

Stomatal conductance correlates with flooding tolerance in *Phragmites australis* and *Sorghum halepense*

Elizabeth F. Waring^{1,2} and Brian R. Maricle²

1. Department of Biological Sciences, Box 43131, Texas Tech University, Lubbock, Texas 79409-3131
2. Department of Biological Sciences, Fort Hays State University, 600 Park St. Hays, Kansas, 67601-4099, Corresponding author: brmaricle@fhsu.edu

Measurements of stomatal conductance and transpiration can provide information on plant carbon gain and water loss, which can easily be used to estimate a plant's ability to tolerate flooding. In this study, flooding-sensitive *Sorghum halepense* and flooding-tolerant *Phragmites australis* were flooded to 8 cm depth or kept dry for 7 days. Transpiration, stomatal conductance, boundary layer conductance, and vapor conductance were measured for each. In *S. halepense*, transpiration was significantly higher in dry treatments compared to flooded treatments. However, in *P. australis* transpiration was significantly higher in flooded treatments compared to dry treatments. Boundary layer conductances were not different between species or treatments. *Phragmites australis* had increased stomatal conductance when flooded, which indicates a high physiological tolerance to waterlogged soils. By contrast, stomatal conductance in *S. halepense* was decreased under flooding, indicating a greater sensitivity to flooding. Based on these differences between *P. australis* and *S. halepense*, leaf-level stomatal conductance appears to serve as a quick indication of flooding tolerance in plants.

Keywords: environmental physics, flooding tolerance, invasive species, stomatal conductance, wetlands

INTRODUCTION

Flooding is one of the most prominent stresses on wetland plants (Mitsch and Gosselink 2007). This stress comes from anoxia, resulting from a displacement of oxygen from soil spaces, and from the limited solubility of oxygen in a dissolved state (Armstrong, Brandle, and Jackson 1994).

Flooding-induced anoxia at the roots can influence stomata in leaves. A lack of O₂ obtained by roots during flooding commonly causes the plant to close stomata (Colmer 2003). Stomatal conductance (g_{vs}) is a measure of stomatal density and the extent individual stomata are open. Such measures are useful when working with stressed plants, as they describe basic leaf physiology and general responses to the environment. Stomata are

important, as they influence both transpiration and photosynthesis. In turn, stomatal conductance measurements are easy to obtain and they can be used to assess the flooding tolerance of a species.

In the present study, *Phragmites australis* (Cav.) Trin. ex Steud. (common reed) and *Sorghum halepense* (L.) Pers. (johnsongrass) were subjected to flooding in greenhouse treatments. Leaf-level physiological responses were measured to determine stomatal activity during flooding. It was hypothesized the high flooding tolerance of *P. australis* (USFWS 1988) would manifest itself as an increase in transpiration, stomatal conductance, and vapor conductance under flooding. By contrast, the higher sensitivity of *S. halepense* to flooding (USFWS 1988) was expected to be measurable as a decrease in transpiration, stomatal

conductance, and vapor conductance under flooding. Such information could become useful in assessing general patterns of flooding tolerance.

Physiological tolerance to flooding can become relevant ecologically. For example, *P. australis* can move oxygen internally from leaves to roots, which allows it to be tolerant of flooding (Colmer 2003; Colmer and Flowers 2008). Internal oxygen transport helps the plant maintain aerobic respiration, which allows the plant to keep stomata open during flooding. A high degree of flooding tolerance has been demonstrated in *P. australis* through extensive work (e.g., Gries, Kappen, and Losch 1990; Armstrong and Armstrong 1990). By contrast, very little work has been done regarding the flooding tolerance of *S. halepense* (Taylor and Smith 2005; Waring and Maricle 2012), making these results novel in terms of the physiology of *S. halepense*. Therefore, the results of this study are intended to expand general knowledge of flooding tolerance in plants, and support the idea that stomatal conductance and transpiration rates are easily obtainable measures that can be used to estimate flooding tolerance.

MATERIALS AND METHODS

Data Collection: *Phragmites australis* and *Sorghum halepense* were obtained near the Wilson Lake Wildlife Area, near Sylvan Grove, KS, USA (38° 49' N, 98° 28' W). Individual plants were planted with potting soil in pots that measured 11 cm × 11 cm × 10 cm and were maintained under greenhouse conditions in Hays, KS, USA for 9 weeks prior to experimentation. Greenhouse conditions consisted of natural lighting, with midday PPFD near 680 μmol quanta m⁻² s⁻¹ and midday temperatures near 31°C during plant establishment. For experimentation, 10 individuals each of *P. australis* and *S. halepense* were selected randomly from plants of similar age and height. Five individuals of

each species were placed in a tub measuring 50 cm × 36 cm and flooded to a depth of 8 cm, enough to submerge the soil completely. The other five individuals of each species were kept on a greenhouse bench in dry conditions; these plants were watered once per week and excess water was allowed to drain from pots. After seven days of treatment, data were collected using the youngest, fully-expanded leaf on each plant. Measured leaves appeared healthy on all plants. All data were collected during midday hours on 12 November 2009 in the Fort Hays State University greenhouse (Hays, KS). Conditions during measurements included natural lighting with a mean PPFD of 202 μmol quanta m⁻² s⁻¹, mean relative humidity was 0.50, and air temperature was 26 °C. There was an average of 10 hours of daylight during the 7 days of treatment. Although autumn light conditions in the greenhouse were lower than summertime values, they still allow comparisons between species in response to experimental flooding.

Wind speed (u) (m s⁻¹) was measured with an AM-4204 hot wire anemometer (Lutron Electronic Enterprise Co., Ltd.; Taipei, Taiwan). Dry bulb (T_a) and wet bulb (T_w) temperatures (°C) in the greenhouse were measured with a digital psychrometer (Extech Instruments RH300; Waltham, MA, USA). Lastly, a SC-1 Leaf Porometer (Decagon Devices, Inc.; Pullman, WA, USA) was used to measure stomatal conductance (g_{vs}) (mmol m⁻² s⁻¹) and leaf temperature (T_l) (°C) near the middle of the abaxial surfaces of leaves. Adaxial g_{vs} was far lower (i.e., near zero) compared to g_{vs} on abaxial leaf surfaces, so abaxial g_{vs} was used for calculations.

Data analysis: Vapor pressure (e_a , kPa) in the greenhouse was calculated after Campbell and Norman (1998) as:

$$e_a = e_s(T_w) - \gamma \times p_a(T_a - T_w) \quad [1]$$

where $e_s(T_w)$ is the saturated vapor pressure at the wet bulb temperature (kPa), γ is the psychrometer constant ($6.66 \times 10^{-4} \text{ }^\circ\text{C}^{-1}$), and p_a is atmospheric pressure (94.05 kPa at the study location).

The mode of exchange between leaf and surroundings was determined to be forced convection (Campbell and Norman 1998). Water vapor conductance of the boundary layer (g_{va} , $\text{mol m}^{-2} \text{ s}^{-1}$) around the leaf was determined for forced convection after Campbell and Norman (1998) as:

$$g_{va} = 0.147 \sqrt{\frac{u}{d}} \quad [2]$$

where u is wind speed (m s^{-1}) and d is the characteristic dimension of the leaf (m), found by multiplying the maximum leaf width by 0.72 (Campbell and Norman 1998). Total conductance to water vapor (g_v , $\text{mol m}^{-2} \text{ s}^{-1}$) for the leaf was determined as:

$$g_v = \frac{1}{\frac{1}{g_{va}} + \frac{1}{g_{vs}}} \quad [3]$$

where g_{vs} was determined with the porometer. Transpiration rate (E , $\text{mol m}^{-2} \text{ s}^{-1}$) was determined after Campbell and Norman (1998) as:

$$E = \frac{g_v [e_s(T_L) - e_a]}{p_a} \quad [4]$$

where $e_s(T_L)$ is saturated vapor pressure at leaf temperature, and p_a is atmospheric pressure (94.05 kPa).

Statistical analysis was performed using SPSS v. 12 (2003, SPSS Inc.; Chicago, IL, USA). Two-way analysis of variances (ANOVA) were used to test for differences among species and treatments ($\alpha=0.05$).

RESULTS AND DISCUSSION

One way to measure physiological responses to environmental changes (including flooding) is to measure stomatal conductance of leaves. Leaf stomatal conductance (g_{vs}) is easy to measure with hand-held porometers, and g_{vs} is fairly sensitive to environmental changes (e.g., Farquhar and Sharkey 1982). Additionally, these results demonstrate the simplicity in measuring stomatal responses and transpiration. Stomatal conductance could be a rapid method to assess general flooding tolerance of plants.

In this study, mean leaf stomatal conductance to water vapor (g_{vs}) ranged from 20 to 61 $\text{mmol m}^{-2} \text{ s}^{-1}$ across species and treatments (Fig. 1a). Similar results were reported by Pagter, Bragato, and Brix (2005) for *Phragmites australis* in native European populations. The present results are somewhat lower than field measures of g_{vs} for plants in wetland settings reported by Oue (2001) and Teal and Kanwisher (1970). Low light levels in the greenhouse in the present study most likely resulted in g_{vs} and E values lower than one would expect under full sunlight. Nonetheless, there was a clear difference in g_{vs} between species in their response to flooding, illustrated by a significant interaction among species and treatment ($p<0.001$). By contrast, there was no difference between species ($p=0.158$) or treatments ($p=0.053$) in g_{vs} . The significant interaction of species and treatment supports the hypothesis that g_{vs} would increase in flooded conditions for *P. australis*, but decrease in flooded conditions for *S. halepense*.

Mean leaf boundary layer conductance to water vapor (g_{va}) ranged from 648 to 799 $\text{mmol m}^{-2} \text{ s}^{-1}$ across species and treatments (Fig. 1b). These figures are far greater than stomatal conductances (g_{vs}), indicating there was sufficient air flow in the greenhouse to allow adequate mixing, and g_{va} was the main influence on total leaf conductance (g_v) and

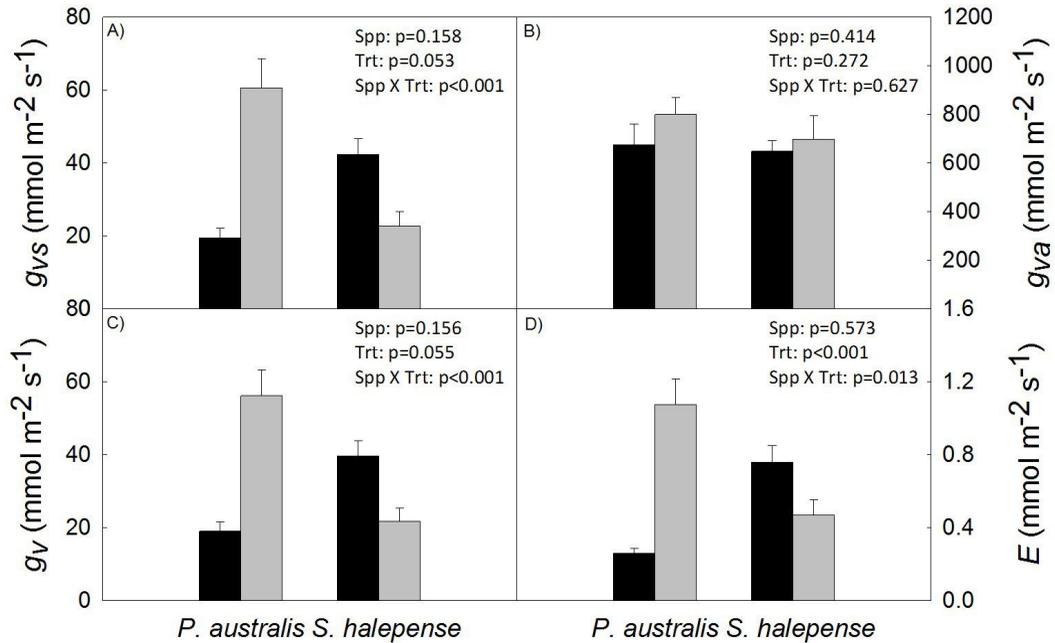


Figure 1. Effects of flooding on transpiration and vapor conductances in *P. australis* and *S. halepense*. (A) stomatal conductance to water vapor, g_{vs} , (B) boundary layer conductance to water vapor, g_{va} , (C) total conductance to water vapor, g_v , and (D) transpiration, E . Bars show the mean of 5 replicates \pm standard error. Black bars indicate dry plants and gray bars indicate flooded plants. Direct statistical effects and interaction terms are indicated for each measure.

transpiration (E). There was no significant difference in g_{va} between treatments ($p=0.272$), species ($p=0.414$), or their interaction ($p=0.627$). Equation 2 demonstrates g_{va} is dependent on wind speed and the characteristic dimension of the leaf. Leaf width in *P. australis* averaged 8.3 mm, whereas *S. halepense* leaves averaged 11.8 mm (data not shown). There was not enough difference in leaf width between species to influence g_{va} statistically.

Mean total leaf conductance to water vapor (g_v) ranged from 19 to 56 $\text{mmol m}^{-2} \text{s}^{-1}$ across species and treatments (Fig. 1c). These numbers are similar to greenhouse measures of g_v in salt marsh grasses by Maricle, Cobos, and Campbell (2007). There were no significant differences in g_v between species ($p=0.156$) or treatments ($p=0.055$). A significant interaction of species and treatment ($p<0.001$) in g_v indicates similar responses to flooding between g_v and

g_{vs} . Flooded conditions increased g_v and g_{vs} in *P. australis*, but decreased g_v and g_{vs} in *S. halepense*.

Mean transpiration rates from leaves (E) ranged from 0.26 to 1.1 $\text{mmol m}^{-2} \text{s}^{-1}$ across species and treatments (Fig. 1d). These numbers are lower than field measures of E in a salt marsh (Teal and Kanwisher 1970) or a prairie wetland (Jacobs et al. 2002), but are only slightly lower than greenhouse measures of salt marsh plants by Maricle, Cobos, and Campbell (2007). A lower value of E in greenhouse studies compared to field studies illustrates the influence of light conditions on stomatal conductance and transpiration. Nonetheless, meaningful differences were detected between *P. australis* and *S. halepense* under greenhouse conditions. In the present study, E was significantly different between treatments ($p<0.001$) and there was a significant interaction of treatment and species

($p=0.013$). By contrast, E was not different between species ($p=0.573$). The significant interaction of treatment and species supports the hypothesis that E would decrease in *S. halepense* and increase in *P. australis* in flooded conditions.

The hypothesis that flooding would increase stomatal conductance and transpiration in *P. australis* was supported. Similarly, the hypothesis that flooding would decrease the stomatal conductance and transpiration in flooded *S. halepense* was also supported (Fig. 1d). There were no significant differences in g_{vs} , g_v , or E between dry *S. halepense* and flooded *P. australis*. However, when considering differences in species as well as treatments, the different adaptations to flooded conditions of *S. halepense* and *P. australis* become apparent in a significant interaction.

Increase of transpiration was correlated with an increase in stomatal conductance. *Phragmites australis* had an increase in E and g_{vs} under flooding, which is related to tolerance of waterlogged soils in field settings. However, decreased E and g_{vs} under flooded conditions in *S. halepense* indicate decreased potential for photosynthesis. The inability of the plants to remove oxygen from the soil caused them to close stomata. However, more research is needed to determine the extent of flooding tolerance in *S. halepense*.

The methods used in this study could easily be performed in field settings. Measurements of stomatal conductance, leaf temperature, leaf width, wind speed, and dry and wet bulb temperatures can be obtained in under a minute for each plant measured. Applying those data to the equations listed earlier is an accurate way to assess the ability of a plant to tolerate flooding. According to previously published work, *P. australis* is flooding tolerant and *S. halepense* is not flooding tolerant. The results of this study support this and give credence to the idea that g_{vs} and E can be used to measure flooding tolerance of other species.

ACKNOWLEDGMENTS

This project was partially funded by the Department of Biological Sciences at Fort Hays State University. The authors thank Susan Eaton for help with plant maintenance.

LITERATURE CITED

- Armstrong, J. and Armstrong, W. 1990. Light-enhanced convective throughflow increases oxygenation in rhizomes and rhizosphere of *Phragmites australis* (Cav.) Trin. ex Steud. *New Phytologist* 114:121-128.
- Armstrong, W., Brandle, R. and Jackson, M.B. 1994. Mechanisms of flood tolerance in plants. *Acta Botanica Neerlandica* 43:307-358.
- Campbell, G.S. and Norman, J.M. 1998. *An Introduction to Environmental Biophysics*. 2nd ed. Springer Science, New York, 286 pp.
- Colmer, T.D. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. *Plant, Cell and Environment* 26:17-36.
- Colmer, T.D. and Flowers, T.J. 2008. Flooding tolerance in halophytes. *New Phytologist* 179:964-974.
- Farquhar, G.D. and Sharkey, T.D. 1982. Stomatal conductance and photosynthesis. *Annual Review of Plant Physiology* 33:317-345.
- Gries, C., Kappen, L. and Losch, R. 1990. Mechanism of flood tolerance in reed, *Phragmites australis* (Cav) Trin ex Steudel. *New Phytologist* 114:589-593.
- Jacobs, J.M., Mergelsberg, S.L., Lopera, A.F. and Myers, D.A. 2002. Evapotranspiration from a wet prairie wetland under drought conditions: Paynes Prairie Preserve, Florida, USA. *Wetlands* 22:374-385.
- Maricle, B.R., Cobos, D.R. and Campbell, C.S. 2007. Biophysical and morphological leaf adaptations to drought and salinity in saltmarsh grasses. *Environmental and Experimental Botany* 60:458-467.
- Mitsch, W.J. and Gosselink, J.G. 2007. *Wetlands*, 4th ed. John Wiley and Sons, Inc, New York, 600 pp.

- Oue, H. 2001. Effects of vertical profiles of plant area density and stomatal resistance on the energy exchange processes within a rice canopy. *Journal of the Meteorological Society of Japan* 79:925-938.
- Pagter M., Bragato, C. and Brix, H. 2005. Tolerance and physiological responses of *Phragmites australis* to water deficit. *Aquatic Botany* 8:285-299.
- Taylor, J.P. and Smith, L.M. 2005. Migratory bird use of belowground foods in moist-soil managed wetlands in the Middle Rio Grande Valley, New Mexico. *Wildlife Society Bulletin* 33:574-582.
- Teal, J.M. and Kanwisher, J.W. 1970. Total energy balance in salt marsh grasses. *Ecology* 51:690-695.
- [USFWS] U.S. Fish and Wildlife Service. 1988. *National list of vascular plant species that occur in wetlands*. U.S. Fish & Wildlife Service Biological Report 88 (26.9).
- Waring, E.F. and Maricle, B.R. 2012. Photosynthetic variation and carbon isotope discrimination in invasive wetland grasses in response to flooding. *Environmental and Experimental Botany* 77:77-86.