Physiological Responses of Ivy Geranium ‘Beach’ and ‘Butterfly’ to Heat Stress

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ABSTRACT. The development of bleaching of the youngest leaves of actively growing ivy geranium (Pelargonium peltatum) has been observed as the season progresses from late spring to summer. Cultivar differences in foliar bleaching in response to elevated air temperature were studied. Ivy geranium ‘Beach’ and ‘Butterfly’ were grown in media containing sphagnum peat and perlite (70:30 v/v) for 6 weeks in modified greenhouse chambers with air temperatures averaging 28/16 or 36/22 °C (day/night). ‘Beach’ had greater plant width, growth index, leaf area, total fresh weight, and total dry weight than ‘Butterfly’ regardless of temperature. Overall, elevated air temperatures severely reduced plant width, plant growth index, leaf area, fresh weight, and dry weight of ivy geraniums. Elevated air temperatures caused foliar bleaching in both cultivars; however, ‘Butterfly’ was more susceptible to bleaching than ‘Beach’. ‘Beach’ had higher chlorophyll (Chl) b and total Chl content than ‘Butterfly’ at ambient air temperature, but they were similar at elevated air temperatures. Regardless of temperature, ‘Beach’ had greater Chl a, carotenoids (Caro), and pheophytins content but lower Chl a:Caro, Chl b:Caro, and total Chl:Caro ratios than ‘Butterfly’. This may contribute to the lower susceptibility to bleaching of ‘Beach’. Elevated air temperatures reduced Chl a, Caro, Chl a:Caro, Chl b:Caro, total Chl:Caro, and pheophytins content of ivy geraniums. In both cultivars, manganese (Mn) content increased with elevated air temperatures, but ‘Beach’ had greater Mn content than ‘Butterfly’. Total iron (Fe) content did not vary with cultivar or temperature. Irrespective of temperature, zinc (Zn) content was greater in ‘Beach’ than ‘Butterfly’, and irrespective of cultivar, Zn content was greater at elevated air temperatures. These results suggest greater chlorophyll, carotenoids, pheophytins, foliar Mn, and Zn contents play a role in reduced susceptibility of ‘Beach’ to foliar bleaching.

High temperatures are a major factor limiting plant growth (Bibi et al., 2008; Xu and Huang, 2008; Zhao et al., 2007). Ivy geranium grows poorly in the heat of southeastern U.S. summers. Optimum temperature for growth of ivy geranium is 20 to 23 °C. Temperatures above 30 °C are quite common during summers in the southeastern states of the United States (Weather Channel, 2008). Elevated air temperatures (heat stress) have been found to cause foliar bleaching in ivy geraniums (Dhir et al., 2011). The newly developing leaves of ivy geranium turn white and lack chlorophyll, are small, and curl upward. Colloquial information and producers’ practices in ivy geranium production indicated heat-induced foliar bleaching could be alleviated using chelated Fe applications.

According to Wahid et al. (2007), heat stress is defined as the rise in temperature beyond a threshold level for a period of time sufficient to cause irreversible damage to plant growth and development. A transient elevation in temperature, usually 10 to 15 °C above ambient, is considered heat stress or heat shock.

Foliar bleaching symptoms resulting from heat stress vary based on species, foliage exposure, and physiological characteristics (Vollenweider and Gunhardt-Goerg, 2005). Heat stress caused leaf yellowing in kentucky bluegrass ([Poa pratensis (He and Huang, 2007)] and rib discoloration in crisphead lettuce [Lactuca sativa (Jenni, 2005)]. Most crops are highly sensitive to heat stress, often resulting in progressively decreasing yields at temperatures above the optimum (Singh et al., 2007). Increased leaf temperatures during the summer are a potential hazard in greenhouses. Low air speed and high humidity during summer can decrease the rate of leaf cooling (Taiz and Zeiger, 2002). Leaf transpiration and internal CO₂ concentration of tomato plants increased with high temperatures (Camejo et al., 2005). Maintenance of transpirational (leaf) cooling was an important factor associated with better performance of kentucky bluegrass under summer heat stress (Bonos and Murphy, 1999).

Temperature stress can lead to inhibition of photosynthesis (Haldimann and Feller, 2005; Sharkey et al., 2001). Temperatures above 30 °C reduced photosynthetic rate as a result of a reduction in photosystem II (PSII) efficiency (Kadir et al., 2006). Photosynthetic response to high temperatures can vary significantly within a species (Reynolds et al., 1990). Net photosynthesis of pea ([Pisum sativum] plants decreased with increasing leaf temperature from 25 to 45 °C (Haldimann and Feller, 2005). Temperatures from 25 to 38 °C slightly decreased photosynthetic rate of lily (Lilium ×formolongo), but temperatures at 44 °C induced a significant reduction in photosynthetic rate (Luo et al., 2008). Rye (Secale cereale) plants growing at
32 °C were deficient in chlorophyll and chloroplastic 70-S ribosomes (Feieraband, 1977). Chlorophyll biosynthesis of cucumber (Cucumis sativus) seedlings was reduced to 60% on heat stress as a result of impairment of Chl biosynthetic enzymes compared with normal growth conditions (Tewari and Tripathy, 1998). The decrease in the ratio of Chl a to Chl b is an indicator of heat bleaching in Euglena gracilis (Thomas and Ortiz, 1995). In addition, phophytin, another Chl, acts as an early electron acceptor in PSII and decreases in concentration inhibit PSII and may indicate heat stress in plants (Groot et al., 1997).

Heat-tolerant genotypes of Phaseolus vulgaris displayed differential responses to high temperatures, suggesting different genetic controls of heat tolerance (Rainey and Griffiths, 2005). ‘Acala’ genotypes of cotton (Gossypium hirsutum) were more tolerant to high temperatures than other genotypes (Bibi et al., 2008). Camellia cultivars originating from C. reticidata and its hybrids had the worst heat tolerance, whereas cultivars of C. sasanqua were more heat-tolerant (Li et al., 2006). Solanum species screened for heat tolerance indicated considerable variation in the degree of chlorosis as a stress response to high temperatures (Reynolds et al., 1990). The higher heat tolerance of Salvia splendens ‘Vista Red’ compared with other S. splendens cultivars tested suggested cultivars with thick, broad leaves and higher stomatal frequency had higher transpirational cooling, gas exchange, and CO2 fixation (Natarajan and Kuehny, 2008). Non-photochemical quenching and antioxidant enzymes were the main mechanisms in seedlings of two lily cultivars, which could effectively protect its photosynthetic apparatus against high temperatures (Luo et al., 2008).

Little is known about foliar bleaching in ivy geraniums and cultivar responses to elevated air temperatures. The objective of the present study was to compare the heat stress responses of two cultivars of ivy geraniums.

Materials and Methods

Plant material, temperature treatment, and sampling. Ivy geraniums ‘Beach’ and ‘Butterfly’ [Fischer Horticulture (Syngenta Flowers), Boulder, CO] were chosen for this study. ‘Butterfly’ has been found to be more susceptible to bleaching than ‘Beach’ (Dhir et al., 2011). Rooted cuttings of ‘Beach’ and ‘Butterfly’ were potted into 1-L containers (15-cm diameter, one plant per container) on 15 Dec. 2006 in sphagnum peat:perlite (70:30 v/v) amended with 0.96 kg m⁻³ gypsum, 7.7 kg m⁻³ limestone, and 0.32 kg m⁻³ wetting agent (SaturAid; Debeco, Tyabb, Australia). Plants were fertigated three times per week with 250 mg L⁻¹ nitrogen from 20N-4.4P-16.6K (Peters Peat-lite 20-10-20; Scotts, Marysville, OH). The plants were grown in a double-layer, inflated, polyethylene greenhouse with 23/20 °C (day/night) venting temperatures for 6 weeks to allow root development.

Six growth chambers (1.37 m width × 1.22 m length × 0.91 m height) were constructed with a single-layer polyethylene (0.1 mm clear) over polyvinyl chloride tubing inside a double-layer polyethylene-covered Quonset greenhouse to maintain treatment temperatures. Two temperature treatments were used: average temperatures of 28/16 °C (day/night, unheated chambers) or 36/22 °C (day/night, heated chambers), with three replications (chambers) per treatment. Heating cables (Gro-Quick Cables; Wrap-On Co., Bedford Park, IL) were run at three levels inside each heated chamber: below, on top of, and 10 cm above the lath benchtop. Heating cables in the heated chambers were controlled using a thermostat (Redi-Heat Model RHT4; Phytotronics, Earth City, MO). Sidewalls of chambers were also raised or lowered to regulate temperature. Air temperature was recorded at hourly intervals with data loggers (WatchDog Model 125; Spectrum Technologies, Plainfield, IL) mounted at canopy level and shielded from the sun. Irradiance inside the growth chambers ranged from 400 to 800 μmol s⁻¹ m⁻² photosynthetic photon flux at canopy level measured between 1000 and 1400 μmol/m²/s from 2 Feb. to 16 Mar. Plants were placed in growth chambers on 2 Feb. 2007 and grown for 6 weeks. Data were collected for plant height (from the rim of the container) and width (an average of widths measured at the widest point and at 90°) at termination of the study, 16 Mar. 2007. Growth index (GI) was calculated as:

\[ GI = \frac{\text{height}}{\text{width}} \times \text{height} \]

Extent of leaf bleaching was determined as a visual rating on a scale of 1 to 7, where 1 = 0%, 2 = 1% to 17%, 3 = 18% to 34%, 4 = 35% to 50%, 5 = 51% to 67%, 6 = 68% to 84%, and 7 = 85% to 100% bleached. Total leaf area was determined using a leaf area meter (LI-3000; LI-COR, Lincoln, NE). For pigment analysis, five 38.5-mm² leaf disks were collected from five recently matured leaves from each plant (one disc per leaf). Each leaf disk was cut into five to six small pieces to aid pigment extraction. Leaf disks from each plant were placed in a vial with 10 mL of 80% acetone. The vials were incubated at 20 °C in the dark for 24 h to allow complete pigment extraction (Chl a, Chl b, and Caro). The pigment concentrations were determined as follows: Chl a = 12.7 × A663nm – 2.69 × A645nm, Chl b = 22.9 × A645nm – 4.80 × A663nm; Caro = (1000 × A700nm – 1.82 × Chl a – 85.02 × Caro) × 1/1.925, where A = absorbance. Pigment concentrations were in micrograms per milliliter. Pigment concentrations were expressed on a leaf area basis (micrograms per square centimeter) using the conversion [chlorophyll (micrograms per square centimeter)] = [chlorophyll (micrograms per milliliter)] × 10/1.925 where 10 is the volume of acetone (milliliters) used for a sample and 1.925 is the total area (square centimeters) of five leaf disks of a sample.

Chlorophyll extracts were acidified with 50 μL of 1 N HCl to measure total pheophytin content of leaves. Eighty percent acetone plus 50 μL of 1 N HCl was used as the control blank. Total leaf pheophytin concentration was determined spectrophotometrically and calculated as follows (Vernon, 1960): pheophytins (micrograms per milliliter) = 6.75 × A665nm + 26.03 × A655nm, where A = absorbance. Pheophytin concentrations were expressed on a leaf area basis (micrograms per square centimeter) using the conversion: [pheophytin (micrograms per square centimeter)] = [pheophytin (micrograms per milliliter)] × 10/1.925 where 10 was the volume of acetone (milliliters) used for a sample and 1.925 was the total area (square centimeters) of five leaf disks of a sample.

Plants were harvested at the substrate line at the end of the experiment and fresh weight was recorded for each plant. Plant tissues were then dried at 60 °C to a constant weight and dry weight was recorded. Dried tissues were ground through a 0.5-mm screen (20 mesh) using a sample mill (Cyclotec; UDY Corp., Fort Collins, CO) for determining total Fe, Zn, and Mn (Crouse, 2001) using inductively coupled plasma atomic emission spectrometry (Optima 4300DV; PerkinElmer Instruments, Norwalk, CT).
Cultivar bentgrass (Agrostis palustris) showing elevated temperatures slowed the growth of creeping bentgrass results (Dhir et al., 2011) and observations in other crops dry weights (Table 1). This is consistent with previously reported air temperatures had smaller plant width, GI, leaf area, and total fresh and dry weights (Table 1). Elevated air temperatures had an adverse effect on ivy geranium growth. Plant width was greater in 'Beach' than 'Butterfly' until the final week (data not shown). This indicated 'Beach' was a more vigorous growing cultivar than 'Butterfly'. There were no differences in fresh to dry weight ratios of 'Beach' or 'Butterfly'. Elevated air temperatures had an adverse effect on ivy geranium (Dhir et al., 2011). Heat stress caused significant bleaching of leaves under the elevated air temperatures (Table 2), confirming elevated air temperatures cause bleaching in ivy geranium (Dhir et al., 2011). Heat stress caused damage to both ivy geranium cultivars, but extent of bleaching was cultivar-specific (Table 2). The greater extent of bleaching in 'Butterfly' suggests greater susceptibility to heat than 'Beach'. Varying cultivar response to high temperatures has been reported in kentucky bluegrass (He and Huang, 2007), grapes (Kadir et al., 2007), and creeping bentgrass (Xu and Huang, 2008) indicating genetic variability for heat tolerance within a species (Kadir, 2006). Different heat tolerance between species and cultivars is believed to be associated with changes in protein abundance and expression (Xu and Huang, 2008).

### Experimental Design and Statistical Analyses

The experiment was a split-plot design, split by temperature with three replications per treatment and two subsamples (one plant/container) per replication. Data were analyzed by analysis of variance using Proc-Glimmix (SAS Version 9.1; SAS Institute, Cary, NC). Fisher’s protected least significant difference test (P = 0.05) was used to indicate significant differences between treatment means.

### Results and Discussion

#### Plant Growth

There were no interactive effects of cultivar and temperature [28/16 and 36/22 °C (day/night)] on plant growth. Plant height, GI, leaf area, and total fresh and dry weights were greater in 'Beach' than 'Butterfly' at termination of the study regardless of the temperature treatment (Table 1). Plant width was greater in 'Beach' than 'Butterfly' until the final week (data not shown). This indicated 'Beach' was a more vigorous growing cultivar than 'Butterfly'. There were no differences in fresh to dry weight ratios of 'Beach' or 'Butterfly'. Elevated air temperatures had an adverse effect on ivy geranium growth. Regardless of cultivar, plants grown under elevated air temperature had smaller plant width, GI, leaf area, and total fresh and dry weights (Table 1). This is consistent with previously reported results (Dhir et al., 2011) and observations in other crops showing elevated temperatures slowed the growth of creeping bentgrass (Agrostis palustris), strawberry (Fragaria xananassa), and pansy (Viola xwittrockiana) (Huang and Gao, 2000; Kadir et al., 2006; Warner and Erwin, 2006), restrict leaf area in strawberry and grapes [Vitis sp. (Kadir et al., 2006, 2007)], decrease fresh weight of sugarcane [Saccharum officinarum] (Gilani et al., 2008), and decrease shoot dry mass in maize [Zea mays (Ashraf and Hafeez, 2004)]. In ivy geranium, temperature did not affect total fresh to dry weight ratio, reducing them proportionally with temperature treatment (Table 1) indicating a uniform reduction in plant mass under heat treatments, unlike in S. splendens in which greater heat tolerance was associated with greater accumulated biomass (Natarajan and Kuehny, 2008).

#### Leaf Bleaching

There were interactive effects of cultivar and temperature on the extent of leaf bleaching (Table 2). ‘Butterfly’ plants started showing bleaching symptoms within a week of elevating air temperatures (observational data); however, at the end of the experiment, both cultivars had significant bleaching of leaves under the elevated air temperatures (Table 2), confirming elevated air temperatures cause bleaching in ivy geranium (Dhir et al., 2011). Heat stress caused damage to both ivy geranium cultivars, but extent of bleaching was cultivar-specific (Table 2). The greater extent of bleaching in ‘Butterfly’ suggests greater susceptibility to heat than ‘Beach’. Varying cultivar response to high temperatures has been reported in kentucky bluegrass (He and Huang, 2007), grapes (Kadir et al., 2007), and creeping bentgrass (Xu and Huang, 2008) indicating genetic variability for heat tolerance within a species (Kadir, 2006). Different heat tolerance between species and cultivars is believed to be associated with changes in protein abundance and expression (Xu and Huang, 2008).

#### Chlorophyll, Carotenoid, and Pheophytins

There were interactive effects of cultivar and temperature on Chl b and total Chl (Table 2). ‘Beach’ had more Chl b and total Chl at ambient air temperature than ‘Butterfly’. Elevated air temperatures decreased Chl b and total Chl content in both ‘Beach’ and ‘Butterfly’, confirming Chl’s sensitivity to supraoptimal temperatures, and was consistent with the findings documented for rye (Feieraband, 1977), winter wheat [Triticum aestivum] (Ristic et al., 2007), and creeping bentgrass (Xu and Huang, 2008). Reduced Chl accumulation in heat-stressed plants may be the result of its impaired synthesis, its increased rate of degradation, or both and could account for the reduced presence of light-harvesting complex photosystem II in heat-stressed cucumber seedlings (Mohanty et al., 2006). Reduced Chl at elevated air temperatures was also linked to reduced photosynthetic rates (Pan et al., 2006).

There were no interactive effects of cultivar and temperature on Chl a, Caro, Chl a:b, Chl a:Caro, Chl b:Caro, total Chl:Caro ratios, or total pheophytins. Cultivar effect was significant on all these pigments and ratios except Chl a:b. ‘Butterfly’ had less Chl a and Caro but greater Chl a:Caro, Chl b:Caro, and total Chl:Caro ratios than ‘Beach’ (Table 3). Greater Chl a content regardless of temperature stress in ‘Beach’ than ‘Butterfly’ suggests
these plants have greater potential photosynthetic rates even under non-stressed conditions. The greater Chl a concentration in ‘Beach’ was not the result of a reduction in dilution factor contributed by lesser growth because ‘Beach’ plants were larger than ‘Butterfly’ plants throughout the experiment. Chl a:b ratio was similar in both cultivars, implying the reduction in Chl a and Chl b concentrations was proportional. ‘Beach’ maintained a greater Caro concentration than ‘Butterfly’, but Chl a:Caro, Chl b:Caro, and total Chl:Caro ratios in ‘Butterfly’ were greater than in ‘Beach’, suggesting a decreased concentration of carotenoids with respect to Chl in ‘Butterfly’. Carotenoids serve as accessory light-harvesting pigments and perform a critical function as antioxidants and non-photochemical quenching. They provide protection against a variety of reactive oxygen species generated primarily during photosynthesis by rapidly quenching the excited state of Chl and dissipating the energy as heat (Guerinot, 2000; Thomas and Ortiz, 1995). The decrease in Caro concentrations may not give sufficient protection to Chl (Feieraband, 1977; Guerinot, 2000; Kirkpatrick et al., 1999). Carotenoids under heat stress can participate in cation competition (Mengel and Kirkby, 1987).

In PSI of green plants, the key photosynthetic reaction consists of the transfer of an electron from the primary donor P680 to a nearby pheophytin molecule (Groot et al., 1997). Total pheophytins content was less in ‘Butterfly’ than ‘Beach’ (Table 3), indicating fewer electron carriers in the photosynthetic electron transport chain in the bleaching-susceptible cultivar Butterfly. A heat-induced block of PSI reaction centers and a heat-induced block of Chl b to Chl a energy transfer resulted in a functional disconnection of the light-harvesting complex from the reaction center complexes (Schreiber and Armond, 1978). Reduced initial pheophytin content may result in a greater response to heat-induced functional disconnection in electron transfer during PSI in the susceptible cultivar Butterfly, especially because pheophytin content decreased under heat stress.

Table 3. Effect of elevated air temperature and cultivar on pigment concentrations and pigment ratios in ivy geranium ‘Beach’ and ‘Butterfly’.

| Cultivar | Chl a | Caro | Chl a:b | Chl a:Caro | Chl b:Caro | Total Chl:Caro | Pheophytins |
|----------|-------|------|---------|------------|------------|---------------|------------|
|          | (µg·cm⁻²) | (µg·cm⁻²) |           | (ratio)    |            |               | (µg·cm⁻²)  |
| Beach    | 23.0 a | 7.3 a | 1.60 a  | 2.63 b     | 1.65 b     | 4.28 b        | 20.4 a     |
| Butterfly| 13.3 b | 5.8 b | 1.61 a  | 2.86 a     | 1.78 a     | 4.64 a        | 12.2 b     |

Temp (°C)

|   | 28   | 36   |
|---|------|------|
| 36 | 19.4 a | 7.6 a |
| 28 | 16.9 b | 5.5 b |
| 36 | 1.54 a | 2.45 b |
| 36 | 1.59 b | 4.04 b |
| 36 | 8.3 b  |      |

*Chl a = chlorophyll a; Chl b = chlorophyll b; Total Chl = total chlorophyll; Caro = carotenoids.

Independent of cultivar, Chl a, Caro, Chl a:Caro, Chl b:Caro, total Chl:Caro, and pheophytins were reduced with elevated air temperatures. However, Chl a:b ratio was unaffected by temperature (Table 3). Leaf Chl content is reduced at elevated temperatures in many crops including creeping bentgrass (Huang and Gao, 2000), wheat (Tahir et al., 2005), and lettuce (Gazula et al., 2005). High temperatures influenced chloroplast development, Chl biosynthesis, and the greening process (Mohanty et al., 2006). Lower
Table 4. Effect of elevated air temperature and cultivar on total foliar manganese (Mn) content of ivy geranium ‘Beach’ and ‘Butterfly’.

| Cultivar | Temp (°C) | Total Mn (mg kg⁻¹) |
|----------|-----------|--------------------|
| Beach    | 28        | 31.4 b c          |
| Beach    | 36        | 67.4 a             |
| Butterfly| 28        | 26.7 c             |
| Butterfly| 36        | 40.2 b             |

Means followed by same letters within column and within cultivar are not different according to Fisher’s protected least significant difference test ($P = 0.05$).

Table 5. Effect of elevated air temperature and cultivar on foliar nutrient (total Fe and Zn) content of ivy geranium ‘Beach’ and ‘Butterfly’.

| Cultivar | Total Fe (mg kg⁻¹) | Zn (mg kg⁻¹) |
|----------|--------------------|--------------|
| Beach    | 71.9 a             | 24.3 a       |
| Butterfly| 51.5 a             | 19.5 b       |

Means followed by same letters within column are not different according to Fisher’s protected least significant difference test ($P = 0.05$).

Crops of Fe or inhibition of its use within ivy geranium plants at elevated air temperatures (Romheld, 2000). ‘Beach’ had greater Zn content than ‘Butterfly’ regardless of temperature (Table 5). Foliar Zn content of ivy geraniums was greater at elevated air temperatures. Greater accumulation of Zn in ‘Beach’ than ‘Butterfly’ indicates the possibility of cultivar differences in nutrient uptake. The accumulation of foliar Zn at elevated air temperatures suggests there may be metabolically mediated uptake at elevated temperatures (Mengel and Kirkby, 1987).

In conclusion, bleaching of ivy geraniums occurs because of heat stress caused by elevated air temperature followed by slower synthesis and/or faster degradation of chlorophyll, carotenoids, and pheophytins. Although elevated air temperatures reduced growth of ivy geraniums, ‘Beach’ had greater growth than ‘Butterfly’ regardless of temperature. ‘Beach’ had decreased susceptibility to bleaching than ‘Butterfly’. The decreased susceptibility of ‘Beach’ may be related to its increased chlorophylls, carotenoids, pheophytins, Mn and Zn content, and decreased Chl:Caro ratios.

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