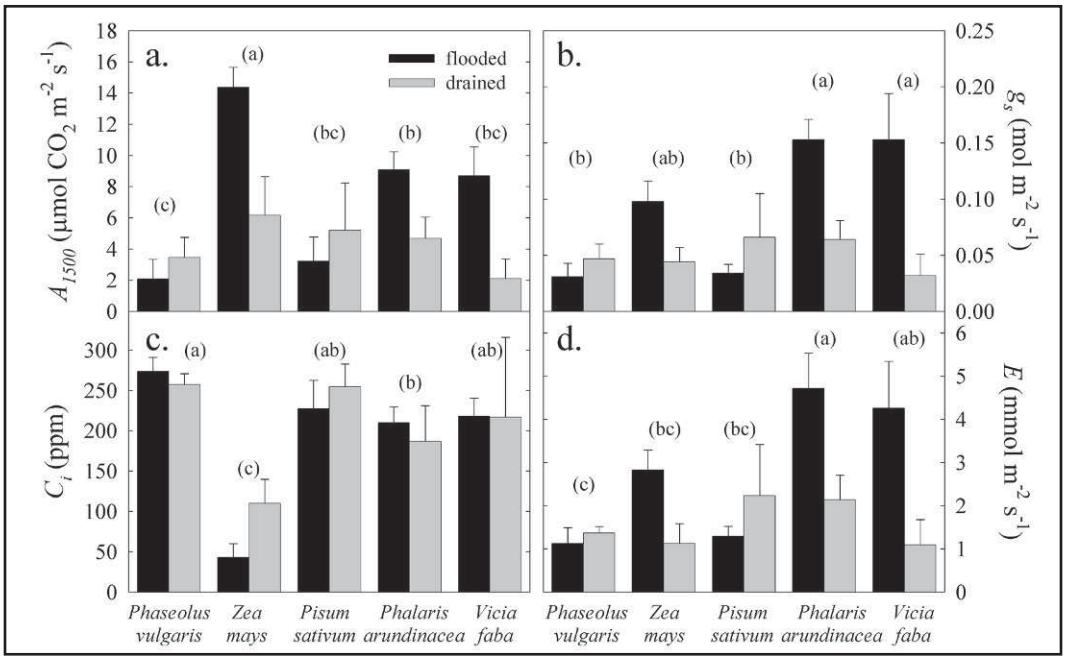

Figure 2. The effect of flooding on (*a*.) CO_2 assimilation (A_{1500}), (*b*.) stomatal conductance (g_s), (*c*.) intercellular CO_2 concentration (C_i), and (*d*.) transpiration (E) at 1500 µmol quanta m^{-2} s^{-1}. Bars are means of 3 to 10 replicate plants ± SE. Letters over the bars indicate significant differences between species at α = 0.05.

from 43 ppm in the C_4 *Z. mays* to 274 ppm in the C_3 *P. vulgaris* (Fig. 2*c*). There was no difference in C_i between drained and flooded treatments (ANOVA, p = 0.606). There was a significant difference in C_i between species (ANOVA, p < 0.001), with the four C_3 species being significantly higher than the C_4 species *Z. mays*. There was no species x treatment interaction for C_i (ANOVA, p = 0.471).

Leaf-level transpiration rates (*E*) ranged from 1.1 to 4.7 mmol H_2O m^{-2} s^{-1} across species and treatments (Fig. 2*d*). Measures of *E* closely tracked measures of g_s, and showed similar differences between species (ANOVA, p = 0.017), treatments (ANOVA, p = 0.007), and a species x treatment interaction (ANOVA, p = 0.033). There was more variability in *E* compared to g_s, but *E* increased with flooding in *Z. mays*, *P. arundinacea*, and *V. faba* and decreased with flooding in *P. sativum* and *P. vulgaris*.

Chlorophyll Fluorescence

Measures of chlorophyll fluorescence also responded to flooding treatments. Steady-state fluorescence in the light (F_s) ranged from 578 to 1274 units across species and treatments (Fig. 3*a*). F_s was significantly higher in *P. vulgaris* compared to all other species and significantly lower in *Z. mays* compared to all other species (ANOVA, p < 0.001). A significant species x treatment interaction (ANOVA, p = 0.028) indicated differential responses to flooding among species. Flooding decreased F_s in *P. sativum* and *P. vulgaris*, but F_s was unchanged in other species.

Minimum light adapted fluorescence (F_0') ranged from 439 to 701 units across species and treatments (Fig. 3*b*). F_0' significantly decreased with flooding (ANOVA, p = 0.037). In a pattern similar to F_s, there was a significant

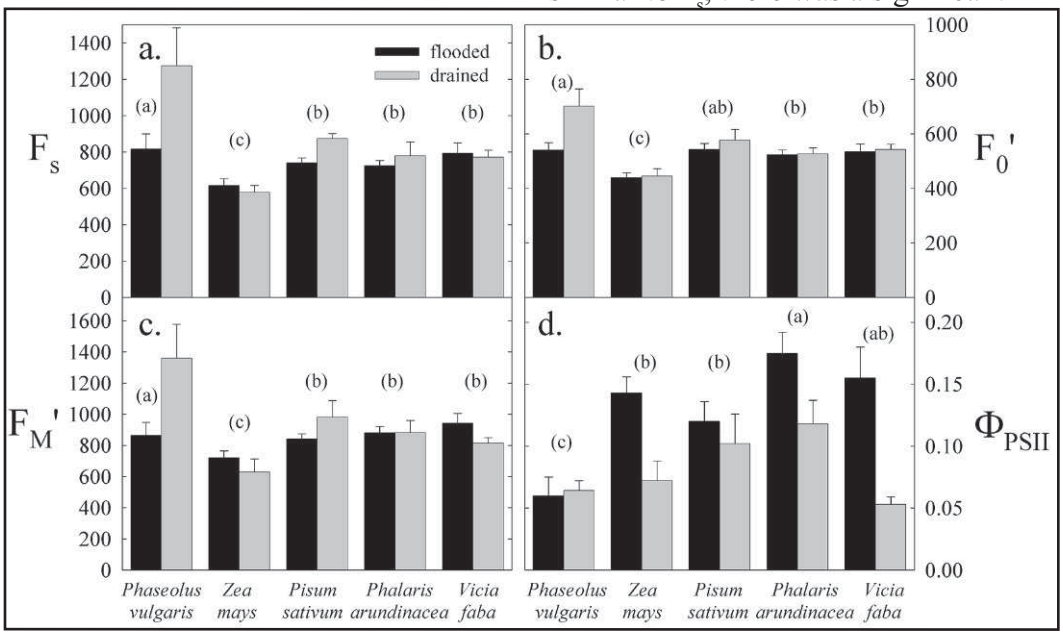

Figure 3. The effect of flooding on (*a*.) steady-state fluorescence (F_s), (*b*.) minimal light-adapted fluorescence (F_0'), (*c*.) maximal light-adapted fluorescence (F_M'), and (*d*.) quantum yield of PSII (Φ_{PSII}) at 1500 µmol quanta m^{-2} s^{-1}. Bars are means of 3 to 10 replicate plants ± SE. Letters over the bars indicate significant differences between species at α = 0.05.

difference in F*0′* between species (ANOVA, $p < 0.001$), with *P. vulgaris* being the highest and *Z. mays* being the lowest. There was no species x treatment interaction for F_0' (ANOVA, $p = 0.065$).

Maximum light adapted fluorescence (F_M') ranged from 630 to 1360 units across species and treatments (Fig. 3*c*). F_M' was not changed with flooding (ANOVA, $p = 0.161$). In a pattern similar to F_s and F_0', F_M' was significantly higher in *P. vulgaris* compared to all other species and F_M' was significantly lower in *Z. mays* compared to all other species (ANOVA, $p < 0.001$). A significant species x treatment interaction (ANOVA, $p = 0.011$) indicated differential responses to flooding among species. F_M' decreased with flooding in *P. sativum* and *P. vulgaris*, but remaining unchanged or increased in *Z. mays*, *P. arundinacea*, and *V. faba*.

The quantum yield of PSII (Φ_{PSII}) ranged from 0.053 to 0.175 across species and treatments (Fig. 3*d*). Mean Φ_{PSII} was significantly higher in flooded plants compared to drained plants (ANOVA, $p < 0.001$). Additionally, Φ_{PSII} was significantly higher in *P. arundinacea* compared to *P. sativum*, *Z. mays*, and *P. vulgaris*. Furthermore, Φ_{PSII} was significantly lower in *Z. mays* compared to all other species (ANOVA, $p < 0.001$). There was no species x treatment interaction for Φ_{PSII} (ANOVA, $p = 0.061$).

The proportion of absorbed light energy allocated to photochemistry (P) ranged from 0.053 to 0.175 across species and treatments (Fig. 4*a*). Mean P closely tracked Φ_{PSII}, and was significantly higher in flooded plants compared to drained plants (ANOVA, $p < 0.001$). Similarly, P was significantly higher in *P. arundinacea* compared to *P. sativum*, *Z. mays*, and *P. vulgaris*. Additionally, Φ_{PSII} was lower in *Z. mays* compared to all other species (ANOVA, $p < 0.001$). There was no species x treatment interaction for P (ANOVA, $p = 0.061$).

The proportion of absorbed light energy lost as heat (D) ranged from 0.545 to 0.737 across species and treatments (Fig. 4b). Mean D complemented P, but was not different between drained and flooded plants (ANOVA, $p = 0.358$). D was significantly higher in *Z. mays*

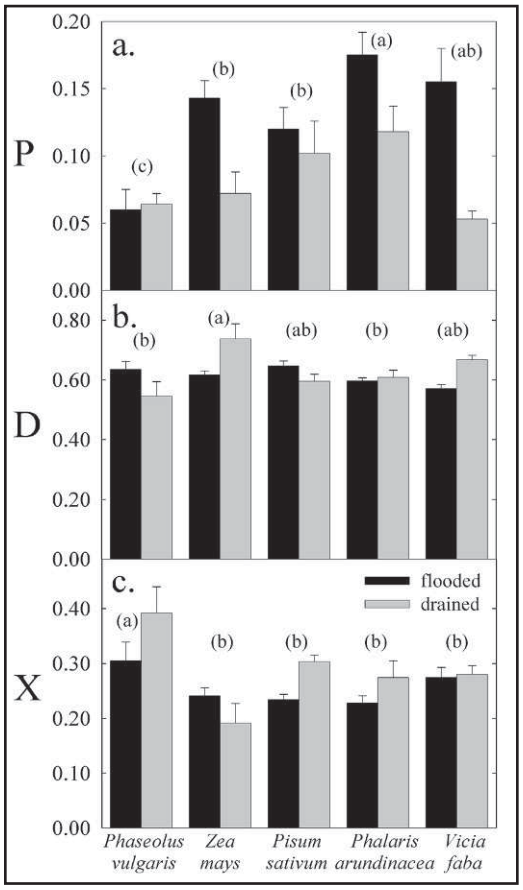

Figure 4. The effect of flooding on the fraction of absorbed light that is allocated to (*a*.) photochemistry (P), (*b*.) heat loss (D), and (*c*.) in excess (X) at 1500 μmol quanta m^{-2} s^{-1}. Bars are means of 3 to 10 replicate plants ± SE. Letters over the bars indicate significant differences between species at α = 0.05.

compared to *P. vulgaris* and *P. arundinacea* (ANOVA, p = 0.014). A significant species x treatment interaction for D (ANOVA, p = 0.002) indicated different responses by species. Specifically, D increased with flooding in *P. sativum* and *P. vulgaris* but remaining unchanged or decreased in *Z. mays*, *P. arundinacea*, and *V. faba*.

The proportion of absorbed light energy in excess of P and D (X) ranged from 0.191 to 0.392 across species and treatments (Fig. 4*c*). X was not different between drained and flooded treatments (ANOVA, p = 0.077). X was significantly higher in *P. vulgaris* compared to all other species (ANOVA, p < 0.001), but there was no species x treatment interaction for X (ANOVA, p = 0.070).

Discussion

Photosynthetic gas exchange, chlorophyll fluorescence, and root oxygen stress were compared in several common plants representing different sensitivities to flooding. From these results, a model of flooding tolerance can be developed regarding respiratory and photosynthetic metabolism.

Respiration in Roots

Most of the physiological effects of flooding are related to a lack of oxygen (Armstrong, Brandle, and Jackson 1994). Respiratory processes are sensitive to oxygen levels, and they can be affected within minutes of flooding (Drew 1997). In this study, root ADH activities increased with flooding in all flooding-tolerant and flooding-sensitive species (Fig. 1), indicating oxygen deficiency following three days of flooding. Traditionally, the greatest increases in root ADH activity under flooding were interpreted to occur in species that are most sensitive to flooding (Crawford 1967). Indeed, in the present study, ADH activities did not increase with flooding in roots of the wetland grass *Phalaris arundinacea*, indicating no oxygen stress following three days of flooding. However, there is now much evidence to suggest flooding tolerant plants can also respond to hypoxia with increased ADH activities (e.g., John and Greenway 1976; Smith and ap Rees 1979; Mendelssohn, McKee, and Patrick 1981). Accordingly, there is more than one way to interpret increased ADH activities under flooding. For example, when sensitive tissues are flooded for extended periods, carbohydrate supplies can become exhausted, and glycolysis collapses as tissues die. This would result in decreased activities of ADH or other associated enzymes (Sairam et al. 2009). Thus, one might expect increased ADH activities in roots of sensitive species shortly after flooding, but then decreased activities thereafter. Measurement of ADH activity after three days of flooding in the present study allowed characterization of oxygen stress and correct interpretation of increased ADH activity in sensitive plants.

There was evidence of increased oxygen stress with flooding across species, with the exception of the wetland grass *P. arundinacea* (Fig. 1). This agrees with data presented by Crawford (1978) for *P. arundinacea*, in support of the hypothesis of low ADH activities in the most tolerant species. This could also provide evidence of the capacity for internal oxygen transport through aerenchyma tissue by *P. arundinacea*, a common practice in wetland species, which could alleviate any oxygen deficiency (Maricle et al. 2006). The advantage of this transport tissue in wetland plants like *P. arundinacea*, compared to upland species, is a likely explanation for high flooding tolerance

and low ADH activity in *P. arundinacea* (Maricle et al. 2006). An increase in ADH activity after short term flooding indicates oxygen stress in all but the most tolerant plants.

Photosynthesis in Leaves

Photosynthesis is the starting point for carbon metabolism, and serves as the entry point for energy in the plant. Consequently, factors that affect photosynthesis are likely to influence plant health and production (Walker 1992). In the present study, photosynthesis responded differently to flooding, based on relative flooding tolerance of each species. The most sensitive species showed decreased A_{1500} under flooding. Photosynthetic limitations can result from a number of factors, as photosynthesis rates are dependent on stomata and the photosynthetic machinery within the leaf, such as chlorophyll, thylakoid membrane complexes, and stromal enzymes for CO_2 reduction (Farquhar and Sharkey 1982; Baker 2008). Differences in gas exchange measures of photosynthesis, stomatal conductance (g_s), and related parameters like transpiration (E) and internal CO_2 concentration (C_i) can elucidate changes in carbon fixation in the plant, and demonstrate how the environment influences the process (Field, Ball, and Berry 1989). Furthermore, pairing CO_2 fixation with measures of chlorophyll fluorescence can also determine effects of environmental stress on light-harvesting machinery in the leaf (Baker 2008). Light harvesting and carbon fixation processes are related, and simultaneous measures of both can provide a comprehensive assessment of energy processing in the leaf (Edwards and Baker 1993; Maricle and Adler 2011).

In the present study, A_{1500} was significantly higher in the C_4 *Z. mays* compared to all other species, which were C_3 plants (Fig. 2*a*). While there were no species-level differences to relate photosynthesis with flooding tolerance, a species x treatment interaction indicates differential effects of flooding between species. The more tolerant *Z. mays*, *P. arundinacea,* and *V. faba* increased A_{1500} in response to flooding, whereas the more sensitive *P. sativum* and *P. vulgaris* decreased A_{1500} with flooding. This suggests A_{1500} is a strong indicator of flooding sensitivity, and that *V. faba* might be more flooding tolerant than previously hypothesized.

Measures of g_s at 1500 µmol quanta m^{-2} s^{-1} occurred in a pattern similar to A_{1500} (Fig. 2*b*). g_s values in the present study increased with flooding in the wetland species *P. arundinacea*, the flooding-tolerant *Z. mays*, and the flooding sensitive *V. faba*. By contrast, g_s decreased with flooding in the flooding sensitive *P. sativum* and *P. vulgaris*. In many cases, flooding causes stomatal closure (Sojka 1992), which often results in decreased photosynthesis (Pezeshki 2001). However, in the present study, stomatal closure did not reduce photosynthesis in any species, as C_i was not affected by flooding (Fig. 2*c*). Consequently, reductions in A_{1500} in the flooding-sensitive species were due to non-stomatal limitations on photosynthesis (Farquhar and Sharkey 1982). Non-stomatal limitations on photosynthesis can include down-regulation of photosynthetic enzymes (Du et al. 1996; Soares-Cordeiro et al. 2009), low foliar nitrogen concentrations (Heckathorn, DeLucia, and Zielinski 1997), or photodamage from excess light energy (Resco et al. 2008; Mehta et al. 2010). Specific limitations on photosynthesis caused by flooding, and how they differentiate flooding-sensitive from flooding-tolerant species, are an intriguing area for future research.

Furthermore, behavior of stomata (and measures of related processes) can provide meaningful assessment of plant sensitivity to flooding. For example, leaf-level *E* at 1500 µmol quanta m^{-2} s^{-1} occurred in a pattern similar to g_s (Fig. 2*d*). With sufficient air flow over the leaf, g_s becomes the main limitation for *E*, where measures of *E* will closely track g_s (Campbell and Norman 1998). Measures in the present study suggest changes in g_s and *E* might be fairly sensitive indicators of flooding tolerance in plants.

Light-adapted chlorophyll fluorescence measures can distinguish flooding sensitive from tolerant plants, but they do not seem to be as sensitive to flooding as gas exchange parameters. In the present study, steady-state fluorescence (F_s) and light-adapted minimal fluorescence (F_0') decreased with flooding (Fig. 3*a*-*b*). Decreases of F_0' indicate down-regulation of PSII (Heckathorn, DeLucia, and Zielinski 1997). Light-adapted maximal fluorescence (F_M') was not changed by flooding, but a significant species x treatment interaction indicates differential responses by species. Dark-adapted F_M often decreases with abiotic stress (Panda, Sharma, and Sarkar 2008). Decreases of F_M' (Fig. 3*c*) also indicate an increase in photochemical quenching in droughted plants, which help prevent damage to PSII (Müller, Li, and Niyogi 2001). Similarly, the proportion of absorbed light energy allocated to photochemistry (P) was highest in the more flooding tolerant species, and lower in flooding sensitive species (Fig. 4*a*). However, there was no clear relationship between flooding tolerance and the proportion of absorbed light energy lost as heat (D). In a manner similar to many other photosynthesis and fluorescence measures, P was significantly higher in flooded plants compared to drained plants in the present study. Short term flooding apparently increases the efficiency of light-harvesting processes.

Measures of photosynthetic light harvesting processes during flooding could help detect photodamage, and potentially distinguish flooding tolerant from flooding sensitive plants. In the present study, down-regulation of PSII and increased photoprotection were most evident in *P. vulgaris*. This could indicate one factor contributing to flooding sensitivity in *P. vulgaris* is susceptibility of PSII to damage during flooding. However, there was no evidence of damage to the PSII reaction center in the present study. These plants all had adequate down-regulation and photoprotection of PSII to prevent damage following three days of flooding. Φ_{PSII} has been shown to decrease beyond 10 days of flooding in sensitive *Citrus* plants, whereas tolerant lines maintained high Φ_{PSII} (Arbona et al. 2009). However, Φ_{PSII} was measured to decrease beyond three days of flooding in tolerant *Myrica cerifera* L. (Naumann, Young, and Anderson 2008). In the present study, Φ_{PSII} increased with flooding across species (Fig. 3*d*), suggesting tolerance to short-term flooding, and an ability to maintain photochemistry despite environmental stress. If high Φ_{PSII} is paired with decreased CO_2 fixation during flooding (e.g., *P. vulgaris* and *P. sativum*), an excess of light energy could result. Indeed, X was significantly higher in the flooding-sensitive *P. vulgaris* compared to all other species. Measures of Φ_{PSII} during longer flooding treatments could help provide insight into susceptibility of PSII to flooding-induced photodamage.

Characters of Flooding Tolerance in Plants

Effects of flooding have broad impacts on plant distributions and production.

Six percent of the world's land area is classified as wetlands (Armstrong, Brandle, and Jackson 1994). Long-term records indicate 15% of U.S. soils are subjected to flooding, and 18.5% of insurance payouts for crop damage in the U.S. from 1939 to 1978 resulted from excess water or flooding (Boyer 1982). Understanding the means by which plants respond to flooding has tremendous importance for ecological and economic reasons.

Flooding tolerant plants are those that are able to maintain photosynthetic CO_2 uptake during flooding, and can maintain respiration in submerged tissues. Stomatal conductance during flooding is also a meaningful measure of plant tolerance to flooding. In fact, the most sensitive measures of flooding response appear to be gas exchange measures on leaves. Stomatal conductance and photosynthesis decreased with flooding in sensitive species, and increased in tolerant species. In the present study, the wetland grass *Phalaris arundinacea* displayed the most flooding-tolerant attributes with increased photosynthetic rates and stomatal conductance and unaffected root respiration in flooding treatments. The flooding-tolerant *Zea mays* and the broad bean *Vicia faba* were the next most flooding tolerant, as indicated by increased photosynthesis rates and stomatal conductance with flooding, but both displayed signs of oxygen stress. An ability to maintain anaerobic respiration in submerged tissues is viewed as an adaptation to flooding (e.g., Mendelssohn, McKee, and Patrick 1981), but the limited ATP production of fermentation can cause respiration to collapse and tissues to die under longer oxygen limitation (Sairam et al. 2009). The flooding-sensitive P*haseolus vulgaris* and *Pisum sativum* were the most impacted by flooding, particularly *P. vulgaris*. Root ADH activities increased in response to flooding, indicating oxygen stress in submerged tissues. Photosynthesis and stomatal conductance decreased in flooding treatments, and there was evidence of increased photoprotection with flooding in *P. vulgaris*. An ability to allocate light energy to photochemistry during flooding also appeared to be related to flooding tolerance. P was higher in the more flooding-tolerant species compared to flooding-sensitive species.

In summary, plant flooding tolerance is mainly determined by an ability to maintain respiratory processes in affected tissues. Tolerance to flooding can be assessed *in situ* with leaf-level measures like photosynthesis and stomatal conductance. To a lesser extent, light-adapted chlorophyll fluorescence can also be used to assess effects of flooding in plants. Many fluorometers are field portable instruments, potentially facilitating use of this measure in natural settings. Tissue-level measures of ADH activity can also provide meaningful comparisons, but ADH activities must be interpreted with caution, as increases or decreases can result for different reasons. Combining photosynthetic and respiratory responses to flooding can provide a broader understanding of how plants are affected by environmental stress.

Acknowledgments

This study was partially supported by the Fort Hays State University Department of Biological Sciences, the College of Health and Life Sciences Tomson Trust, the Li-Cor LEEF program, and the FHSU Office of Scholarships and Sponsored Projects Undergraduate Research Experience (URE) program.

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Abbreviations

A_{1500} = photosynthetic CO_2 uptake at 1500 µmol quanta m^{-2} s^{-1} (µmol CO_2 m^{-2} s^{-1}); ADH = alcohol dehydrogenase; C_i = internal (intercellular) concentration of CO_2 (ppm); D = proportion of absorbed light energy lost to thermal dissipation; E = transpiration from leaf (mmol H_2O m^{-2} s^{-1}); F_s = steady-state PSII fluorescence in the light; F_M' = maximum fluorescence yield from PSII following a saturating pulse of light in a light-adapted plant; F_0' = minimum light-adjusted fluorescence after a brief dark period in a light-adapted plant; g_s = stomatal conductance to water vapor (mol m^{-2} s^{-1}); P = proportion of absorbed light energy allocated to photochemistry; PPFD = photosynthetic photon flux density (400-700 nm); PSII = photosystem II; q_P = photochemical quenching, a measure of the proportion of oxidized electron carriers in thylakoids; Φ_{PSII} = quantum yield of PSII, calculated as $(F_M' - F_s)/F_M'$.