Greenhouse Carbon Dioxide Supplementation with Irrigation and Fertilization Management of Geranium and Fountain Grass

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Abstract. Increase in ambient carbon dioxide (CO₂) concentration is beneficial for plant growth due to increased photosynthesis and water use efficiency. A greenhouse study was conducted to investigate how supplemented CO₂ influences optimal irrigation and fertilization management for production of two ornamental plants. Two identical greenhouses were used, with one having CO₂ supplementation and the other serving as the control with ambient CO₂ concentration. Tensiometer-based irrigation treatments were applied at soil tensions of –5, –10, and –15 kPa with 0-, 3-, 6-, or 9-g controlled-release fertilizer rates applied in factorial with irrigation treatments. Plugs of geranium ‘Pinto Premium Rose Bicolor’ and fountain grass were grown under experimental conditions for 12 and 16 weeks, respectively. The results showed that CO₂ supplementation increased the dry weight of geranium ‘Pinto Premium Rose Bicolor’ and fountain grass by 35% and 39%, respectively. Under the two driest irrigation regimes (–10 and –15 kPa), photosynthesis of geranium ‘Pinto Premium Rose Bicolor’ increased with CO₂ supplementation compared with the ambient condition. Similarly, for fountain grass, the moderately watered (–10 kPa) treatment had a greater rate of photosynthesis with greater fertilizer rates of 6 or 9 g. CO₂ supplementation resulted in increased water use efficiency of both species, whereas rate of transpiration was lower only in fountain grass. Among different fertilizer rates, 6- or 9-g fertilizer rates had greater values for dry weight, number of flowers, and stomatal conductance in both species. Therefore, it can be concluded that CO₂ supplementation can help in efficient use of water for greenhouse production of ornamental plants.

In the terrestrial ecosystem, carbon is added to plants through fixing of atmospheric CO₂ by the process of photosynthesis (A_n), which can be defined as an oxidation-reduction reaction activated through light absorbed by chlorophyll, resulting in the evolution of oxygen from water and in the formation of reduced carbon compounds (e.g., carbohydrate) from CO₂ (Whittingham, 1952). The amount of CO₂ present inside a plant leaf (internal CO₂) depends on available CO₂ surrounding the plant, which plays a significant role in the rate of photosynthetic assimilation (Nederhoff, 1994). Therefore, air CO₂ concentration is reported to be an imperative climate variable in greenhouse

production with its great effect on plant photosynthetic assimilation (Sánchez-Guerrero et al., 2005). Although the CO₂ concentration in the atmosphere is reported to be increasing at a rapid rate of 2 ppm or more addition each year (National Oceanic and Atmospheric Research Administration, 2017), the scenario for CO₂ concentration in a greenhouse environment is the opposite due to reduced air exchange rates and uptake of plants (Frantz, 2011; Hughes and Bazzaz, 2001). In low-cost greenhouses, CO₂ supply is reported to be lower than that in ambient conditions due to poor ventilation systems (Slack and Hand, 1985).

A greenhouse can develop a critically low CO₂ concentration, particularly during winter production cycles under low radiation conditions (Singh et al., 2020). Slack and Hand (1985) reported that 200 ppm CO₂ in an unventilated greenhouse significantly reduced growth and yield of cucumber (*Cucumis sativus* L.). Rubisco, the carboxylation enzyme of A_n , has an 80 times higher affinity to bind with CO₂ compared with oxygen (O₂)

(Pessarakli, 2016). However, under low CO₂ concentration conditions, rubisco may catalyze oxygenation of ribulose 1,5-bisphosphate (RuBP) instead of carboxylation by incorporating CO₂. This oxygenation of RuBP initiates a pathway called photorespiration, which results in reduced net A_n . Thus, ambient CO₂ concentration, relative to O₂ concentration, can influence the occurrence of photorespiration, particularly in C₃ plants. However, C₄ plants use the enzyme PEP-carboxylase, which binds HCO₃[–] to concentrate CO₂ within the plant so that photorespiration is reduced even under diminishing ambient CO₂ concentrations. To provide optimal growing conditions for many vegetables and ornamental plants, Mortensen (1987) and Nederhoff (1994) each suggested the use of supplemental CO₂, particularly in sealed and nonventilated greenhouses.

Carbon dioxide supplementation or CO₂ fertigation is a process of adding CO₂ into the plant growing environment for the purpose of increasing plant photosynthetic activity and growth. The source of CO₂ in greenhouse supplementation systems is typically a hydrocarbon compound that, when burned, releases CO₂ into the environment (Laumb et al., 2013). For example, common sources can include natural gas or other petroleum products. Ghannoum et al. (2000) reported that doubling the ambient CO₂ level could enhance A_n by 40% to 45% and 10% to 20% in C₃ and C₄ plants, respectively. Generally, a CO₂ level of 600 to 900 ppm is desirable for greenhouse crop production (Arp et al., 1998; Sánchez-Guerrero et al., 2009). In a review of supplemental CO₂ practices for greenhouse-grown ornamentals and vegetables, supplementing CO₂ led to an increase in growth and early flowering (Mortensen, 1987).

In contrast, studies in fig (*Ficus benjamina* L.) (Papenhagen, 1983) and philodendron (*Philodendron chelonoides* Schott.) (Schmidt and Brundert, 1984) reported a decrease in plant size when grown at 1000 to 1500 ppm of CO₂. Arp et al. (1998) reported that plant response to elevated CO₂ is highly influenced by other environmental factors. The moisture status, nutrient level, light level, disease, and pest presence might affect the response of plants under elevated CO₂ (Arp et al., 1998; Baligar et al., 2017; Penuelas et al., 1995). A wide range of plant responses in terms of growth, biomass production, morphological, and physiological processes have been observed under elevated CO₂ and varying growth environments. A proper understanding of the interaction of CO₂ with other environmental factors is crucial to provide an optimal growing environment for plants.

Water stress is a major problem affecting plant growth in the ornamental industry, which significantly affects plant size, flower number, and plant quality (Farooq et al., 2009). Under drought stress, a plant’s stomatal aperture closes to regulate moisture loss, resulting in a lower rate of transpiration (E). Thus, prolonged stomatal closure under drought stress can result in a lower internal

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CO₂ concentration and A_n , resulting in decreased plant growth (Chaves et al., 2009; Flexas et al., 2006). The negative impact of drought stress could be ameliorated to some extent through supplemental CO₂. Morison and Gifford (1983) reported that the stomatal conductance (g_s) of plants decreases under elevated CO₂, whereas the internal concentration of CO₂ increases and net assimilation rate is either unaffected or increased (Mott, 1988). Others have reported that this greater A_n in combination with a decrease in g_s can result in lower E (Reddy et al., 2005). Therefore, this increased A_n and decreased E results in increased water use efficiency (WUE), which can be defined as the A_n divided by E (Hatfield and Dold, 2019). Various studies also reported $\approx 40\%$ increase in WUE as a result of greenhouse CO₂ supplementation in vegetable and ornamental crops (Sánchez-Guerrero et al., 2009).

Soil nutrient availability can also interact with CO₂ concentration to affect plant growth rate (Peñuelas et al., 1995). It has been reported that growth rate of maize (*Zea mays* L.) was increased even with a lower nitrogen (N) level under elevated CO₂ (Hocking and Meyer, 1991). Similarly, Wong (1979) also reported 1.5 times increase in rate of CO₂ assimilation in cotton (*Gossypium hirsutum* L.) plants in CO₂-supplemented conditions, particularly at low-level N nutrition. In nutrient-stressed plants, elevated CO₂ is reported to increase N use efficiency, which can be defined as dry mass productivity per unit N taken up from soil (Bowes, 1993). Similarly, increased N use efficiency under elevated CO₂ concentrations was reported by Norby et al. (1986) during a growth chamber study of white oak (*Quercus alba* L.). A recent report by Easlon et al. (2015) also showed better plant growth and A_n in arabidopsis [*Arabidopsis thaliana* L. (Heynh.)] lines under N-limitation under elevated CO₂ conditions compared with sufficient N supply. The objective of the study was to evaluate the effect of CO₂ supplementation with precision irrigation and fertilizer management of greenhouse-grown ornamentals. We hypothesized that CO₂ supplementation would help in increasing water use efficiency of ornamental species and the response of CO₂ supplementation would be greater in the C₃ plant geranium (*Pelargonium hortorum* L.H. Bailey 'Pinto Premium Rose Bicolor') compared with the C₄ plant fountain grass (*Pennisetum alopecuroides* sp. L.) (Siebke et al., 2003).

Materials and Methods

Experimental setup and treatments. The study was conducted at the Oklahoma State University Department of Horticulture and Landscape Architecture Research Greenhouses (36.13602913, -97.08619773; 1301 N. Western Rd, Stillwater, OK 74075), in Spring 2016 and was repeated in Spring 2017. The experiment was arranged as a split-split plot design with 10 replications per treatment. Two identical greenhouses

(10.97 × 24.38 m; whole main plots) were used to provide two CO₂ levels described as either supplemented CO₂ or ambient CO₂. The greenhouse with supplemented CO₂ was fitted with a natural gas-burning CO₂ generator (Johnson Gas Appliance Company, Cedar Rapids, IA) in the middle of the house (Fig. 1). The generator was set to turn on from 6:00 AM to 2:00 PM for the first 3 months starting from 24 Feb. 2016 (first-year experiment) and 20 Feb. 2017 (second-year experiment) and then reduced to 6:00 AM to 12:00 PM at the end of growth cycle as temperatures increased causing the ventilation to come on around 8:00 AM instead of 10:00 AM. An Extech CO210 CO₂ Monitor (FLIR Commercial System Inc. Nashua, NH) was used to measure the CO₂ concentration every hour.

Within each CO₂ treatment, irrigation and fertilizer rates were arranged as a 3 × 4 factorial. Three irrigation levels (subplots) of -5, -10, and -15 kPa were assigned to three separate benches with irrigation supplied by drip emitters and scheduled to run

using an automated tensiometer (Field Scout hand held TDR 100 sensor) (Irrometer, Riverside, CA). The tensiometer was inserted to a depth of 10 cm in between the edge and center of one container located centrally within each bench for each irrigation level. The tensiometer was connected to a solenoid valve, which turned on the irrigation when the tensiometer reached the respective set point. The -5, -10, and -15 kPa irrigation thresholds were $\approx 50\%$, 40%, and 30% volumetric water content, respectively (Fig. 2). For fertilizer rates, a controlled-release fertilizer (Osmocote Plus 15N-3.9P-9.9K, 3- to 4-month release, Everris NA Inc., Dublin, OH) was applied at rates of 0, 3, 6, and 9 g per plant based on manufacturer recommendations. The fertilizer was split applied, half top-dressed at the time of transplanting and the remaining half applied 40 d after treatment. All fertilizer treatments were randomized within irrigation treatments. The experimental unit was a single pot and each

Fig. 1. Average daily value of ambient and supplemental CO₂ concentration measured in Oklahoma State University Department of Horticulture and Landscape Architecture Research Greenhouses (Stillwater, OK) during the years (A) 2016 and (B) 2017.

fertilizer treatment consisted of 10 pots per irrigation level. Main plot, subplot, and sub-subplot consisted of 120, 40, and 10

pots, respectively. The direction of greenhouses and plots was from north to south. Therefore, each CO₂ treatment consisted of

120 pots. All this experimental setup was replicated twice by repeating it in next year.

Plant materials and growth conditions. On 26 Feb. 2016 and 20 Feb. 2017, plug trays of geranium and fountain grass were obtained from Park Seed (Greenwood, SC). These trays were kept on a mist bench until pots and media were ready for transplanting. The plants were manually misted twice a day. Plugs were transplanted into azalea pots (Landmark Plastic Corporation, Akron, OH) having 15.24 cm diameter and 1.33-L maximum liquid volume capacity containing ≈450 g of 902 MetroMix Media (Sun-Gro Horticulture, Bellevue, WA). Pots were moved to their respective benches, and the greenhouse was set at 21 °C/18 °C (day/night) in both greenhouses. The daily light integral ranged from 14.6 to 16.3 mol·m⁻²·d⁻¹ from March through June, and temperatures ranged from 23.8 to 30.0 °C. Plants were grown under natural photoperiods.

Growth analyses. Number of flowered umbels, number of flowers in an umbel on day of harvesting, and days to first flowering were measured in geranium ‘Pinto Premium Rose Bicolor’. For fountain grass, measurements were made for days to first flowering and spike numbers. Geranium ‘Pinto Premium Rose Bicolor’ and fountain grass had growth cycles of 12 and 16 weeks, respectively. Shoots, stem cut at the media level, were harvested in both species at the end of the growing cycle and oven-dried at 60 °C for 72 h to determine shoot dry weight.

The parameters A_n , E , g_s , and WUE (defined as the ratio of net A_n to E) were measured using a LI-6400 portable photosynthesis system (LI-COR Biosciences, Lincoln, NE) on 24 May 2016 and 16 May 2017 for geranium ‘Pinto Premium Rose Bicolor’ (flowering stage) and 16 June 2016 and 11 June 2017 for fountain grass. Due to time constraints, only five replicates of the fountain grass were evaluated for gas exchange parameters. The LI-6400 was fitted with a 6400-02B LED light source chamber, and

Fig. 2. Volumetric water content (VWC) of geranium ‘Pinto Premium Rose Bicolor’ and fountain grass measured by a Field Scout handheld TDR 100 sensor (Spectrum Technologies, Inc., Aurora, IL) when plants were grown under -5-, -10-, and -15-kPa irrigation regimes in soilless 902 MetroMix Media (Sun Gro Horticulture, Bellevue, WA).

Table 1. Interaction and main effects of CO₂ (ambient at 400 ppm and supplemented at an average of 800 ppm), irrigation (-5, -10, and -15 kPa tensiometer settings), and fertilizer (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 month) rates on geranium ‘Pinto Premium Rose Bicolor’ and fountain grass.

Effects	Growth index	Dry wt (g)	No. of inflorescences ^a	Days to first flower	No. of flowers per umbel	No. of tillers	A_n (μmol CO ₂ ·m ⁻² ·s ⁻¹)	E (mmol H ₂ O·m ⁻² ·s ⁻¹)	g_s (mmol·m ⁻² ·s ⁻¹)	WUE (mmol CO ₂ ·mol ⁻¹ H ₂ O)
Geranium ‘Pinto Premium Rose Bicolor’										
CO ₂	NS	*	NS	**	NS	—	NS	NS	NS	***
Irrigation	NS	NS	NS	NS	*	—	*	***	***	*
Fertilizer	***	***	***	***	***	—	***	NS	NS	***
CO ₂ × irrigation	*	NS	NS	NS	NS	—	*	*	NS	NS
CO ₂ × fertilizer	NS	NS	*	NS	NS	—	*	NS	*	***
Irrigation × fertilizer	NS	NS	NS	NS	NS	—	NS	*	*	NS
CO ₂ × irrigation × fertilizer	NS	NS	NS	NS	NS	—	NS	NS	NS	NS
Fountain grass										
CO ₂	NS	**	***	NS	—	*	***	**	NS	*
Irrigation	**	NS	**	NS	—	*	***	NS	NS	NS
Fertilizer	***	***	***	NS	—	***	***	***	*	**
CO ₂ × irrigation	*	NS	NS	NS	—	*	***	NS	NS	NS
CO ₂ × fertilizer	***	NS	**	NS	—	**	NS	**	NS	NS
Irrigation × fertilizer	***	NS	*	NS	—	NS	NS	NS	NS	NS
CO ₂ × irrigation × fertilizer	NS	NS	NS	NS	—	NS	*	**	NS	*

^aNumber of inflorescence indicates number of umbels in geranium ‘Pinto Premium Rose Bicolor’ and number of spikes in fountain grass.

NS, *, **, ***Nonsignificant or significance at $P < 0.05$, 0.01, or 0.001, respectively.

A_n = photosynthetic rate; g_s = stomatal conductance; E = transpiration; WUE = water use efficiency.

measurements were made from 9:00 AM to 3:00 PM. The instrument reference CO₂ was kept at 400 ppm for the ambient treatment and at 800 ppm for the supplemented CO₂

condition. The light level was fixed at 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ photosynthetic photon flux density, and block temperature was set at 28 °C. One of the first five young leaves was mea-

sured in situ on each plant for geranium 'Pinto Premium Rose Bicolor'. For fountain grass, three leaves were combined within the chamber for a single measurement due to the narrow leaf width.

Statistical analyses. All data were subjected to analyses of variance using SAS 9.4 (SAS Institute Cary, NC). The analyses were made considering four factorial treatments of CO₂ concentration, irrigation, fertilizer, and species in a split-split plot design. The second-year study was a replication of the first-year study. The MIXED procedure was used to perform tests of significance for main effect and interaction. When treatments showed a significant difference, a macro program, pdmix800, was used to separate means using Tukey-Kramer test. All statistical tests used a significance level of $P \leq 0.05$.

Results

Dry weight. There was no significant effect of irrigation or any higher order interactions for either geranium 'Pinto Premium Rose Bicolor' or fountain grass (Table 1). Thus, data were pooled to estimate the main effects of CO₂ ($P < 0.05$) and fertilizer ($P < 0.001$) in each species. Among fertilizer rates, geranium 'Pinto Premium Rose Bicolor' dry weight production in 3, 6, and 9 g fertilizer rates were statistically similar but greater than the control ($P < 0.05$) (Table 2). Geranium 'Pinto Premium Rose Bicolor' grown under supplemented CO₂ had 34.7% greater dry weight compared with plants grown under the ambient condition ($P < 0.05$) (Table 2). Fountain grass under supplemented CO₂ also showed a significant increase in dry weight (38.5%) over the control ($P < 0.05$) (Table 2). Fountain grass receiving fertilizer had significantly greater dry weight than the control, whereas the 9-g rate resulted in 21.0% greater dry weight than the 3-g rate and 79.6% greater dry weight compared with the control treatment ($P < 0.05$) (Table 2).

Inflorescences count. For geranium 'Pinto Premium Rose Bicolor', only the CO₂ $\times$ fertilizer interaction was significant for umbels per plant ($P < 0.05$) (Table 1). Under ambient CO₂ treatment, number of umbels produced per plant were significantly greater in the 6- and 9-g fertilizer rates compared with the control treatment, but this was not significantly different from that produced in the 3-g fertilizer rate. In contrast, the supplemented CO₂ treatment resulted in greater umbels per plant for the 6- and 9-g rate compared with the 3-g rate (Fig. 3A). Significant irrigation ($P < 0.05$) and fertilizer ($P < 0.001$) main effects were observed for flower counts per umbel (Table 1). For the fertilizer main effect, flower counts per umbel increased with increasing fertilizer rate such that $9\text{ g} > 6\text{ g} > 3\text{ g} > 0\text{ g}$ ($P < 0.05$) (Table 3). For the irrigation main effect, geranium 'Pinto Premium Rose Bicolor' grown under -10 kPa irrigation level had a significantly greater flower counts per umbel as compared with other irrigation treatments ($P < 0.05$) (Table 3).

Table 2. Main effect of CO₂ (ambient at 400 ppm and elevated at an average of 800 ppm) treatments and different fertilizer (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 month) rates on dry weight production of geranium 'Pinto Premium Rose Bicolor' and fountain grass.

Fertilizer (g)	Geranium dry wt (g/plant)	Fountain grass dry wt (g/plant)	CO ₂ levels	Geranium dry wt (g/plant)	Fountain grass dry wt (g/plant)
0	20.3 b ²	41.7 c	Ambient	30.8 b	51.7 b
3	38.1 a	61.9 b	Elevated	41.5 a	71.6 a
6	41.6 a	68.2 ab			
9	44.5 a	74.9 a			

²Means (n = 120) within a column followed by same letters are not significantly different at $P \leq 0.05$.

Fig. 3. Interaction between CO₂ (ambient at 400 ppm and supplemented at an average of 800 ppm) and fertilizer rates (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 month) on (A) number of umbels per plant in geranium 'Pinto Premium Rose Bicolor', (B) number of inflorescences in fountain grass, (C) interaction between irrigation treatments (-5-, -10-, and -15-kPa tensiometer settings) and fertilizer rates (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 month) on number of inflorescences in fountain grass. Means with the same letter are not significantly different at $P \leq 0.05$.

In fountain grass, the $\text{CO}_2 \times \text{fertilizer}$ ($P < 0.001$) and irrigation $\times$ fertilizer ($P < 0.05$) interactions were significant for inflorescence count, but no three-way interaction was observed (Table 1). The $\text{CO}_2 \times \text{fertilizer}$ interaction resulted in a significantly greater inflorescence count for supplemented CO_2 treatments, but this only occurred under the 6- and 9-g fertilizer rates ($P < 0.05$) (Fig. 3B). In contrast, the ambient CO_2 treatment did not demonstrate increased inflorescence count with increasing fertilizer rate beyond the 3-g rate. For the irrigation $\times$ fertilizer interaction, inflorescence count was significantly greater for the -10-kPa irrigation treatment compared with the -5- and -15-kPa treatments, but this only occurred at the highest fertilizer rate (i.e., 9 g) ($P < 0.05$) (Fig. 3C). As fertilizer rate decreased, the effect of irrigation became diminished, with no differences detected at the 0- or 3-g rates.

Days to flower initiation. The main effects of fertilizer ($P < 0.001$) and CO_2 ($P < 0.01$) were significant for flower initiation in geranium 'Pinto Premium Rose Bicolor' (Table 1). For the fertilizer main effect, geranium 'Pinto Premium Rose Bicolor' flowered earlier under 6- or 9-g fertilizer rates compared with other fertilizer rates (Table 3). For the CO_2 main effect, supplemented CO_2 resulted in flower initiation at 57.7 d, which was significantly greater ($P < 0.05$) than for the ambient CO_2 treatment (53.2 d) (Table 3). In fountain grass, no main or interaction effects were detected for flower initiation (data not shown).

Stomatal conductance. In geranium 'Pinto Premium Rose Bicolor', the $\text{CO}_2 \times \text{fertilizer}$ and irrigation $\times$ fertilizer interactions were significant for g_s ($P < 0.05$) (Table 1). For the $\text{CO}_2 \times \text{fertilizer}$ interaction, supplemental CO_2

reduced ($P < 0.05$) g_s compared with ambient CO_2 treatment but only at the 6-g fertilizer rate. A similar pattern was seen at the 9-g rate, although this difference was not significantly different (Fig. 4A). In the irrigation $\times$ fertilizer interaction, g_s was significantly greater ($P < 0.05$) for the -15-kPa irrigation level at the 0- and 9-g fertilizer rates compared with the -5-kPa irrigation level for the same fertilizer rate (Fig. 4B).

For fountain grass, only the fertilizer main effect was significant for g_s ($P < 0.05$) (Table 1). Stomatal conductance for the 9-g fertilizer treatment was significantly greater ($P < 0.05$) than the 0-g control but similar to the 3- and 6-g rates (Table 4).

Water use efficiency. In geranium 'Pinto Premium Rose Bicolor', the $\text{CO}_2 \times \text{fertilizer}$ interaction and the irrigation main effects were significant ($P < 0.05$) for WUE (Table 1). For the irrigation main effect, WUE of geranium 'Pinto Premium Rose Bicolor' was greater under the -10-kPa irrigation level compared with the -15-kPa level with the -5-kPa level being intermediate and statistically similar to each other treatment (Table 4). For the $\text{CO}_2 \times \text{fertilizer}$ interaction, the supplemented CO_2 treatment at the 6- and 9-g fertilizer rates resulted in significantly greater ($P < 0.05$) WUE compared with other treatment combinations. The WUE increased with increasing fertilizer rate up to the 6-g rate for both ambient and supplemented CO_2 treatments (Fig. 5). Furthermore, the supplemental CO_2 increased WUE at each fertilizer rate, with the exception of the 0-g control.

In fountain grass, WUE showed a significant ($P < 0.05$) three-way interaction. Water use efficiency for each irrigation and fertilizer treatment combinations under the supplemental CO_2 treatment was greater compared with the ambient CO_2 treatment, with the exception of 6- and 9-g fertilizer rates under the -10-kPa irrigation level (Table 5). Among all three-way combinations, the greatest WUE was observed with supplemental CO_2 plus the 6-g fertilizer rate and the -15-kPa irrigation level (Table 5).

Photosynthesis. For geranium, the $\text{CO}_2 \times \text{irrigation}$ and $\text{CO}_2 \times \text{fertilizer}$ interactions were significant ($P < 0.05$) for A_n (Table 1). For the $\text{CO}_2 \times \text{irrigation}$ interaction, A_n was significantly greater in the -10- and -15-kPa irrigation treatments supplemented with CO_2 compared with all other treatment combinations ($P < 0.05$) (Fig. 6A). For the $\text{CO}_2 \times \text{fertilizer}$ interaction, the 6- and 9-g fertilizer rates supplemented with CO_2 had significantly greater A_n compared with all other treatments ($P < 0.05$) (Fig. 6B).

In fountain grass, the three-way interaction was significant for A_n ($P < 0.05$). Results showed there was no effect of irrigation on A_n except for at the 9 g fertilizer rate supplemented with CO_2 , which demonstrated greater ($P < 0.05$) A_n for the -10-kPa treatment compared with the -5- or -15-kPa treatments (Table 5). For each irrigation and CO_2 treatment combination, the fertilizer effect showed similar patterns, with the 6- and 9-g rates having a significantly greater A_n than the 0-g treatment. The only exception to this

Table 3. Main effect of CO_2 treatments (ambient at 400 ppm and supplemented at an average of 800 ppm) treatments, different fertilizer (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 month) rates and different irrigation treatments (-5-, -10-, -15-kPa tensiometer settings) on flower counts per umbel and days to flower initiation in geranium 'Pinto Premium Rose Bicolor'.

Irrigation (kPa)	Flower counts per umbel	Fertilizer (g)	Days to flower initiation	Flower counts per umbel	CO_2 levels	Days to flower initiation
-5	61.4 b ^z	0	58.1 a	47.8 d	Ambient	53.2 b
-10	67.7 a	3	56.3 b	61.1 c	Supplemented	57.7 a
-15	62.3 b	6	53.8 c	69.6 b		
		9	53.8 c	76.8 a		

^zMeans (n = 10) within a column followed by same letters are not significantly different at $P \leq 0.05$.

Fig. 4. (A) Interaction between CO_2 (ambient at 400 ppm and supplemented at an average of 800 ppm) and fertilizer rates (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 per month) and (B) interaction between CO_2 (ambient at 400 ppm and supplemented at an average of 800 ppm) and irrigation treatments (-5-, -10-, and -15-kPa tensiometer settings) for stomatal conductance (g_s) in geranium 'Pinto Premium Rose Bicolor'. Means with the same letter are not significantly different at $P \leq 0.05$.

pattern was at the -5-kPa treatment plus supplemental CO₂, for which there was no effect of fertilizer rate.

Transpiration. The two-way interaction of CO₂ × irrigation and irrigation × fertilizer were significant ($P < 0.05$) for E in geranium 'Pinto Premium Rose Bicolor' (Table 1). For the irrigation × fertilizer interaction, the -15-kPa irrigation treatment increased E for the 0- and 9-g fertilizer rates compared with the -5- and -10-kPa treatments, although a similar pattern was not observed for the 3- or 6-g rates (Fig. 7A). For the CO₂ × irrigation interaction, E for geranium 'Pinto Premium Rose Bicolor' was greatest ($P < 0.05$) at the -15-kPa irrigation level in both ambient and supplemented CO₂ treatments (Fig. 7B). Fur-

thermore, supplemental CO₂ reduced E at the -5-kPa irrigation level but showed no effect in other treatment combinations.

In fountain grass, the three-way interaction was significant ($P < 0.01$) (Table 1). Results showed that under ambient CO₂ treatment, E was greatest in the 9-g fertilizer rate at the -15-kPa irrigation level, although this mean was similar to E at the fertilizer rate of 3 g and irrigation level of -5 kPa, 6-g fertilizer rate at irrigation level of -15 kPa, and 9-g fertilizer rate at irrigation level of -10 kPa. Lower E was observed under supplemented CO₂ treatment compared with ambient CO₂ treatment in all irrigation and fertilizer combinations (Table 5).

Discussion

Dry mass production and flowering. In general, plant growth is reported to be favored under supplemented CO₂ treatment due to greater photosynthate accumulation in most plants (Springer and Ward, 2007). Previous studies with supplemented CO₂ reported a greater increase in dry weight of C₃ plants (40% to 45%) compared with that in C₄ plants (10% to 20%) (Ghannoum et al., 2000; Prior et al., 1997). This difference in dry weight accumulation between C₃ and C₄ plants can be explained by differences in A_n which is reported to increase by ≈58% for C₃ plants under a doubling of the atmospheric CO₂ concentration, whereas for C₄ plants, A_n is nearly saturated at current CO₂ concentrations (Hamim, 2005). This response of C₃ and C₄ plants to supplemented CO₂ concentration can be modified due to an interaction with some environmental factors such as irrigation or fertilization.

In the current study, supplementing CO₂ increased shoot dry weight of geranium 'Pinto Premium Rose Bicolor' by 34.7% and fountain grass by 38.5%, which is within the range reported by other studies for geranium but greater than those reported for fountain grass. The greater response of fountain grass can be explained because some immature C₄ leaves have C₃-like A_n mechanisms and therefore are more responsive to enhancement of A_n under supplemented CO₂ concentrations (Dai et al., 1995; Ziska and Bunce, 1999). Others have suggested that growth of C₄ plants in controlled environments with confined root systems, such as observed in potted plant production, may result in different responses to CO₂ than field production (Ainsworth et al., 2003; McLeod and Long, 1999).

In addition to dry matter accumulation, greenhouse CO₂ supplementation is reported to impact flowering time in ornamental plants. (Simpson et al., 1999). However, the mechanism behind the effect of supplemented CO₂ on flowering is still unclear as 63% showed accelerated flowering times, whereas 29% did not change, and 8% had delayed flowering among a meta-analysis of 24 crop species (Springer and Ward, 2007). The results from the current study were also individualistic for each species, with a significant delayed flowering with CO₂ supplementation in geranium 'Pinto Premium Rose

Table 4. Main effect of different fertilizer rates (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 month) on stomatal conductance (g_s) in fountain grass and different irrigation treatments (-5-, -10-, -15-kPa tensiometer settings) on water use efficiency in geranium 'Pinto Premium Rose Bicolor'.

Fertilizer (g)	g_s ($\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) ^a	Irrigation (kPa)	Water use efficiency ($\text{mmol CO}_2\cdot\text{mol}^{-1}\text{H}_2\text{O}$)
0	0.2 b ^y	-5	3.3 ab
3	0.22 ab	-10	3.4 a
6	0.23 ab	-15	3.1 b
9	0.26 a		

^aStomatal conductance was an average of five samples.

^yMeans ($n = 10$) within a column followed by same letter are not significantly different at $P \leq 0.05$.

Fig. 5. Interaction between CO₂ (ambient at 400 ppm and supplemented at an average of 800 ppm) and fertilizer rates (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 month) for water use efficiency in geranium 'Pinto Premium Rose Bicolor'. Means with the same letter are not significantly different at $P \leq 0.05$.

Table 5. Interaction effect of CO₂ (ambient at 400 ppm and supplemented at an average of 800 ppm), irrigation treatments (-5, -10, -15 kPa using a tensiometer) and fertilizer rates (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 month) in physiology of fountain grass.

CO ₂ levels	Fertilizer (g)	Photosynthesis ($\mu\text{mol CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)			Transpiration ($\text{mmol H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)			Water use efficiency ($\text{mmol CO}_2\cdot\text{mol}^{-1}\text{H}_2\text{O}$)		
		-5 kPa	-10 kPa	-15 kPa	-5 kPa	-10 kPa	-15 kPa	-5 kPa	-10 kPa	-15 kPa
Ambient	0	14.9 i ^z	17.1 e-i	15.5 hi	3.5 b-f	2.8 ef	4.7 b-f	5.3 c-g	4.5 d-g	3.9 fg
	3	18.9 c-i	15.3 hi	16.8 f-i	5.2 a-e	2.9 ef	4.1 b-f	4.2 fg	5.5 b-g	4.4 e-g
	6	20.3 c-h	23.3 a-c	19.3 c-i	4.8 b-f	3.3 c-g	5.9 a-c	5.2 c-g	7.2 a-c	5.1 c-g
	9	21.5 b-g	23.5 a-c	21.8 b-f	4.6 b-f	5.8 abd	7.9 a	5.3 c-g	6.5 a-f	3.0 g
Supplemented	0	17.7 d-i	21.5 b-g	16.5 g-i	2.1 f	3.4 b-f	2.6 ef	8.5 ab	6.4 a-f	6.5 a-f
	3	19.2 c-i	23.9 a-c	19.3 c-i	2.6 ef	3.2 b-f	2.6 ef	7.4 a-d	7.6 a-c	7.5 a-c
	6	21.9 b-f	25.4 ab	22.9 bc	2.6 ef	3.5 b-f	2.7 ef	8.4 ab	7.4 a-e	8.8 a
	9	22.3 b-e	28.1 a	22.6 b-d	3.3 b-f	3.5 b-f	2.9 d-g	7.4 a-d	7.9 a-c	7.7 a-c

^zMeans ($n = 5$) within a parameter followed by same letters are not significantly different at $P \leq 0.05$.

Fig. 6. (A) Interaction between CO₂ (ambient at 400 ppm and supplemented at an average of 800 ppm) and irrigation treatments (–5-, –10-, and –15-kPa tensiometer settings) and (B) interaction between CO₂ (ambient at 400 ppm and supplemented at an average of 800 ppm) and fertilizer rates (0, 3, 6, and 9 g of 15N–3.9P–9.9K Osmocote Plus 3–4 month) for photosynthesis rate (A_n) in geranium ‘Pinto Premium Rose Bicolor’. Means with the same letter are not significantly different at $P \leq 0.05$.

Bicolor’ flowering date, whereas there was no effect on fountain grass. Two C₃ ornamental plants, gerbera (*Gerbera jamesonii* L.) (Berkel, 1984) and impatiens (*Impatiens repens* L.) (Reimherr, 1984), also showed delayed flowering under supplemented CO₂. In contrast, several studies have reported accelerated flowering of ornamental plants by 2 to 15 d under supplemented CO₂ (Cleland et al., 2006; Mortensen and Ulsaker, 1985; Reekie et al., 1997). The possible reason for these varying results for flowering under supplemented CO₂ treatment can be that supplemented CO₂ can have interaction with some other growth factors, such as temperature, nutrient availability, and light, thus altering the effect of supplemented CO₂ on flowering.

Increased fertilizer rates have been reported to enhance photosynthate accumulation, and thereby result in early flowering (El-Naggar and El-Nasharty, 2009). Asrar et al. (2014) reported a similar finding for chrysanthemum (*Dendranthema grandiflora* L.) using a controlled release fertilizer. In the current study, this fertilizer response

was evident for geranium but not the C₄ species, fountain grass. This finding is in agreement with Leakey et al. (2006) who reported no effect of CO₂ and water in flowering time of a C₄ maize plant. Also, fertilizer treatments in a C₄ plant, sugarcane (*Saccharum officinarum* L.), had no significant effect in days to panicle initiation (Brunkhorst, 2003).

In contrast to flower initiation timing, the number of inflorescences (umbels per plant in geranium ‘Pinto Premium Rose Bicolor’ and spike per plant in fountain grass) in this study were greatly influenced by CO₂ supplementation for fountain grass but not geranium. The importance of fertilizer rate on flower count was evident in both species, but the effect was apparently enhanced for fountain grass under supplemental CO₂. This likely was due to the increased tillering observed under the same treatments for fountain grass. Also, greater number of inflorescences can be attributed to increase in photosynthates due to greater availability of the nutrients and CO₂ under supplemented CO₂ treatment and at the greatest fertilizer rate (Jablonski et al.,

2002). Thus, growing fountain grass under supplemented CO₂, greater fertilizer rate, and adequate water might initiate an earlier reproductive phase resulting in a greater number of flowering primordia and flower number (He et al., 2005; Teng et al., 2006). The lower flower counts for the –15-kPa irrigation level also suggest that underwatering may reduce inflorescences formation in fountain grass.

Stomatal conductance and transpiration. In general, the decreased g_s is reported in response to CO₂ supplementation (Ainsworth and Rogers, 2007; Ji et al., 2015), but its magnitude depends on environmental variables and different species (Ainsworth and Rogers, 2007; Haworth et al., 2013; Medlyn et al., 2001; Ward et al., 2012). Different studies conducted in greenhouses or growth chambers reported almost 40% decreases in g_s at double rate of ambient CO₂ concentration (Xu et al., 2016). The decrease in g_s can be beneficial because it would decrease E but at same time, because stomata act as a path for CO₂ uptake for photosynthesis, it would decrease A_n . However, a recent experiment indicated that, with supplemented CO₂, pigeon pea (*Cajanus cajan* L.) leaves were able to maintain higher A_n and lower g_s (Sreeharsha et al., 2019). Nackley et al. (2014) reported a reduction in g_s and E under supplemented CO₂ treatment in giant reed (*Arundo donax* L.). Therefore, the prior research is in agreement with the findings of the current study for geranium.

The greatest E (8.7 mmol H₂O·m^{–2}·s^{–1}) and greatest g_s (0.68 mol·m^{–2}·s^{–1}) was observed at the 9-g fertilizer rate with irrigation level of –15 kPa. Bower (2008) reported similar results of increased transpiration rate under greater fertilizer rate in peppers (*Capiscum annuum* L.). Increasing CO₂ concentration may help in reducing the drought effect under water-deficit conditions (Nackley et al., 2014). Xu et al. (2016) reported a significant decrease in the stomatal index (ratio of stomata to epidermal cells) under supplemented CO₂ in many species, which might result in lower E in a supplemented CO₂ condition. Similarly, Kang et al. (2002) found that doubling CO₂ concentration reduced E by 17.4%, 22.1%, and 5.6% under high soil water treatment for spring wheat, maize, and cotton, respectively. Thus, increased stomatal index under ample fertilizer, and irrigation in ambient CO₂ might have resulted in greater g_s in fountain grass compared with supplemented CO₂ treatment.

Photosynthesis and water use efficiency. In geranium ‘Pinto Premium Rose Bicolor’, either slightly dry or moderately watered irrigation treatments showed greater A_n under supplemented CO₂. Likewise, A_n increased in supplemented CO₂ when fertilizer rate was greater. Arp et al. (1998) also reported increased A_n in sorrel (*Rumex obtusifolius* L.) when grown under well-watered and greater nitrogen conditions. Explanation for this increase in C₃ species can be provided by increases in activity of rubisco enzyme for carboxylation reactions and suppressing

Fig. 7. (A) Interaction between fertilizer rates (0, 3, 6, and 9 g of 15N-3.9P-9.9K Osmocote Plus 3-4 month) and irrigation treatments (-5-, -10-, and -15-kPa tensiometer settings) and (B) CO₂ (ambient at 400 ppm and supplemented at an average of 800 ppm) and irrigation treatments (-5-, -10-, and -15-kPa tensiometer settings) for transpiration (E) in geranium 'Pinto Premium Rose Bicolor'. Means with the same letter are not significantly different at $P \leq 0.05$.

competition for oxygenation of RuBP (photorespiration), thereby resulting in increased A_n in C₃ species (Lara and Andreo, 2011). Although the mechanism for stimulation of A_n is clear in C₃ plants, stimulation in C₄ species is still not clear (Leakey et al., 2009). In a review, Kimball (2016) reported that some C₄ species might respond well to CO₂ supplementation under ample moisture and nitrogen status. These concepts are in agreement with the current study as fountain grass also exhibited greater A_n in supplemented CO₂ under greater fertilizer and -10-kPa irrigation treatment.

In general, an increase in A_n should result in increased WUE if there is a reduced or similar E . Li et al. (2003) also reported an 10% to 12% increase in WUE through increased A_n and decreased E in wheat by doubling the CO₂ concentration. Similarly, in the current study, greater A_n and reduced g_s and E under supplemented CO₂ treatment resulted in increased WUE in geranium 'Pinto Premium Rose Bicolor'. However, the present study suggests sufficient fertilizer is needed to make these responses detectable.

Conclusion

It can be concluded that greenhouse CO₂ supplementation resulted in increase in dry weight of geranium 'Pinto Premium Rose Bicolor' and fountain grass by 35% and 39%, respectively. The effect of CO₂ supplementa-

tion on flowering time was species dependent, whereas number of inflorescences were increased in both species under CO₂ supplemented conditions. Therefore, greenhouse CO₂ supplementation may help achieve greater yield production in ornamental crops, although it may not be appropriate for species in which early flowering is desirable. Additionally, CO₂ supplementation resulted into increase in A_n of geranium 'Pinto Premium Rose Bicolor' in two driest irrigation regimes (-10 and -15 kPa). Similarly, for fountain grass, the moderately watered (-10-kPa) treatment had a greater rate of A_n under greater fertilizer rates (6 or 9 g). Also, CO₂ supplementation helped in lowering E in both species. Therefore, greenhouse CO₂ supplementation can result in increased WUE by lowering E and increasing A_n as well as dry weight production.

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