Effects of Carbon Dioxide and Photosynthetic Photon Flux on Mineral Content in Chrysanthemum Allowing for Growth as a Covariate

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Abstract. The effect of CO₂ concentration (330 and 675 $\mu\text{L}\cdot\text{L}^{-1}$) and photosynthetic photon flux (PPF) (mean daily peaks of 550–1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) on total mineral contents in shoots was studied in chrysanthemum [*Dendranthema × grandiflorum* (Ramat) Kitam ‘Fiesta’] during three times of the year. Growth (as measured by shoot dry weight) and shoot mineral contents (weight of nutrient per shoot) of hydroponically grown plants were analyzed after 5 weeks. There was a positive synergistic interaction of CO₂ concentration and PPF on growth with the greatest growth at high PPF (1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) with high CO₂ (675 $\mu\text{L}\cdot\text{L}^{-1}$). When growth was not used as a covariate in the statistical model, both CO₂ concentration and PPF significantly affected the content of all eight nutrients. However, after growth was included as a covariate in the model, nutrients were classified into three categories based on whether CO₂ concentration and PPF level were needed in addition to growth to predict shoot nutrient content. Neither CO₂ concentration nor PPF level was needed for Mg, Fe, and Mn contents, whereas PPF level was needed for N, P, K, and Ca contents, and both CO₂ concentration and PPF level were required for B content.

An increase in ambient CO₂ concentration generally results in increased growth and a greater demand for nutrients (Campbell

and Sage, 2002; Hagedorn et al., 2002; Jin et al., 2015; Taiz and Zeiger, 2010). Elevated ambient CO₂ concentration has been shown to result in increased phosphate uptake due to changes in root morphology and depth as well as quantity and composition of root exudates (Jin et al., 2015). Reich et al. (2006) observed a 20% to 25% increase in plant biomass in perennial grassland species when an increase in ambient CO₂ concentration was accompanied by an enriched N supply vs. an 8% to 12% increase when only the CO₂ concentration was increased. Similar responses to increased fertility have been observed in other species grown under high levels of CO₂ (Knecht and O’Leary, 1983; Patterson and Flint, 1982; Sionit et al., 1981).

Similarly, an increase in PPF also yields improved growth and greater nutrient demand (Taiz and Zeiger, 2010). Frantz (2013) reported that increasing light from 53% to full ambient in a greenhouse resulted in increased phosphate uptake without a change in tissue P concentration in *Vinca* [*Catharanthus roseus* (L.) G. Don] and *Zinnia* (*Zinnia elegans* Jacq.). This indicated that phosphate uptake kept pace with increased growth. Increased light and temperature resulted in enhanced macronutrient uptake in lettuce (Albomoz and Leith, 2015). Baligar et al. (2017) observed increased macronutrient uptake in five tropical legume cover crop species with increasing PPF in the range of 100–450 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. Chrysanthemum and *Rosa hybrida* L. grown under supplemental PPF responded to higher levels of fertility (Armitage and Tsujita, 1979; Hughes and Tsujita, 1982).

Nutrient levels have very often been presented as concentrations (i.e., percentage or $\text{mg}\cdot\text{kg}^{-1}$); however, nutrient content (weight per whole plant or plant part) provides additional useful information. Content has been used to assess total accumulation, distribution of specific nutrients, or both during or after a growth period in several species (Al-Tarhah et al., 2008; Frantz, 2013; Mishra et al., 2012; Niedziela et al., 2015; Overdieck, 1993; Somda et al., 1999; Subramanian et al., 2011; Zhu et al., 2016).

Whereas elevated CO₂ and PPF have been reported to result in increased nutrient contents, decreased nutrient concentrations have frequently been associated with these nutrient content increases. Overdieck (1993) observed increased contents and decreased concentrations of macro- and micronutrients in four herbaceous and two woody plant species. Zhu et al. (2016) found increased N, P, and K contents along with decreased concentrations in annual wormwood (*Artemisia annua* L.). Eng et al. (1985) examined the nutrient status of 3-week-old chrysanthemums grown under supplementary PPF and CO₂ enrichment and reported larger plants but decreased nutrient concentrations.

One explanation for decreased N concentration in plants under increased CO₂ is dilution by increased photosynthetic C accumulation (Taub and Wang, 2008). Fangmeier et al. (2002) noted that although total accumulation of macro- and micronutrients increased in potato tubers grown under increasing CO₂ level, nutrient concentrations decreased because of greater increases in biomass than nutrient uptake. Kuehny et al. (1991) found that increased CO₂ concentrations and PPF resulted in increased concentrations of starch and decreased concentrations of 11 nutrient elements in chrysanthemum shoots. When the concentrations of the 11 nutrients were recalculated by subtracting the weight of starch from the shoot dry weight, shifts in shoot concentrations of seven nutrients due to increases in CO₂ concentration and PPF treatments no longer occurred. These studies suggested

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that a component of growth more directly controlled shoot nutrient concentration than the ambient CO₂ concentration or PPF.

Willits et al. (1992) modeled nutrient uptake in chrysanthemum as a function of growth rate by using relative growth rate to predict relative accumulation rates for 11 elements. The Willits et al. (1992) report addressed the overall effect of CO₂ and PPF on nutrient uptake in which growth stimulation was a main effect but did not break out the effects of CO₂ or PPF above and beyond the additional growth these two factors caused. The objective of our study was to determine the effects of ambient CO₂ concentration and PPF on the shoot content of eight mineral nutrients in chrysanthemum and to assess the relative influence of ambient CO₂ concentration and PPF vs. growth on these shoot contents. In other words, we were interested in treatment effects on shoot dry weight (growth) in a covariance analysis with shoot dry weight as a covariate to assess whether there was an effect on shoot nutrient content over and above any effects associated with weight differences.

Materials and Methods

Treatments. Three greenhouse experiments, each 5 weeks in duration, were conducted during separate times of the year: 25 Apr. to 30 May, 8 Nov. to 12 Dec., and 29 Jan. to 5 Mar. Treatments in each experiment included a combination of two target CO₂ concentrations (330 and 675 $\mu\text{L}\cdot\text{L}^{-1}$) and three target PPF levels (830, 1100, and 1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the April to May and January to March experiments and 550, 830, and 1100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ in the November to December experiment). The upper CO₂ concentration selected was based on the yield response in cucumber, which was optimum at that concentration when tested in a closed-loop rock-storage bed system in the same facilities (Peet and Willits, 1987). The four target PPF levels of 550, 830, 1100, and 1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ were designated as extra-low, low, medium, and high, respectively. The high (1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) and low (830 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$) PPFs were selected based on expected maximum irradiance at 1200 HR on 21 June and 21 Dec., respectively. However, daily variations in cloud cover prevented the continuous maintenance

of the selected maximum irradiance levels. Also, CO₂ concentrations were modestly higher than set points because of respiration of workers in closed greenhouses. Minimum day and night temperature set points were 21 and 17 °C, respectively. Actual average daily CO₂ concentration during daylight hours, daily total PPF, and day and night air temperatures were as indicated in Table 1.

Greenhouse facilities. This study was conducted in Raleigh, NC (lat. 35°47'N, long. 78°42'W at an elevation of $\approx$ 121.9 m above sea level). It was conducted in four free-standing, double-polyethylene, gutter-connected style greenhouses (5.2 m wide $\times$ 6.1 m long). Each house contained a natural gas-fired heater, a two-speed fan, and an evaporative pad cooling system. Two of the greenhouses had attached rock-storage beds (3.1 m wide $\times$ 5.4 m long $\times$ 1.8 m high), which served as energy sinks during daylight hours and energy sources at night. In addition, the rock-storage beds provided closed-loop cooling which allowed CO₂ to be injected during a large part of the year (Peet and Willits, 1987; Willits and Peet, 1987). Rock-storage beds were given priority for heating and cooling; however, when the rock-storage beds were unable to meet the demand, conventional heating and cooling methods were used.

Liquid CO₂ was used for CO₂ enrichment during daylight hours. A conductimetric controller of the type developed by Kimball and Mitchell (1979) was used to monitor and control CO₂ within the greenhouses. PPF was set using a combination of natural solar radiation, shade frames (with various layers of cheesecloth), and supplemental high-pressure sodium lamps. PPF at the top of each leaf canopy were monitored with six LI-COR Model 190 quantum sensors (LI-COR, Lincoln, NE) rotated throughout each location on a weekly basis.

Cultural procedures. Unrooted cuttings of 'Fiesta' pot chrysanthemum were placed in aerated tanks of distilled water (60 plants per 20-L tank with a top measurement of 35 cm $\times$ 45 cm) for 1 week followed by half-strength complete nutrient solution for 3 d, and then full-strength solution for the remainder of the 2-week rooting period before each experiment. The full-strength nutrient solution

contained the following macronutrients (mM): 2 NH₄⁺, 14 NO₃⁻, 1 HPO₄²⁻, 6 K⁺, 4 Ca²⁺, 2 Mg²⁺, and 2 SO₄²⁻; and micronutrients (μM): 72 Fe²⁺, 18 Mn²⁺, 1.5 Zn²⁺, 1.6 Cu²⁺, 93 BO₃³⁻, and 0.1 MoO₄²⁻. Nutrient sources were (NH₄)₂HPO₄, KNO₃, Ca(NO₃)₂·4H₂O, MgSO₄·7H₂O, FeDTPA, MnCl₂·4H₂O, ZnCl₂, CuCl₂·2H₂O, H₃BO₃, and Na₂MoO₄·2H₂O. Nutrient solutions were formulated in distilled water. To prevent flowering during this 2-week rooting period, a short-night photoperiod was achieved with incandescent light applied at an intensity of 2 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ from 0000 to 0200 HR.

On the first day of the experiment, plants were transferred to larger fiberglass culture tanks with tops measuring 45 cm $\times$ 60 cm. Forty-two liters of full-strength nutrient solution was added to each tank and adjusted to a pH of 6.7. In each tank, 35 plants were supported by placing stems in hollow plastic stoppers that were suspended through holes in the tank lid. The solution in each tank was aerated at a rate of 5 L·min⁻¹ through two fritted glass diffusers connected to an air pump.

Six 42-L tanks were placed in each of four greenhouses (two houses at the high CO₂ concentration and two at low concentration) for a total of 24 culture tanks. Within each greenhouse, two tanks were placed under each of three PPF treatments. The nutrient solution was changed weekly for the duration of the experiment. Distilled water was added daily to each tank as necessary to replace transpirational losses. A short-night photoperiod was continued during the first week of the experiment. Thereafter, a long-night photoperiod of at least 12 h of uninterrupted darkness was maintained to initiate and promote floral bud development. When the natural photoperiod exceeded 12 h, plants were covered with black plastic from $\approx$ 1930 to 0730 HR. Also, at the start of long nights, the terminal end of each shoot was removed to encourage lateral branch development.

Sampling and analysis of tissue. Shoots, including leaves and stems, from five plants from each tank were taken initially and at weekly intervals on the morning of and before nutrient solution change. The five shoots were combined, washed for 1 min in 0.2 N HCl, rinsed in distilled water, dried for

Table 1. Overall means and sds^z of mean daily CO₂ concentration during daylight hours, daily total photosynthetic photon flux (PPF), and mean day and night air temperatures at the top of the plant canopy over 5 weeks in three experiments.

Expt. ^y	CO ₂ ($\mu\text{L}\cdot\text{L}^{-1}$) ^y				Daily total PPF ($\text{mol}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$) ^x								Air temp (°C) ^w			
	Low		High		Extra-low		Low		Medium		High		Day		Night	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
April to May	368	14	558	26	—	—	11.9	1.4	17.9	2.2	24.5	2.9	26.2	0.4	20.3	0.8
November to December	422	43	645	14	5.8	1.0	8.3	1.3	15.8	1.4	—	—	24.0	1.1	18.6	0.6
January to March	423	10	693	15	—	—	7.0	2.0	12.7	2.9	20.7	1.6	22.8	0.6	17.7	0.1

^zsds were of the weekly means and thus represent the variability of the associated factor from growth interval to growth interval to growth interval for each experiment.

^yTarget low and high CO₂ treatment levels were 330 and 675 $\mu\text{L}\cdot\text{L}^{-1}$, respectively.

^xTarget PPF levels for extra-low, low, medium, and high PPF treatments (maximum noontime irradiance) were 550, 830, 1100, and 1400 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$, respectively.

^wMinimum day and night temperature set points were 21 and 17 °C, respectively.

^vA short-night photoperiod was applied during the first week for vegetative growth followed by a long-night photoperiod for flowering in each experiment.

24 h at 70 °C, weighed, and ground in a stainless-steel Wiley Mill (Thomas Scientific, Philadelphia, PA) to a particle size of 1 mm or less. Tissue was dry-ashed at 500 °C, dehydrated in HCl, and finally dissolved in 0.5 N HCl for P, K, Ca, Mg, Fe, and Mn analyses. Tissue was handled in a similar manner for B analyses except that it was ashed in the presence of a solution of Mg (NO₃)₂ in MeOH. Total N was determined by a Kjeldahl procedure which included a salicylic acid pretreatment to aid reduction of NO₃⁻ (Eastin, 1978). Analyses for K, Ca, Mg, Fe, and Mn were carried out by atomic absorption spectrophotometry (AAAnalyst 100; Perkin-Elmer, Norwalk, CT). Colorimetric analyses were performed for P (Jackson, 1958) and B (Grinstead and Snider, 1967) using a Perkin-Elmer Lambda 3 ultraviolet/VIS spectrophotometer (Norwalk, CT). Resulting nutrient concentrations were converted to nutrient content per shoot (mg/shoot and µg/shoot for macro- and micronutrients, respectively).

Experimental design and statistical analysis. The experimental design was an unbalanced split-split plot with three experiments (April to May, November to December, and January to March) as main plots. Within each experiment, there were two target CO₂ concentrations (330 and 675 µL·L⁻¹) replicated twice each in four greenhouses (two greenhouses at each CO₂ concentration). Within each greenhouse, there were three target *PPF* levels (830, 1100, and 1400 µmol·m⁻²·s⁻¹ in the April to May and January to March experiments and 550, 830, and 1100 µmol·m⁻²·s⁻¹ in the November to December

experiment). There were two replications within each target *PPF* level. Experimental units were 42-L hydroponic tanks (72 in total).

Analysis of variance (ANOVA) was performed on shoot dry weight and nutrient content using PROC MIXED (SAS 9.4; SAS Inst., Cary, NC). At first, the experiment was used as a source of variation in the analysis. Because there were no significant interactions of experiment by CO₂, experiment by *PPF*, and experiment by CO₂ by *PPF* ($\alpha = 0.05$), data were reanalyzed treating experiments as blocks. Finally, shoot dry weight was used as the covariate of the nutrient contents to determine the additive effects of CO₂ concentration and *PPF* levels beyond the effect of growth (as measured by differences in shoot dry weight) on nutrient content. Means were compared using the Tukey–Kramer method. If the ANOVA for a nutrient was significant and the test for shoot dry weight as a covariate was also significant, then shoot dry weight was used as a covariate for the Tukey–Kramer analysis. Terms of each model were tested using *F* values and significance level $\alpha = 0.05$. To further investigate the nature of a CO₂ by *PPF* interaction that occurred for B content when shoot dry weight was used as a covariate, simple effects (differences of least squares means) of CO₂ within the different levels of *PPF* were computed using an LSMEANS statement with the SLICE option. The SLICE option enabled detection of any trends in the strength of evidence (*F* value) for CO₂ concentration separation over *PPF* levels.

Results and Discussion

There was a significant CO₂ concentration by *PPF* interaction for growth, as measured by shoot dry weight (Table 2A). Generally, growth increased with increasing *PPF* and increased CO₂ concentration (Table 3). The combination of increasing both independent variables together had a greater effect on growth than each individually. Specifically, growth was lowest at extra-low *PPF* (550 µmol·m⁻²·s⁻¹) regardless of CO₂ concentration or low *PPF* (830 µmol·m⁻²·s⁻¹) with low CO₂ concentration (330 µL·L⁻¹). The next greatest growth occurred at low *PPF* with high CO₂ concentration (675 µL·L⁻¹) or medium *PPF* (1100 µmol·m⁻²·s⁻¹) with low CO₂ concentration. Growth increased further at medium *PPF* with high CO₂ concentration or high *PPF* (1400 µmol·m⁻²·s⁻¹) with low CO₂ concentration. The greatest growth was recorded at high *PPF* with high CO₂ concentration. The positive synergistic effects of increasing light and raising ambient CO₂ on growth in chrysanthemum have been previously reported (Eng et al., 1983, 1985; Stephanis and Langhans, 1982).

Using the statistical model with experiment blocked, but without shoot dry weight as a covariate, there were significant main effects of CO₂ concentration and *PPF* for all the nutrients tested (Table 2A). In addition, Mg had a CO₂ concentration by *PPF* interaction using this model. After shoot dry weight (growth) was included in the model as a covariate, shoot dry weight was significant for all nutrients, and the main effect of target CO₂ concentration was no longer significant

Table 2. Results of two-way factorial analyses of variance on the effects of two CO₂ and four photosynthetic photon flux (*PPF*) levels on shoot dry weight (growth) and content of eight mineral nutrients in chrysanthemum shoots after 5 weeks: (A) without and (B) with shoot dry weight as a covariate in the model.

	Shoot dry wt	N	P	K	Ca	Mg	Fe	Mn	B
Source	<i>P</i> > <i>F</i> ^z								
(A) ANOVA without shoot dry wt as a covariate									
CO ₂	0.0002	0.0002	0.0045	0.0001	0.0008	0.0040	0.0006	0.0062	0.0316
<i>PPF</i>	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
CO ₂ × <i>PPF</i>	0.0294	0.1600	0.2517	0.1074	0.1088	0.0325	0.0777	0.5983	0.5582
(B) ANOVA with shoot dry wt as a covariate									
CO ₂	—	0.4544	0.9236	0.2303	0.9750	0.2042	0.9560	0.2451	0.0003
<i>PPF</i>	—	0.0166	<0.0001	0.0035	<0.0001	0.1550	0.0911	0.0291	0.0624
CO ₂ × <i>PPF</i>	—	0.3370	0.4270	0.1352	0.0869	0.4735	0.0663	0.1591	0.0002
Shoot dry wt	—	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

^zNumbers bolded were significant ($\alpha = 0.05$).

ANOVA = analysis of variance.

Table 3. The interactive effects of CO₂ and photosynthetic photon flux (*PPF*) on shoot dry wt and main effect of *PPF* on nitrogen, phosphorus, potassium, calcium, and manganese content in chrysanthemum shoots after 5 weeks.^z

Target <i>PPF</i> ^y (μmol·m ⁻² ·s ⁻¹)	Shoot dry wt (g/shoot)		N	P	K	Ca	Mn (μg/shoot)
	Low CO ₂ ^x	High CO ₂	(mg/shoot)				
550	4.20 a	5.06 a	415 a	66 a	648 a	80 a	1,778 a
830	6.03 a	8.48 b	462 b	83 b	698 b	94 b	1,900 a
1,100	9.30 b	12.13 c	501 bc	98 c	750 c	99 b	1,791 a
1,400	11.33 c	15.03 d	526 c	100 c	782 c	101 b	1,897 a

^zMeans were compared using the Tukey–Kramer method. Shoot dry weight (growth) was a covariate in the model used for mean separation in the five mineral nutrients. Numbers for each dependent variable with the same letter were not significantly different ($\alpha = 0.05$).

^yMean daily total *PPF* at the target *PPF* levels of 550, 830, 1100, and 1400 µmol·m⁻²·s⁻¹ were 5.8, 9.1, 15.5, and 22.6 mol·m⁻²·d⁻¹, respectively, when measured values from Table 1 were averaged across the three experiments.

^xTarget low and high CO₂ treatment levels were 330 and 675 µL·L⁻¹, respectively.

Table 4. The simple effects of CO₂ within photosynthetic photon flux (PPF) levels on boron content in chrysanthemum shoots after 5 weeks.^z

Target <i>PPF</i> ^y (μmol·m ⁻² ·s ⁻¹)	B (μg/shoot)		Difference in CO ₂		<i>t</i>	<i>P</i> > <i>t</i>
	Low CO ₂ ^x	High CO ₂	Low vs. high			
550 (extra-low)	591	582	9		0.36	0.7236
830 (low)	572	514	58		3.12	0.0039
1,100 (medium)	533	473	60		3.02	0.0049
1,400 (high)	539	386	153		6.16	<0.0001

^zMeans were compared across CO₂ concentrations within the different levels of PPF by using an LSMEANS statement with the SLICE option. Shoot dry wt (growth) was a covariate in the model used for mean separation.

^yMean daily total PPF at the target PPF levels of 550, 830, 1100, and 1400 μmol·m⁻²·s⁻¹ were 5.8, 9.1, 15.5, and 22.6 mol·m⁻²·d⁻¹, respectively, when measured values from Table 1 were averaged across the three experiments.

^xTarget low and high CO₂ treatment levels were 330 and 675 μL·L⁻¹, respectively.

for all nutrients except B (Table 2B). However, the main effect of PPF remained significant for N, P, K, Ca, and Mn, and there was also a CO₂ concentration by PPF interaction for B. All subsequent discussions concerning nutrient contents will use the results of this latter model that included growth as a covariate.

Although the covariate shoot dry weight was significant for Mg and Fe, there were no effects of CO₂ or PPF (Table 2B). The elimination of significant treatment effects for Mg and Fe by including shoot dry weight (growth) as a covariate in the model indicated that differences in these two nutrients could be explained by the differences in shoot dry weight without additional input from CO₂ or PPF levels. In other words, changes in these two nutrients paralleled changes in growth. The mean Mg and Fe contents for all treatments in the three experiments were 23.2 mg/shoot and 1067 μg/shoot, respectively.

There was a significant main effect of PPF on shoot N content (Table 2B). The N content generally increased with increasing PPF (Table 3). The shoot N content at extra-low PPF (550 μmol·m⁻²·s⁻¹) was lower than that at the other treatment levels. Shoot N content at low PPF (830 μmol·m⁻²·s⁻¹) was similar to that at medium PPF (1100 μmol·m⁻²·s⁻¹) but lower than that at high PPF (1400 μmol·m⁻²·s⁻¹).

There was a main effect of PPF on shoot P and K contents (Table 2B). Phosphorus and K had a similar response patterns. Phosphorus and K contents, such as N, tended to increase with increasing PPF (Table 3). However, the shoot P and K contents were least at extra-low PPF (550 μmol·m⁻²·s⁻¹), intermediate at low PPF (830 μmol·m⁻²·s⁻¹), and greatest at medium and high PPF (1100 and 1400 μmol·m⁻²·s⁻¹, respectively).

There was a main effect of PPF on shoot Ca content (Table 2B). Calcium content was lower at extra-low PPF (550 μmol·m⁻²·s⁻¹) than that at the other three PPF levels, which were similar to each other (Table 3).

Whereas the ANOVA showed a significant main effect of PPF on Mn content (Table 2B), the Tukey–Kramer analysis did not exhibit significant mean separation (Table 3). The mean Mn content for all treatments in the three experiments was 1850 μg/shoot.

The response of B to CO₂ and PPF differed from the other nutrients tested. Increased CO₂ and PPF caused a decrease in

shoot B content rather than the increase with increasing PPF that occurred for N, P, K, and Ca, or the lack of response to CO₂ or PPF that occurred for Mg, Fe, and Mn (Table 3). There was a significant CO₂ concentration by PPF interaction for shoot B content (Table 2). The SLICE option analysis provided insight into the simple effects of CO₂ within the PPF levels on B content (Table 4). There was no effect of CO₂ concentration at the extra-low PPF level (550 μmol·m⁻²·s⁻¹), a moderate effect at the low and medium PPF levels (830 and 1100 μmol·m⁻²·s⁻¹, respectively), and a strong effect at the high PPF level (1400 μmol·m⁻²·s⁻¹). Mishra et al. (2012) likewise reported lower leaf B content and B uptake rate in seed geranium (*Pelargonium xhortorum*) when grown at 700 vs. 370 μL·L⁻¹ CO₂. During the early research of CO₂ enrichment in greenhouses, B deficiencies were noted in flowering crops grown in high concentrations of CO₂ (Lindstrom, 1964).

In our study, increased PPF from low to optimal levels likely caused increases in leaf temperature which may have increased growth and uptake of many nutrients. Jia et al. (2015) found that growth and uptake of N, P, and K increased with increasing temperature in gerbera seedlings. Other studies have shown increases in influx of ammonium in tomato (Bloom et al., 1998), net ammonium or nitrate uptake in barley (Bloom, 1985) and tomato (Smart and Bloom, 1988), and potassium uptake in barley roots (Hoagland and Broyer, 1936) with increasing temperature. The *Q*₁₀ values in those studies ranged from 1.8 to 2.0. However, leaf temperature measurements were not available from our study to assess the impact of leaf temperature on nutrient accumulation either through temperature-enhanced growth or temperature effects above and beyond growth. These results emphasize the importance of providing an adequate supply of nutrients at high CO₂ concentrations and PPF levels.

Conclusions

The eight nutrients tested can be allocated to three groups with respect to the role CO₂ and PPF levels play beyond growth measurement in prediction of their content in chrysanthemum shoots. For Mg, Fe, and Mn, levels of CO₂ and PPF were not required for prediction of their content. The level of PPF,

but not CO₂, was needed for prediction of N, P, K, and Ca content. Finally, both CO₂ and PPF levels were required for prediction of B content.

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