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Physiological Response of Wax Begonia to Heat and Light Stress

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Summary

Wax begonia (*Begonia ×semperflorens-cultorum*) is a common ornamental plant used in flower beds for its diverse flower coverage to beautify public spaces. The intense Florida summers can increase its greenhouse production costs and hinder its year-round landscape potential, especially in full sun conditions. The physiological response of wax begonia to stress associated with heat, drought and light is not well understood but necessary for plant selection and improvement. Experiments were conducted to compare physiological plant responses (i.e., photosynthesis, fluorescence, and ion leakage) of four different wax begonia genotypes (FB08-059, OPGC 5104, 'Sprint White' and 'Cocktail Vodka') grown under light 35/22.5 °C or shade (30/22.5°C) conditions for 41 d. Results showed that when stressed (nonshaded and hot) FB08-059 (a noncommercial red genotype) had greater stomatal conductance (0.23 compared to 0.12-0.16 mol m^{-2} s^{-1}), greater chlorophyll fluorescence (0.7-0.75 compared to 0.45-0.64), and less ion leakage (11.91% compared to 20.34%-33.72%) than the other three genotypes. Results of this study combined with subsequent mor-

phological data are foundational for breeding enhanced abiotic stress tolerance in wax begonia. Additional studies are in place to measure physiological stress and severe drought tolerance of these same genotypes.

INTRODUCTION

Commonly called wax begonia, *Begonia ×semperflorens-cultorum* refers to a group of cultivated hybrid begonias derived from *Begonia cucullata* and *Begonia schmidtiana* (Hvoslef-Eide and Munster, 2007; Neale et al., 2006). These fibrous-rooted begonias are primarily used as bedding plants for lining walkways, roads, and flowerbeds. The market for begonias has increased to over $130 million with production primarily in Florida (USDA, 2020). Plants thrive in cooler parts of the year but decline noticeably in the summer months of southern states due to heat stress. The development of new cultivars withstanding high temperatures and intense sunlight is much needed to expand the year-round utilization of wax begonia in warm climates of the U.S. and beyond.

With global temperatures expected to increase between 1.8 and 4 °C in the next eighty years (Hasanuzzaman et al., 2013), it is essential to better understand plant response to heat and intense UV light as a consequence of climate change. The negative effects of heat stress and elevated UV light on agricultural crop production are already evident, with significant yield losses that may lead to global food insecurity (Christensen and Christensen, 2007). Despite their economic and ecological importance, how heat stress and/or elevated UV light physiologically affect ornamental plants is less studied compared to row crops. Under stress conditions, physiological responses in plants are triggered to protect against stress-induced damage (Abdelmageed and Gruda, 2009). Carbon assimilation, indicative of photosynthetic efficiency, is highly sensitive to various environmental stresses. For example, Urban et al. (2017b) found that carbon assimilation of poplar (*Populus deltoide*s × *nigra*) and loblolly pine (*Pinus taeda*) was significantly reduced under high temperatures (40 °C), a response due to increased stomata closure and photorespiration (Farquhar and Sharkey, 1982; Urban et al., 2017a). While heat stress responses of agronomic crops have been extensively studied, research on the effect of such environmental stress on ornamentals including the top bedding plant, wax begonia, is lacking. As such, the following study was conducted to understand heat stress responses in wax begonia. Specific objectives were to compare photosynthesis, chlorophyll fluorescence and ion leakage among four wax begonia genotypes grown under shade and non-shaded conditions.

MATERIALS AND METHODS

Plant Material and Light Treatments. Four different wax begonia genotypes, two with red foliage and two with green foliage, were propagated from cuttings and grown for ten weeks in a low light greenhouse (PAR value of 750 μmol m^{-2} s^{-1}) with day/night temperatures of 28/23 °C (**Fig. 1**). FB08-059 is a putatively heat-tolerant begonia with dark green/red foliage in low light environments (Pounders et al., 2015); OPGC 5104 is a bright-green wild begonia

that was collected from Hawaii. 'Sprint White' and 'Cocktail Vodka' are two commercial cultivars with green and red foliage, respectively. Plugs were transplanted to 1-gal. pots filled with a bark and peat- based soilless media (Premium Nursery and Veg Mix, Reliable Peat Company, Leesburg, FL) supplemented with 5gm/qt 14N-14P-14K Osmocote slow- release fertilizer two weeks prior to the commencement of the experiment. The plants were randomly assorted into two experimental groups, non-shaded and shaded.

The non-shaded treatment had a peak PAR value of 2100 µmol m^{-2} s^{-1} and day/night temperatures of 35/22.5 °C while the shaded treatment PAR value peaked at 750 µmol m^{-2} s^{-1} with day/night temperatures of 30/22.5 °C. Relative humidity fluctuated between 65% and 100% depending on time of day in both treatment groups. Water was consistently available through either rain or above ground sprinklers to eliminate the effect of drought conditions on plant response.

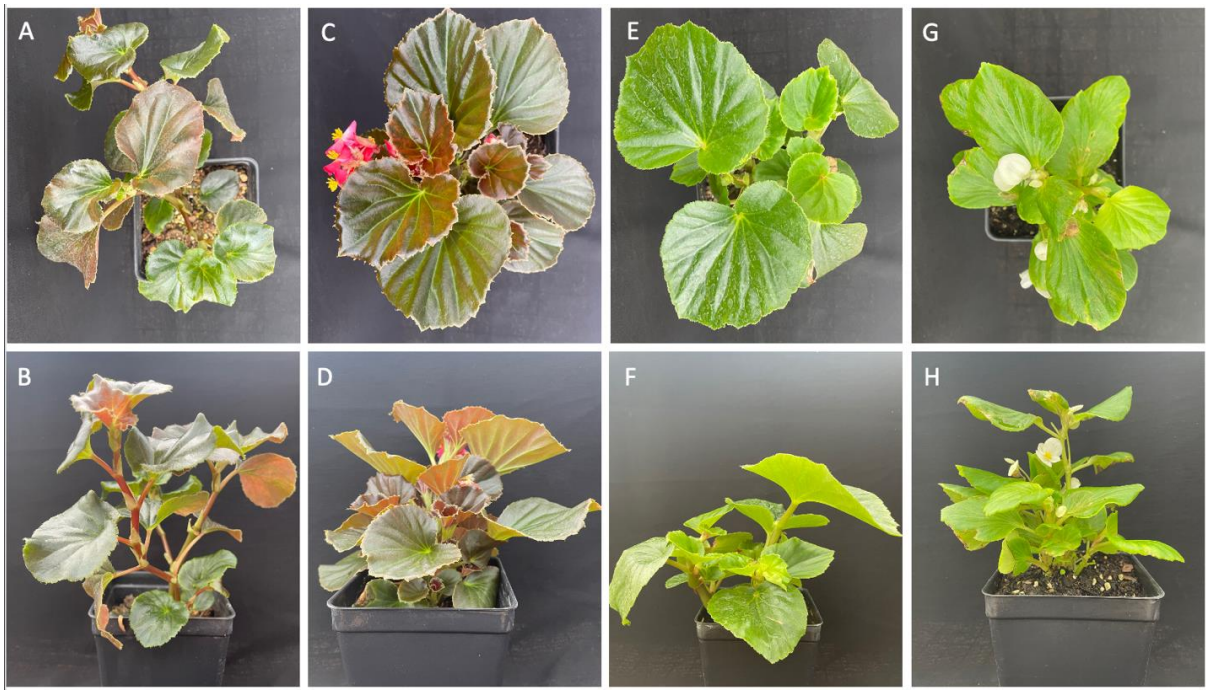

Figure 1. Top and side views of wax begonia genotypes FB08-059 (A,B), 'Cocktail Vodka' (C,D), OPGC 5104 (E,F), and 'Sprint White' (G,H) utilized to examine stress tolerance under shaded and non-shade conditions. Images were taken ten weeks after initial cutting, prior to commencement of the study.

Measurements of Stress Response. Carbon assimilation, stomatal conductance, and transpiration were measured throughout the 41-d study using a LiCOR-6800 Portable Photosynthesis System (Lincoln, NE). After stabilization, three readings were taken (30 sec. apart) for each plant at peak daylight hours, between 12pm and 4pm, to ensure stability in the chamber. The Fv/Fm measurements were taken with an OS30p chlorophyll fluorometer (Opti-Sciences, Hudson, NH). All plants were measured two hours prior to dawn to allow the plant to enter a dormant state overnight prior to taking measurements. Readings were taken from three fully expanded

leaves per plant, with which mean Fv/Fm was recorded. Leaves were handled gently to avoid any damage. For measurement of ion leakage, three leaf disks from each leaf sample were placed each in 5mL of deionized water for four hours. Initial readings for each leaf disc were taken using an Orion Star A215 conductivity meter (Waltham, MA). Second measurements were performed with the same samples after being autoclaved for 20 min. and allowing to cool for 15 min.

Statistical Analysis. A completely random block design with three replications for each block and four plants per genotype for each replication were utilized for each light treatment. Mean value with the standard error (n=12) was calculated for each measured trait at each time interval. Final data collected at day 41, were subjected to a two-way analysis of variance (genotype x light) and when appropriate significant means were separated using Tukey's honestly significant difference test at $P \leq 0.05$ (Agricolae package in R studio, Boston, MA). Images for leaf folding were taken with a Nikon and leaf angle determined using ImageJ. ROS accumulation leaf images were modified using GIMP (version 2.10) followed by quantification in ImageJ (Bethesda, MD).

RESULTS

Photosynthetic Parameters. The interaction between genotype x light was nonsignificant for carbon assimilation (P=0.1772), and significant for both transpiration (P=0.0025) and stomatal conductance (P=0.0006). For the non-shaded plants, response in photosynthesis was nonsignificant among genotypes, remaining steady at 9-10 μmol m^{-2} s^{-1} for much of the experiment (**Fig. 2A**). Transpiration of non-shaded genotypes was also nonsignificant (**Fig. 2B**), yet stomatal conductance of FB08-059 (red, noncommercial genotype) was 1.6 times greater compared to all other genotypes **(Fig. 2C**).

When shaded, commercial genotypes had noticeably higher carbon assimilation by day 12 than noncommercial genotypes; and at 41 days 'Sprint White' assimilated 1.1 times more carbon than OPGC 5104 (Fig. 2A). Under the same shaded conditions, stomatal conductance was similarly high among the commercial genotypes ('Sprint White and 'Cocktail Vodka'), being 43% greater than the OPGC 5104 genotype (green, noncommercial) (**Fig. 2C**).

Chlorophyll Fluorescence. There was a nearly significant genotype effect (P=0.0519) and a strong significant light effect (P=0.0012) on Fv/Fm, with a nonsignificant genotype x light interaction (P=0.2512). At each time interval (days after treatment), chlorophyll fluorescence (Fv/Fm) was greater among genotypes when shaded compared to non-shaded treatments (**Fig. 2B**). At day 41, Fv/Fm of shaded genotypes was 1.08 times greater than non-shaded genotypes. For the duration of the experiment (days 3-41), Fv/Fm values were between 0.45 and 0.71 when non-shaded and 0.68 and 0.76 when shaded. Under direct sunlight and heat, the effect of these stressors on Fv/Fm was much more prominent, resulting in very low Fv/Fm at days 3-6 of the treatment (**Fig. 2B**). However, the Fv/Fm in non-shaded FB08-059 and OPGC 5104 plants gradually increased to levels comparable to the shaded treatment after 20 d.

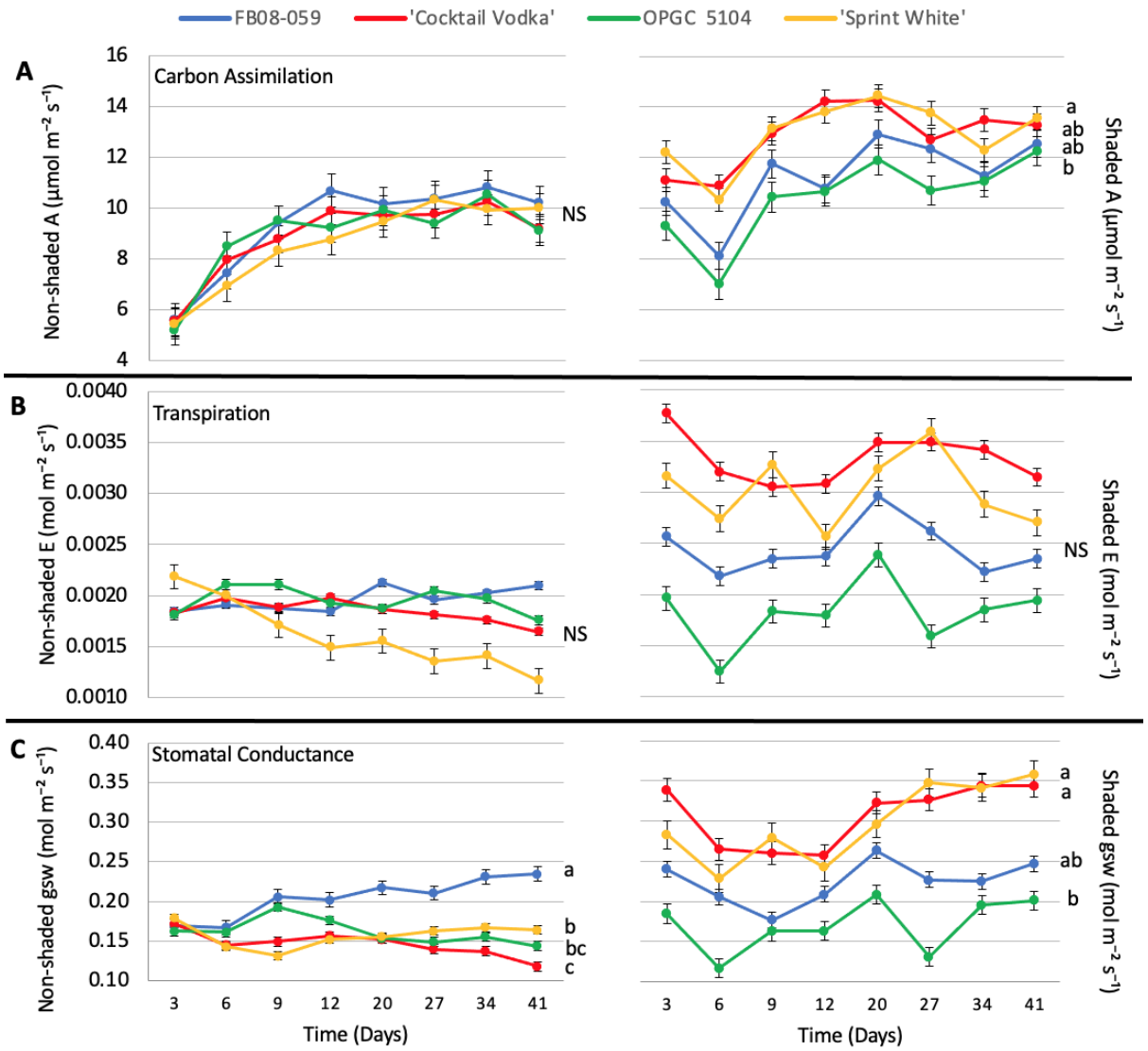

Figure 2. Carbon assimilation (A), transpiration (B), and stomatal conductance (C) responses of wax begonia genotypes (FB08-059, OPGC 5104, 'Sprint White' and 'Cocktail Vodka') grown under non-shaded (graphs to the left) or shaded (graphs to the right) conditions for 41 d. Plants were measured during the peak light and heat intensity between 12pm and 3pm. Bars represent mean ± standard error (n=12). Means followed by a different letter are significantly different according to a Tukey's HSD test, at $P \leq 0.05$.

Ion Leakage. Both light (P<0.0001) and genotype (P<0.0001) affected plant response to stress indicated by K+ leakage with a non-significant interaction (**Fig. 4**; P=0.1859). Percentages of ion leakage in non-shaded genotypes FB08-059 (11.91%) and 'Sprint White' (33.72%) were higher than their corresponding shaded treatments (9.65% and 21.49%, respectively), revealing the detrimental effect inflicted by direct sunlight and heat (**Fig. 4**). In general, non-commercial genotypes had less K+ leakage than commercial genotypes. In particular, FB08-059 had 2.32 times less K+ leakage when non-shaded, and 1.89 times less K+ leakage when shaded compared to all other genotypes in respective treatments.

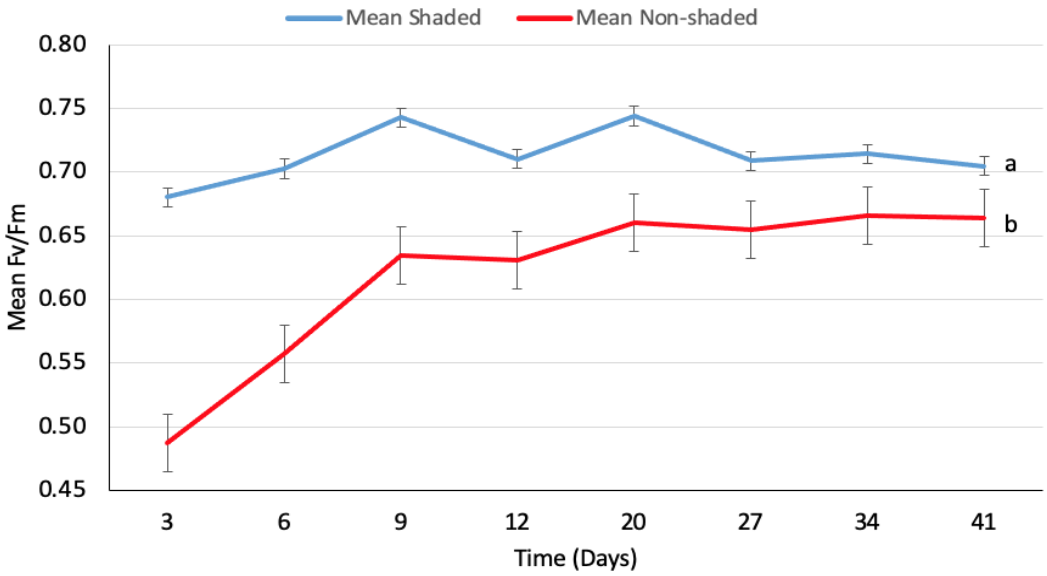

Figure 3. Mean chlorophyll fluorescence (Fv/Fm) responses of four wax begonia genotypes (FB08-059, OPGC 5104, 'Sprint White' and 'Cocktail Vodka') grown under shaded or non-shaded conditions for 41 d. Fv/Fm values at 41d did not differ by genotype (P=0.0519) and there was no genotype × light interaction (P=0.2512). Therefore, main treatment effects of light (P=0.0012) are reported for the combined genotypes. As a reference, Fv/Fm values above 0.70 are indicative of plants not under stress. Bars represent mean ± standard error (n=12). Means followed by a different letter are significantly different according to a Tukey's HSD test, at $P \leq 0.05$ for the day 41 measurements.

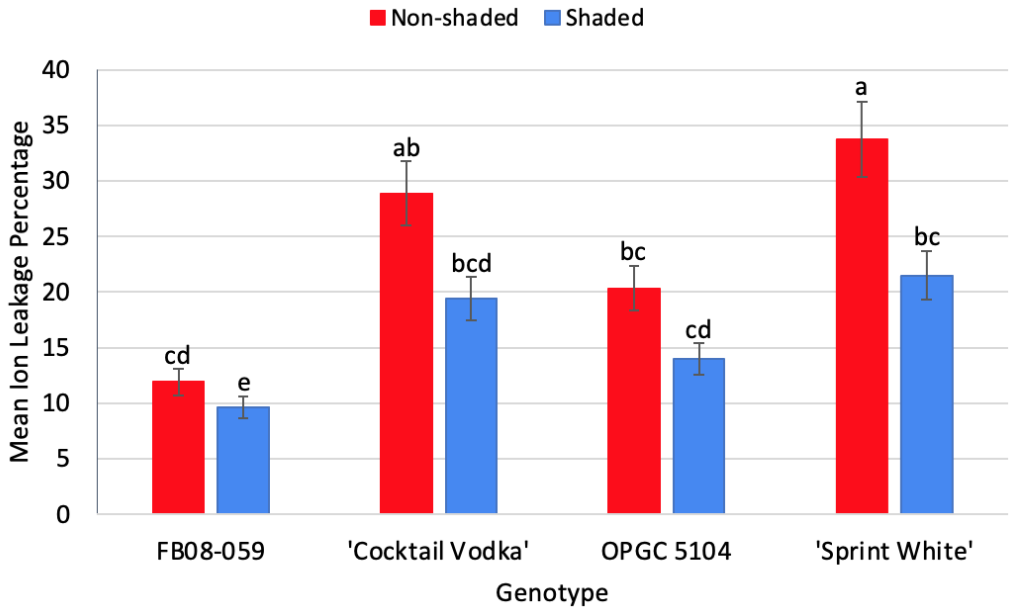

Figure 4. Ion leakage of four wax begonia genotypes (FB08-059, OPGC 5104, 'Sprint White' and 'Cocktail Vodka') grown under shaded or non-shaded conditions for 41 d prior to sample collection. Means followed by the same letter are not significantly different according to Tukey's HSD at P≤0.05. Bars represent mean ± standard error (n=12).

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DISCUSSION

This study compared heat stress tolerance of four wax begonia genotypes grown under shaded and non-shaded conditions and found the non-commercial genotypes to be overall more tolerant to stress (light and heat) than the commercial genotypes. In particular, the FB08-059 genotype grown in full sun had higher stomatal conductance and less K+ leakage than other genotypes grown in the same conditions revealing its pronounced ability to tolerate stress. Comparatively, when shaded (non-stressed), 'Sprint White' (green commercial genotype) had higher carbon assimilation and stomatal conductance than OPGC 5104 (green, non-commercial genotype). It is of interest to consider the effect of foliage color on physiological responses in plants such as anthocyanin and chlorophyll content, cuticle thickness and leaf folding angle. Darker colored leaves of begonia have been shown to have enhanced photoprotective properties, enabling them to better withstand negative environmental stressors (Zhang et al., 2010).

Chlorophyll fluorescence is an important indicator of plant stress well before there are morphological responses. A Fv/Fm between 0.75-0.80 implies a plant is functioning at optimal performance, implying slight stress was present for the shaded treatment but not at a level that negatively impacted the functioning of the plants. It should be noted that, although Fv/Fm was nearly significant among genotypes (P=.0519) there was a general trend where the commercial red and green genotypes ('Sprint White' and 'Cocktail Vodka') had relatively low Fv/Fm values, suggesting they were less tolerant to stress than the red and green non-commercial genotypes (FB08-059 and OPGC 5104).

Ion leakage caused by irreversible membrane damage is another commonly used method to examine plant response to various stresses. In this study, genotypes responded similarly to light treatments where ion leakage was greater for non-shaded plants compared to shaded plants. Also, the non-commercial red and green genotypes (FB08-059 and OPGC 5104, respectively) generally had less ion leakage than the commercial red and green genotypes ('Sprint White' and 'Cocktail Vodka') indicating they were able to tolerate more stress. Interestingly, OPGC 5104 originated from the Hawaiian Islands where it grows in full sunlight and reproduces by seed freely. It's success in a similar environment could explain why this cultivar does not succumb to the same levels of stress response as its commercial counterpart.

CONCLUSION

The results presented herein show genotypic responses to abiotic stress under different environmental conditions. This data along with ongoing studies provide the necessary framework to uncover the physiological and morphological basis of abiotic stress tolerance in begonia, knowledge that is essential to plant breeding programs.

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