THESIS

CHARACTERIZING ACCLIMATION OF PANSY AND PETUNIA TO CO_2 ENRICHMENT FOR CONTROLLED ENVIRONMENT PRODUCTION

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ABSTRACT

CHARACTERIZING ACCLIMATION OF PANSY AND PETUNIA TO CO_2 ENRICHMENT FOR CONTROLLED ENVIRONMENT PRODUCTION

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While crops often respond immediately to enriched CO_2 concentrations (e.g., increased photosynthesis), this initial response is often not sustained throughout production, reducing the benefit of this input. For horticulture species, the timing and extent of these acclimation responses is still widely uncertain. Therefore, the objective of this research was to determine species-specific acclimation responses to enriched CO_2 concentrations for pansy (*Viola ×wittrockiana* 'Matrix Blue Blotched Improved') and petunia (*Petunia ×hybrida* 'Dreams Midnight) during both propagation and finishing.

To investigate the effects of enriched CO_2 concentrations on pansy and petunia during finishing production, seedlings were transplanted into 11.5-cm pots and placed in growth chambers with air temperature, relative humidity, and radiation intensity setpoints of 21 °C, 55%, and 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Carbon dioxide treatments were established using the two growth chambers with setpoints of either 400 (ambient) or 1000 $\mu mol \cdot mol^{-1}$ (enriched) maintained during a 16-h photoperiod. In addition to data collected through destructive harvest, rate of photosynthesis (A) in response to increasing internal leaf CO_2 concentration (A-C_i) and ambient CO_2 concentration (A-C_a) were measured weekly with a portable leaf photosynthesis system at saturating (A-C_i; 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or production (A-$C_a$; 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) radiation

intensities. For both pansy and petunia, plants grown under the enriched CO_2 concentration produced higher total shoot dry mass compared to ambient after 4 weeks. However, decreased maximum rate of photosynthetic electron transport (J_{max}), maximum rate of Rubisco carboxylase (V_{cmax}), and similar photosynthesis at operating C_i concentration were observed under the enriched CO_2 concentration after 4 weeks. Additionally, A measured at 1000 and 400 μmol·mol^{-1} was lower for both pansy and petunia grown under the enriched compared to ambient CO_2 concentration based on A-C_a responses after 1 week, further indicating quick physiological acclimation to this input. This indicates little benefit of elevated CO_2 to increase plant quality during the finishing stage of production in pansy and petunia, however there is possible marginal benefit due to increased biomass with no effect on overall plant size.

To evaluate the impact of CO_2 enrichment at varying timing and duration during propagation, pansy and petunia seeds were sown in 128-cell trays and placed in growth chambers with air temperature, relative humidity, and radiation intensity setpoints of 21 °C, 55%, and 250 μmol·m^{-2}·s^{-1}, respectively. Carbon dioxide treatments were established using the two growth chambers with setpoints of either 400 (ambient) or 1000 μmol·mol^{-1} (enriched) maintained during a 16-h photoperiod. Treatments consisted of seedlings grown for 28 days at ambient (Amb_{28}), 28 days at elevated (Elv_{28}), 14 days at ambient then 14 days at elevated (Amb_{14}:Elv_{14}), and 14 days at elevated then 14 days at ambient CO_2 concentration (Elv_{14}:Amb_{14}). Harvest data was collected weekly, and four weeks after germination seedlings were transplanted into the greenhouse to determine impacts on finishing quality and flowering. Pansy and petunia produced higher total dry mass (roots + leaves + stem) under Elv_{28} and Amb_{14}:Elv_{14} compared to Amb_{28} after 4 weeks, but showed no difference in leaf area. Additionally, plants grown under Elv_{28} and Amb_{14}:Elv_{14} produced higher leaf mass area than Amb_{28} and Elv_{14}:Amb_{14} for both species. Pansy

showed decreased days to flower under Elv_{28}, but no difference in biomass or size after transplant into the greenhouse. Therefore, elevated CO_2 during seedling production may influence days to flower but does not contribute to growth rate long term after transplant. Likewise, similar morphological responses can be achieved with elevated CO_2 being applied during the last two weeks of seedling production compared to elevation throughout the propagation stage.

These results provide useful information regarding the timing and extent of physiological acclimation in response to enriched CO_2 concentrations for pansy and petunia. However, due to physiological acclimation potentially occurring within one week of treatment initiation, additional research is needed to best understand how this input can be further optimized for controlled environment production.

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CHAPTER 1. INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction to Annual Bedding Plant Production and CO_2 Enrichment

The 2018 wholesale value for floriculture crops in the US is estimated at $4.77 billion. Approximately a third of that is represented by annual bedding plants, with over 423 million square feet of greenhouses used for floriculture production (U.S. Dept. Agr., 2019). The production of high quality bedding plant seedlings (plugs) is essential to the industry, with desired criteria including a compact habit, high root and shoot biomass, and low leaf area to reduce mutual shading (Craver, 2018; Oh et al., 2010; Pramuk and Runkle, 2005; Randall and Lopez, 2014). These plug characteristics facilitate processing, shipping, and transplanting (Pramuk and Runkle, 2005). However, challenges regarding plug uniformity, consistency, and overall quality are common due to the time of the year when production generally occurs (Both et al., 2017; Erwin and Gesick, 2017; Mortensen, 1987).

Production of plugs from seed for the ornamental annual bedding plant market typically occurs in the winter and early spring (Styer, 2003). While the average ambient CO_2 concentration is approximately 400 $\mu mol \cdot mol^{-1}$, it is not uncommon for concentrations in the greenhouse to fall as low as 200 $\mu mol \cdot mol^{-1}$ during the winter months (Both et al., 2017; Erwin and Gesick, 2017; Mortensen, 1987). This generally happens on sunny, cold days when the greenhouse is full of plants, but the ventilation is too low to replenish CO_2. Crop demand for CO_2 becomes greater than the supply, limiting photosynthesis and similarly, plant growth (Both et al., 2017; Erwin and Gesick, 2017). Even with proper ventilation, greenhouse concentrations are still commonly 250 to 300 $\mu mol \cdot mol^{-1}$ (Mortensen, 1987). Injecting CO_2 into the greenhouse may benefit young plant production to help growers maintain uniformity and quality during these

times of the year when controlled environment conditions may be unfavorable. However, CO_2 can be used to not only replenish depleted concentrations but possibly increase plant quality or shorten production time through enrichment (Mortensen and Moe, 1992; Mortensen, 1987; Prior et al., 2011).

Enriching greenhouse environments with elevated CO_2 is a well-known method of enhancing plant growth (Mortensen and Moe, 1992; Mortensen, 1987). Current atmospheric CO_2 concentrations are too low for maximum photosynthetic capacity, mainly due to competition between CO_2 and O_2 fixed by the enzyme Ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco) which results in possible photorespiration and a loss in carbon (Mortensen, 1987). CO_2 levels near 900 $\mu mol \cdot mol^{-1}$ almost eliminates O_2 inhibition of photosynthesis due to an increased CO_2/O_2 ratio (Gunderson and Wullschleger, 1994; Mortensen, 1987). Numerous studies have shown that CO_2 concentrations between 800 to 1200 $\mu mol \cdot mol^{-1}$ have the potential to increase plant growth, while further increases above this range have limited benefit (Both et al., 2017). Common practice in commercial vegetable production greenhouses is injecting 800 to 1000 $\mu mol \cdot mol^{-1}$ CO_2 to increase yield (Erwin and Gesick, 2017). Concentrations of 600 to 1000 $\mu mol \cdot mol^{-1}$ CO_2 are often the most practical during conditions requiring limited ventilation, and fall within the optimal range for most species (Mortensen, 1987). Generally, elevated CO_2 concentrations have the greatest effect on increasing photosynthesis from the first 100 $\mu mol \cdot mol^{-1}$ above ambient (400 to 500 $\mu mol \cdot mol^{-1}$) and incrementally decrease in benefit with further increases (Both et al. 2017; Erwin and Gesick, 2017). Early short-term studies concluded that increasing the CO_2 concentration in greenhouses was an economically efficient way to enhance growth in ornamental and vegetable crops (Mortensen, 1987; Prior et al., 2011). However, many of these early experiments were designed to convince growers about the benefits

of CO_2 rather than provide a comprehensive evaluation of sustained plant responses under these conditions (Mortensen, 1987).

Many short-term experiments have been conducted in controlled environments using both hydroponic and soilless substrate production methods. These short-term studies retain an important role due to the ease of investigating CO_2 responses under reduced co-limitations compared to free-air concentration enrichment (FACE) studies (Kirschbaum and Lambie, 2015).

1.2 Free-Air Concentration Enrichment (FACE) Studies

Alongside early studies examining the economic benefits of using CO_2 enrichment as an input of production, FACE experiments established the effects of rising atmospheric CO_2 concentrations on agronomic crops and natural ecosystems. These FACE studies help confirm plant responses to elevated CO_2 concentrations, both beneficial and detrimental, especially for arguments of species-specific responses (Drake et al., 1997; Prior et al., 2011). For example, 40 species across 12 FACE studies showed a 20% reduction in stomatal conductance in response to elevated CO_2 (Ainsworth and Long, 2004). Meanwhile, biomass generally increased with exposure to elevated CO_2 concentrations, but the extent of the increase was varied across species, growing season, and experimental conditions (Ainsworth and Long, 2004). Further studies examined crop yield responses. For example, while cotton (*Gossypium hirsutum* L.) showed a 42% increase in yield with 550 $\mu mol \cdot mol^{-1}$ CO_2 compared to ambient during a full growing season, rice (*Oryza sativa*), wheat (*Triticum aestivum*), and sorghum (*Sorghum bicolor*) showed no response to 500 – 800 $\mu mol \cdot mol^{-1}$ CO_2 compared to ambient (Ainsworth and Long, 2004; Mauney et al., 1994).

A review of these early FACE studies indicated that exposure to elevated CO_2 resulted in a 31% increase in the light-saturated leaf photosynthetic rate when averaged across all

experiments and species (Ainsworth and Long, 2004). However, these FACE studies showed the greatest photosynthetic stimulation to elevated CO_2 concentrations came from trees, followed by shrubs and herbaceous plants, establishing a possible sink-source relationship to photosynthetic acclimation and variation amongst species (Ainsworth and Long, 2004). Further reviews confirm an immediate plant response of increased photosynthesis in response to CO_2 concentrations above ambient (Drake et al., 1997; Prior et al., 2011). Similar to controlled environment studies, FACE studies concluded that elevation in CO_2 concentration increased photosynthetic rate by increasing carboxylation and inhibiting oxygenation activity of Rubisco in the short-term (Drake et al., 1997). However, net photosynthetic rate failed to meet predicted values across agronomic and natural species in the long-term, indicating possible acclimation to elevated CO_2 concentrations (Ainsworth and Long, 2004).

1.3 Short-Term Responses to Elevated CO_2

Short duration experiments can be analyzed by comparing biomass between plants grown at elevated CO_2 and at an ambient concentration, with the amplification of biomass increase in the elevated treatments being small at first but becoming quantitatively important over time (Kirschbaum and Lambie, 2015). Elevated CO_2 stimulates photosynthesis leading to increased carbon uptake and assimilation, increasing plant growth (Prior et al., 2011). Species-specific increases in photosynthetic rate were found to vary between 33% and 40% for C_3 plants in FACE studies (Prior et al., 2011; Prior et al., 2003). Ornamental horticulture varieties tend to show a reduced increase in photosynthetic rate, closer to 15% to 25%, possibly due to limited root area and lack of carbon sinks with containerized production (Mortensen, 1991, 1994; Prior et al., 2011). In another survey by Drake et al. (1997) encompassing 60 experiments, the authors reported overwhelming evidence that photosynthetic rate increased immediately by up to 58% under increased CO_2 concentrations compared to plants under ambient conditions. Other short-

term responses to elevated CO_2 concentrations observed broadly across species include reduction in stomatal conductance and transpiration, improved water-use efficiency (WUE), and increased light-use efficiency (Ainsworth and Long, 2004; Anderson et al., 2001; Drake et al., 1997). For example, a survey conducted by Mortenson (1987) showed an increase in CO_2 concentration can improve WUE by 30% across multiple species. Elevated CO_2 can improve plant water relations with slowed transpiration due to partial closure of stomatal guard cells (Prior et al., 2011). This reduction in transpiration combined with an increased photosynthetic rate contributes to increased WUE, although the production of ornamental species in controlled environments is rarely limited by water (Prior et al., 2011). A higher WUE coupled with increased short-term photosynthetic rate can ameliorate drought stress, although the effect is dampened with increasing leaf area or whole plant size (Prior et al., 2011). While this may indicate that plants in the greenhouse can be watered less frequently in elevated CO_2, frequency may need to be maintained as plants increase in size with a restricted root zone (Prior et al., 2011). In food crops like lettuce (*Lactuca sativa* 'Grand Rapids'), production time can be shortened using CO_2 enrichment while also reducing other inputs such as heating, supplemental lighting, and water from improved WUE (Both et al., 2017; Frantz, 2011).

1.4 Long-Term Acclimation Responses to Elevated CO_2

However, acclimation in the form of reduced photosynthetic rate to elevated CO_2 concentrations can occur over time, with this response linked to a number of metabolic processes, morphological responses, and physiological changes (Arp, 1991; Both et al., 2017; Drake et al., 1997; Mortensen, 1987; Mortensen and Moe, 1983; Prior et al., 2011). Acclimation for the purpose of this review can be defined as any biochemical or physiological changes that result from growth under elevated CO_2 concentrations (Eamus and Jarvis, 1989; Gunderson and Wullschleger, 1994).

Immediate photosynthetic measurements (survey) on a broad range of natural species indicates a down regulation of net photosynthesis after sustained exposure to elevated CO_2 concentrations (Dillon et al., 2018). The same trend was detected when assessing overall net photosynthesis taken from A-C_i curves (Dillon et al., 2018). This coincided with an overall decrease in biochemical processes of photosynthesis. The duration of exposure to elevated CO_2 concentration is correlated to reduction in photosynthetic capacity, showing diminishing positive response to this input long term (Arp, 1991; Drake et al., 1997; Mortensen, 1987).

Photosynthetic rates of plants grown in elevated CO_2 are often lower than the rates of plants grown at ambient concentrations when measured at the same C_i (Arp, 1991). Additionally, plants grown at high levels of CO_2 show larger reductions in photosynthetic capacity compared to lower but still elevated levels (Arp, 1991). For example, young lettuce 'Black-Seeded Simpson' plants exposed to elevated CO_2 displayed a more than two-fold increase in net photosynthetic rate compared to ambient conditions after short-term exposure. However, with longer exposure (3 weeks) the net photosynthetic rate declined well below those grown at ambient (Giri et al., 2016). FACE experiments indicated similar acclimation to elevated CO_2, especially in C_3 species (Ainsworth and Long, 2004; Rogers and Humphries, 2000).

There are several proposed mechanisms for photosynthetic acclimation. Many FACE studies attribute an acclimated photosynthetic rate to decreased carboxylation rate and reduced investment in Rubisco (Ainsworth and Long, 2004; Rogers and Humphries, 2000). Acclimation of plants to elevated CO_2 concentrations is attributed in part to accumulation of carbohydrates, decreased stomatal conductance, and reduced activity and decreased regeneration of Rubisco (Mortensen, 1987). Long-term decline of photosynthetic capacity can be attributed to a decrease in Rubisco in the leaves, limiting the rate of carbon assimilation and carbon fixing efficiency

(Ainsworth and Long, 2005; Anderson et al., 2001, Arp, 1991; Giri et al., 2016; Moore et al., 1999). Reduction in photosynthetic rate after exposure to elevated CO_2 concentrations can also be attributed to carbohydrate accumulation after enhanced supply (Arp, 1991). For example, other proposed mechanisms of photosynthetic acclimation include an increased concentration of sucrose resulting in a negative feedback on sucrose synthesis enzymes, which in turn induces higher rates of starch synthesis (Arp, 1991; Herold, 1980). If starch and sucrose levels exceed the maximum rate of synthesis, photophosphorylation can be affected and photosynthesis becomes insensitive to the O_2 and CO_2 concentrations (Arp, 1991; Sharkey, 1985). Other possible acclimation responses may be due to suppressed gene expression due to accumulation of hexoses derived from the high levels of sucrose, causing limitation in the photosynthetic apparatus (Makino and Mae, 1999; Moore et al., 1999; Rolland et al., 2002). Regulation of the expression of photosynthetic genes, via increased soluble carbohydrate concentration, may underlie acclimation to growth in elevated CO_2 (Drake et al., 1997; Moore et al., 2002). Finally, accumulation of starch in the leaf can directly affect photosynthesis by damaging or changing the structure of the chloroplasts and impact CO_2 diffusion into the chloroplasts (Arp, 1991; Cave et al., 1981; Makino and Mae, 1999; Moore et al., 1999; Mortensen, 1987; Wulff and Strain, 1982).

1.4.1 Sink Limitations

Plants grown in containers, like much of the floriculture industry, respond to elevated CO_2 with significant preference for carbon partitioning to belowground biomass, contributing to sink-limiting acclimation (Arp, 1991; Cotrufo and Gorissen, 1997; Drake et al., 1997; Kirschbaum, 2011; Mauney et al., 1994; Morgan et al., 2001; Prior et al., 2011). Limited root area in restricted root zones like containers has been shown to decrease the positive response of CO_2 enrichment (Arp, 1991; Both et al., 2017; Drake et al., 1997; Kirschbaum, 2011;

Kirschbaum and Lambie, 2015; Prior et al., 2011). The long-term response of plants to CO_2 is partially related to sink size and the limited ability for the plant to metabolize fixed carbon (Anderson et al., 2001; Arp, 1991; Frantz and Ling, 2011; Makino and Mae, 1999; Rogers et al., 1998). Photosynthesis is dampened when carbon cannot be fully metabolized or stored. In response, plants may alter their carbon allocation in the short term to increase sink size, like number of leaves, number of branches, or number of flowers (Frantz and Ling, 2011; Mortensen, 1987). Plants grown in sink-limiting containers exhibit higher leaf mass area and show quick photosynthetic acclimation when grown at elevated CO_2 concentrations in controlled environments compared to field grown or natural plants (Ainsworth and Long, 2004; Anderson et al., 2001; Arp, 1991). For example, FACE studies often did not see photosynthetic acclimation due to abundant sinks in natural settings (Anderson et al., 2001). In a study on pansy (*Viola ×wittrockiana*), smaller container sizes resulted in fewer growth difference between plants grown at an 800 $\mu mol \cdot mol^{-1}$ CO_2 or an ambient concentration (Both et al., 2017). Other studies across multiple species indicate the size of the rooting volume will influence the magnitude and speed of the sink-limitation response (Drake et al., 1997; Kirschbaum, 2011). Due to this sink-limitation response, only certain types of production may benefit from elevated CO_2, like short-term production of young plants, semi-open root zone environments (e.g., hydroponics), large containers, or stock plant production where timeframe or creation of new sinks reduce permanent limitations (Frantz and Ling, 2011).

1.4.2 Rubisco and Chlorophyll Content

A common response to elevated CO_2 concentration is a significant decrease in the nitrogen (N) content of leaves (Morgan et al., 2001). A survey by Drake et al. (1997) showed that across 8 studies on 11 species there was a 15% reduction in Rubisco content and 24% reduction in Rubisco activity. Rubisco accounts for 25% of leaf N, resulting in a substantial

decrease in leaf N content for plants grown under elevated CO_2 concentrations (Drake et al., 1997; Makino and Mae, 1999). Reduction in leaf N content could also be attributed to repartitioning of Rubisco to plant sinks like new leaves and roots (Kirschbaum, 2011; Moore et al., 1999). Elevated CO_2 forces plants to devote less N to carbon fixation and more to Ribulose 1,5-bisphosphate (RuBP) regeneration, resulting in a decreased capacity for carboxylation (Anderson et al., 2001; Makino and Mae, 1999). Results from FACE studies suggest that the decrease in Rubisco is specific and not part of a general decrease in leaf protein (Ainsworth and Long, 2004). Additionally, multiple studies suggest a selective loss of Rubisco content without reduction in RuBP regeneration (Arp, 1991; Drake et al., 1997; Kirschbaum, 2011). For example, elevated CO_2 concentrations resulted in decreased Rubisco activation in kale (*Brassica oleracea* 'Toscano', 'Winterbor', and 'Red Russian'), spinach (*Spinacea oleracea* 'Melody', 'Harmony', and 'Bloomsdale LS'), and swiss chard (*Beta vulgaris* 'Rhubarb', 'Fordhook Giant', 'Bright Yellow', and 'Bright Lights') (Erwin and Gesick, 2017). Petunia (*Petunia ×hybrida* 'Madness White') grown at 800 $\mu mol \cdot mol^{-1}$ CO_2 compared to ambient (400 $\mu mol \cdot mol^{-1}$ CO_2) had a higher N concentration after 5 weeks as well as all other nutrients with the exception of Cu, contrary to the most commonly observed responses in many CO_2 studies investigating N supply (Frantz and Ling, 2011; Taub and Wang, 2008). However, after 7 weeks, plants grown at an elevated CO_2 concentration had a decreased N concentration (Frantz and Ling, 2011).

While there is commonly a reduction in Rubisco content, plants grown at elevated CO_2 concentrations also invest fewer resources into the production of chlorophyll, depending on species (Ainsworth and Long, 2004; Gunderson and Wullschleger, 1994). Long-term exposure to elevated CO_2 can cause starch accumulation that can inhibit or even breakdown chlorophyll in the leaves (Arp, 1991; Cave et al., 1981; Makino and Mae, 1999; Mortensen, 1987; Wulff and

Strain, 1982). For example, Zhang et al. (2012) found that New Guinea impatiens *Impatiens hawkeri*) decreased chlorophyll content by 18% after exposure to 760 μmol·mol^{-1} CO_2 for ten weeks compared to ambient. For many species, there is no change in relative chlorophyll content as a result of long-term elevated CO_2 (Ainsworth and Long, 2004; Giri et al., 2016; Gunderson and Wullschleger, 1994). For example, leaf total chlorophyll content in lettuce 'Black-Seeded Simpson' and spinach 'Bloomsdale Long Standing' was not affected by 700 μmol·mol^{-1} CO_2 compared to an ambient of 400 μmol·mol^{-1} CO_2 (Giri et al., 2016). However, a shift in the chlorophyll α and chlorophyll β ratio can occur under elevated CO_2 concentrations, possibly as a shade response due to thicker leaves (Arp, 1991). For example, Perez-Lopez et al. (2015) found that the concentration of chlorophyll-b increased by 64% in lettuce 'Blond of Paris Batavia' and 52% in lettuce 'Oak Leaf' after exposure to 700 μmol·mol^{-1} CO_2 compared to an ambient (400 μmol·mol^{-1} CO_2). Plant responses in the form of changes to chlorophyll content appear to be highly species-specific (Ainsworth and Long, 2004; Arp, 1991; Giri et. al., 2016; Gunderson and Wullschleger, 1994).

1.4.3 Carbohydrate Concentration

Plants grown at elevated CO_2 concentrations have high accumulations of starch and sucrose (Kirschbaum, 2011; Morgan et al., 2001). A survey across a dozen studies shows an average 60% increase in sucrose and 160% increase in starch of plants grown under elevated CO_2 concentrations (Drake et al., 1997). For example, carbohydrate concentration was significantly higher for petunia 'Madness White' after 3, 5, and 7 weeks of being grown at a CO_2 concentration of 800 μmol·mol^{-1} compared to an ambient of 400 μmol·mol^{-1} CO_2 (Frantz and Ling, 2011). This indicates that fixed carbon remained in the leaves possibly as a result of persistent sink limitations (Frantz and Ling, 2011). Acclimation of photosynthesis to elevated CO_2 was highly correlated to an increase in soluble saccharide concentration in kale, spinach,

and swiss chard (Erwin and Gesick, 2017). Evidence suggests that elevated CO_2 concentrations increase net photosynthetic rate of light-limited leaves in the lower canopy, contributing to whole plant carbohydrate concentrations (Drake et al., 1997).

1.4.4 Leaf Morphology and Stomatal Conductance

Evidence suggests physiological and morphological changes to elevated CO_2 concentrations first occurs at the leaf level (Gunderson and Wullschleger, 1994). Leaf-level changes in response to CO_2 elevation include leaf size, anatomy, and stomatal features (Gunderson and Wullschleger, 1994). For example, leaf mass area has been found to increase under elevated CO_2 concentrations due to starch accumulation, accumulation of non-structural carbohydrates, and an increase in palisade cell thickness (Arp, 1991; Giri et al., 2016; Gunderson and Wullschleger, 1994). While many long-term studies have shown no change in total leaf area in response to elevated CO_2 across many species (Drake et al., 1997), a reduction in leaf area and leaf number has also been reported (Drake et. al., 1997; Erwin and Gesick, 2017; Giri et. al., 2016). For example, a commonly observed response to elevated CO_2 is an increase in leaf number, while the overall size of leaves is smaller (Mortensen and Ulsaker, 1985). Increase in total leaf area and number of leaves may be a consequence of accelerated ontogeny rather than a direct response to elevated CO_2 in some species (Gunderson and Wullschleger, 1994). Finally, other studies show that some species respond to increased CO_2 concentrations with increased number of lateral shoots, leaf area, and plant height (Gislerod and Nelson, 1989). Thus, it is apparent that morphological responses to sustained elevated CO_2 concentrations are highly species-specific. However, accumulated carbohydrates results in heavier leaves, which can contribute to an ineffective transformation of photosynthetic carbon gain into new growth (Kirschbaum, 2011; Poorter 1993). Additionally, morphological changes, such as increased leaf

thickness, are not reversible and may continue to affect photosynthetic capacity after termination of CO_2 enrichment (Arp, 1991).

Long-term exposure of plants to elevated CO_2 concentrations often has implications for stomatal density. In a combined study across 100 different species, CO_2 enrichment reduced stomatal density by an average of 14.3% compared to ambient conditions (Woodward and Kelly, 1995). Reduced stomatal density is a common response to elevated concentrations but is species-specific and may be less of a limitation for photosynthesis compared to a reduction in stomatal conductance (Drake et al., 1997). However, species-specific changes to stomatal density in response to elevated CO_2 concentration ultimately affect the maximum values of stomatal conductance (Drake et al., 1997; Woodward and Kelly, 1995). Similarly, the effect on stomatal conductance from the decrease in stomatal density has been observed to be less than the effect of decreased aperture, based on the observation that the decline in conductance was greater in older leaves developed before the CO_2 exposure began (Gunderson and Wullschleger, 1994).

Stomatal conductance is often reduced under elevated CO_2 concentrations (Arp, 1991). For example, after three weeks at a CO_2 concentration of 700 $\mu mol \cdot mol^{-1}$, stomatal conductance of kale, lettuce, and spinach was reduced by nearly 60% and 65%, respectively, compared to ambient (Erwin and Gesick, 2017; Giri et al., 2016). This could be a contributing factor for decreased photosynthetic rate as stomatal conductance (in conjunction with reduced leaf area) may influence the influx of CO_2 into the leaves (Drake et al., 1997; Erwin and Gesick, 2017; Giri et al., 2016). Similarly, stomatal conductance decreased for multiple species after elevation of CO_2 in field chamber studies (Anderson et al., 2001). In 41 observations covering 28 species, average reduction in stomatal conductance was 20% when subjected to elevated CO_2 concentrations, while some species displayed no change (Drake et al., 1997). Reduction of

stomatal conductance and aperture further explains the reduction in transpiration for plants grown at elevated CO_2 (Drake et al., 1997; Gislerod and Nelson, 1989; Gunderson and Wullschleger, 1994). Due to stomatal conductance being mediated by changes in photosynthesis, reduced photosynthetic capacity resulting in lower stomatal conductance is expected (Drake et al., 1997).

1.4.5 Biomass Accumulation

As discussed previously, many species display positive responses to elevated CO_2 concentrations such as increased above and below ground biomass, height, number of leaves, and lateral branching (Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011). For example, in lettuce 'Black-Seeded Simpson' and spinach 'Bloomsdale Long Standing', elevated CO_2 increased total dry mass by 18% compared to ambient conditions (Giri et al., 2016). Similarly, Frantz and Ling (2011) observed a 10% increase in leaf mass was observed for petunia 'Madness White' when grown at a CO_2 concentration of 800 $\mu mol \cdot mol^{-1}$ compared to ambient after five weeks. However, these authors observed no influence of elevated CO_2 concentration on any biomass measurement after 7 weeks of exposure at 800 $\mu mol \cdot mol^{-1}$ for petunia 'Madness White', suggesting that the initial increase in photosynthetic rate was not sustained and resulted in no long-term benefit to plant biomass (Frantz and Ling, 2011). Mortensen and Ulsaker (1985) observed a similar response in begonia (*Begonia* ×*hiemalis* 'Schwabenland') with a 35% increase in total dry mass after five weeks of growth at an elevated CO_2 concentration (1500 $\mu mol \cdot mol^{-1}$) compared to ambient (350 $\mu mol \cdot mol^{-1}$). Root dry mass and total dry mass were significantly increased by 58% and 71%, respectively, at an elevated CO_2 concentration (940 $\mu mol \cdot mol^{-1}$) in chrysanthemum (*Chrysanthemum* ×*morifolium* 'Fiesta') after six weeks compared to ambient (Gislerod and Nelson, 1989). Similarly, elevated CO_2 at 600 $\mu mol \cdot mol^{-1}$ increased dry weight by 10-30% in pansy 'Delta Yellow Blotch' and 'Delta Primrose Blotch'

compared to ambient (Niu et al., 2000). However, perennial C_3 grasses showed acclimation to an elevated CO_2 concentration of 700 $\mu mol \cdot mol^{-1}$ after 32 days, showing no significant difference in biomass between elevated and ambient treatments (Cotrufo and Gorissen, 1997).

1.4.6 Gas Exchange

Many species show no acclimation to elevated CO_2 after 1 to 6 days, while long-term exposure beyond this timeframe leads to a steady decrease in net photosynthetic rate (Gunderson and Wullschleger, 1994). After long-term exposure to elevated CO_2, photosynthetic rate is commonly reduced with indicated responses including change in soluble sugar content and negative feedback inhibition from excess carbohydrates (Kirschbaum and Lambie, 2015; Mortensen and Ulsaker, 1985). Photosynthetic acclimation is often accompanied by higher carbohydrate accumulation, decreased Rubisco content and efficiency, and inhibition to photosynthetic capacity (Drake et al., 1997). For example, the rate of mitochondrial respiration (R_d) decreases ~20% when the CO_2 concentration is doubled due to enzyme inhibition of the mitochondrial electron transport chain (Drake et al., 1997). This decline reflects decreased demand for energy to sustain growth and indicates acclimation of respiration to high CO_2 concentrations (Drake et al., 1997). As discussed previously, acclimation of photosynthesis reduces tissue nitrogen content, which may reduce the demand for energy generated by respiration (Bunce, 1994). Mitochondrial oxygen uptake and electron transport associated with R_d is inhibited by CO_2 elevation (Farquhar et al., 1980). R_d inhibition is limited by the disruption of the activity of two key enzymes of the mitochondrial electron transport chain, cytochrome c oxidase (Cytox) and succinate dehydrogenase (Azcón-Bieto, 1994; Bunce, 1994; Drake et al., 1997; Gonzàlez-Meler, 1997; Wullschleger et al., 1994). This is at least partly due to accumulation of starch in the plant, which does not require a high amount of metabolic energy

but increases total biomass (Poorter, 1993). Generally, plants grown at elevated CO_2 relative to those grown at ambient CO_2 often exhibit increased growth and photosynthesis, lower transpiration, and inhibited respiration (Zhang et al., 2012). One other common indicator is that the light compensation point is lowered by increased CO_2 concentration (Mortensen and Moe, 1983; Mortensen, 1987).

Another indicator of photosynthetic acclimation is changes in the rate of Rubisco carboxylase (V_{cmax}) and rate of photosynthetic electron transport (J_{max}). A review of FACE studies across 109 species showed that V_{cmax} was reduced on average by 13% and J_{max} by 5% when exposed to 500-600 $\mu mol \cdot mol^{-1}$ CO_2 compared to an ambient of 350 $\mu mol \cdot mol^{-1}$ CO_2 (Ainsworth and Long, 2005; Kirschbaum, 2011). In similar surveys, V_{cmax} was lower for plants grown at elevated CO_2 regardless of species, indicating a decrease in either the amount, activity, or kinetic properties of Rubisco (Azcón-Bieto, 1994; Bunce, 1994; Drake et al., 1997; Gonzàlez-Meler, 1997; Wullschleger, 1994). Generally, changes in J_{max} tend to mirror those associated with V_{cmax} (Gunderson and Wullschleger, 1994).

Decreased triose phosphate utilization (TPU) rate is another measurable consequence of elevated CO_2 concentration. TPU-limited photosynthesis occurs when accumulated carbon cannot be processed fast enough, associated with the accumulation of hexose sugars and starch (Dahal and Vanlerburghe, 2018; Lombardozzi et. al., 2018; Yang et. al., 2016). For example, eucalyptus (*Eucalyptus camaldulensis*) showed a TPU decrease of 38% in response to elevated CO_2 concentrations, indicating acclimated photosynthetic rate (Dillon et. al., 2018). Tobacco (*Nicotiana tabacum*) also showed a significant reduction in TPU after exposure to elevated CO_2, showing both acclimated photosynthesis and respiration (Dahal and Vanlerburghe, 2018).

It is interesting to note that the photosynthetic rate of plants grown at elevated CO_2 and then transferred to ambient conditions is generally lower than plants continuously grown at ambient (Moore et al., 1999). Specifically, when averaged across multiple species, there is a 21% reduction in photosynthetic potential (Gunderson and Wullschleger, 1994). However, loss of photosynthetic capacity from plants at elevated CO_2 is rapidly reversible upon return to ambient concentrations (Moore et al., 1999). For example, when plants were transferred to ambient CO_2 after long-term exposure to elevated CO_2 (1000 $\mu mol \cdot mol^{-1}$), accumulated carbohydrates in leaves disappeared after three days and the photosynthetic rate recovered to the level of plants grown in ambient CO_2 (Arp, 1991).

1.4.7 Flowering

Number of flowers and buds has been found to increase with CO_2 enrichment across multiple studies (Mortensen and Ulsaker, 1985). Additionally, elevated CO_2 has been found to reduce time to flower in some species (Mortensen and Moe, 1992). For example, cyclamen (*Cyclamen* sp.) and nasturtium (*Tropaeolum* sp.) showed increased dry weight and greater flower yield when exposed to elevated CO_2 (Cummings and Jones, 1918; Prior et al., 2011). Similarly, in a study by Mortensen and Ulsaker (1985), begonia 'Schwabenland' flowered four days earlier and produced 13% more flowers at the CO_2 concentration of 1500 $\mu mol \cdot mol^{-1}$ compared to ambient. However, time to flower was not affected by elevated CO_2 for rose (*Rosa* L. 'Frisco' and 'Kiss') (Mortensen and Moe, 1992). Similarly, petunia 'Madness White' displayed no difference in time to flower between ambient and an elevated CO_2 treatment at 800 $\mu mol \cdot mol^{-1}$ (Frantz and Ling, 2011). The concentration of CO_2 also did not influence time to flower or flower development for pansy 'Delta Yellow Blotch' and 'Delta Primrose Blotch' (Niu et al., 2000). It's been proposed that some species may increase flowering to better utilize excess carbohydrates, specifically increasing sinks in the form of developing flowers and fruit

(Kirschbaum, 2011). Studies have found an increase in sinks occurs when plants shift from vegetative to reproductive growth; thus, annual plants may possess sink limitations during vegetative growth but possible source limitations during the reproductive stage (Arp, 1991; Kirschbaum, 2011).

1.4.8 Species-Specific Acclimation

Many studies have concluded that plant responses to elevated CO_2 concentrations are not only species-specific, but possibly cultivar-specific. For example, above- and below-ground biomass generally increases with exposure to elevated CO_2, but the magnitude of this response has been found to be highly species-specific (Ainsworth and Long, 2004; Cotrufo and Gorissen, 1997). Species-specific responses to elevated CO_2 have been found to include leaf area, biomass, and stomatal density (Frantz and Ling, 2011; Woodward and Kelly, 1995). Similarly, studies across many varieties of lettuce, kale, spinach, and swish chard found that photosynthetic and morphological responses to elevated CO_2 varied significantly across cultivars of the same species (Erwin and Gesick, 2017; Giri et al., 2016; Perez-Lopez et al., 2015). It has been proposed that species may not only have unique responses to elevated CO_2 concentrations, but have their own optimal CO_2 concentrations; genetics and provenance lead to species- and cultivar-specific responses to elevated CO_2 (Dillon et al., 2018; Mortensen, 1987). Acclimation to CO_2 is also dependent on species due to differing inherent relative growth rates (Gunderson and Wullschleger, 1994; Kirschbaum, 2011). FACE studies showed an extreme range of species-specific responses, with one large source of variation being acclimation of photosynthetic rate through up- or down-regulation in photosynthetic biochemistry (Anderson et al., 2001).

1.5 Intermittent CO_2

Previous studies evaluated intermittent CO_2 elevation and possibly bypassing the acclimation response of photosynthesis (Frantz and Ling, 2011; Kirschbaum and Lambie, 2015;

Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2010). These early studies examined reducing CO_2 elevation to a few hours a day compared to continuous application (Mortensen, 1987). Mortensen (1986 and 1987) found in African violet (*Saintpaulia ionantha* 'Nicole', 'Lena', and 'Rosa Roccoco'), soybean (*Glycine max* 'Fiskeby V'), and tomato (*Lycopersicum esculentum* 'Virosa') that the reduced application over several hours showed no benefit compared to continuous elevated application. Meanwhile, Frantz and Ling (2011) suggest that to keep the benefits while bypassing detrimental effects of long-term elevated CO_2 exposure, short-term exposure in the form of several days to a few weeks at a time may be effective. While elevated CO_2 research of short-term oscillation between days or weeks is lacking, a possible timing component to acclimation is suggested by previous studies, as a five-day break from elevated CO_2 in soybeans returned the photosynthetic rate to 75% above plants grown at ambient upon re-exposure (Jones et al., 1985). More research is needed on various stages of plant development and responses to short-term CO_2 elevation, especially in ornamental species (Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2010).

1.6 Further Research

Environmental studies investigating CO_2 enrichment have been performed on only a handful of greenhouse crops, likely as a result of complex experimental design (Frantz and Ling, 2011). Additionally, multiple factor interaction studies are still needed (Frantz and Ling, 2011; Mortensen, 1987). While the effects of elevated CO_2 on plants is well known, horticulture species have received much less attention than agronomic and forest species (Prior et al., 2011). While it is conjectured that horticulture species will benefit from elevated CO_2 in production, research is lacking to support this contention (Prior et al., 2011). Therefore, further research is

needed to develop management strategies both in response to rising atmospheric CO_2 concentrations and the use of CO_2 as an input for production (Prior et al., 2011; Mortensen, 1987).

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CHAPTER 2. THE IMPACT OF LONG-TERM CO_2 ENRICHMENT ON PANSY AND PETUNIA GROWTH AND PHYSIOLOGY FOR CONTROLLED ENVIRONMENT PRODUCTION

2.1 Summary

While elevated CO_2 concentrations often beneficially effect plants immediately (e.g., increased photosynthesis), the initial responses commonly do not persist throughout production, which may reduce the benefit of CO_2 as an input. These acclimation responses in horticulture species, specifically their timing and extent, are still widely uncertain. Therefore, the objective of this research was to determine species-specific acclimation responses to enriched CO_2 concentrations for pansy (*Viola ×wittrockiana* 'Matrix Blue Blotched Improved') and petunia (*Petunia ×hybrida* 'Dreams Midnight) during finishing production. To evaluate to responses of pansy and petunia to enriched CO_2 concentrations, seedlings were transplanted into 11.5-cm pots and placed in growth chambers with air temperature, relative humidity, and radiation intensity setpoints of 21 °C, 55%, and 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Carbon dioxide treatments were established using the two growth chambers with setpoints of either 400 (ambient) or 1000 $\mu mol \cdot mol^{-1}$ (enriched) maintained during a 16-h photoperiod. In addition to data collected through destructive harvest, rate of photosynthesis (A) in response to increasing internal leaf CO_2 concentration (A-C_i) and ambient CO_2 concentration (A-C_a) were measured weekly with a portable leaf photosynthesis system at saturating (A-C_i; 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$) or production (A-$C_a$; 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$) radiation intensities. For both pansy and petunia, elevated CO_2 produced greater total shoot dry mass compared to ambient after 4 weeks. However, other observations after 4 weeks included decreased maximum rate of photosynthetic electron transport (J_{max}), maximum rate of Rubisco carboxylase (V_{cmax}), and similar photosynthesis at operating C_i

concentration. Similarly, A measured at 1000 and 400 μmol·mol^{-1} was reduced for both pansy and petunia grown under the enriched compared to ambient CO_2 concentration based on A-C_a responses after 1 week, indicating quick physiological acclimation to this input. These results provide useful information regarding the timing and extent of physiological acclimation in response to enriched CO_2 concentrations for pansy and petunia. However, due to physiological acclimation potentially occurring within one week of treatment initiation, additional research is needed to best understand how this input can be further optimized for controlled environment production.

2.2 Introduction

Floriculture crops in the US had an estimated wholesale value of $4.77 billion in 2018, with annual bedding plants making up approximately one third of this total in over 423 million square feet of greenhouses (U.S. Dept. Agr., 2019). While the average ambient carbon dioxide (CO_2) concentration is currently 409.8 μmol·mol^{-1}, concentrations in the greenhouse environment commonly drop below 200 μmol·mol^{-1} during production in the winter and early spring (Both et al., 2017; Erwin and Gesick, 2017; Lan et al., 2020; Mortensen, 1987; Styer, 2003). This typically happens when limited ventilation cannot replenish CO_2 on sunny, cold days with a greenhouse full of plants; meaning crop demand for CO_2 is greater than supply, limiting photosynthesis and growth (Both et al., 2017; Erwin and Gesick, 2017). Even well-ventilated greenhouses show canopy concentrations falling between 250-300 μmol·mol^{-1}, well below ambient (Mortensen, 1987).

Meanwhile, the current atmospheric concentration does not maximize photosynthetic capacity, so there is potential for enhancing plant growth by enriching greenhouse environments with CO_2 (Mortensen, 1987; Mortensen and Moe, 1992). Studies have shown that CO_2

concentrations between 800 to 1200 μmol·mol^{-1} have great potential to increase plant growth, with concentrations above 900 μmol·mol^{-1} nearly eliminating photorespiration (Both et al., 2017, Erwin and Gesick, 2017; Gunderson and Wullschleger, 1994; Mortensen, 1987). However, previous studies have established that the most practical range for most species is CO_2 concentrations within 600 to 1000 μmol·mol^{-1} (Mortensen, 1987). Early short-term studies showed that an economically efficient way to enhance ornamental plant growth in greenhouses is to increase the CO_2 concentration within this target range (Mortensen, 1987; Prior et al., 2011).

Short duration experiments concluded that elevated CO_2 immediately stimulates photosynthetic rate up to possibly 58%, leading to increased carbon assimilation and increasing plant growth (Drake et al., 1997; Kirschbaum and Lambie, 2015; Prior et al., 2003; Prior et al., 2011). For instance, Mortensen and Moe (1983) found that net photosynthetic rate increased by 50% in chrysanthemum (*Chrysanthemum morifolium* 'Horim') when grown at 900 μmol·mol^{-1} CO_2 for five days compared to plants grown at ambient. Additional commonly observed short-term responses to elevated CO_2 concentrations include reduced stomatal conductance and reduced transpiration, leading to improved water-use efficiency (WUE) (Ainsworth and Long, 2004; Anderson et al., 2001; Drake et al., 1997). For example, Mortensen (1987) concluded that an elevated CO_2 concentration can improve WUE by 30%, possibly due to reduced stomatal aperture and transpiration (Arp, 1991; Erwin and Gesick, 2017; Giri et al., 2016; Gislerod and Nelson, 1989; Gunderson and Wullschleger, 1994; Prior et al., 2011). Similarly, Drake et al. (1997) showed that stomatal conductance was reduced by an average of 20% across 41 observations covering 28 species. These short-term responses to elevated CO_2 show potential for enhancing plant growth while possibly contributing to fewer production inputs (Both et al.,

2017). However, the benefit to increased photosynthetic rate seen with elevated CO_2 is often not realized throughout production.

Long-term exposure to elevated CO_2 concentrations results in many morphological and physiological responses. Many plants display a reduction in chlorophyll content due to enriched CO_2 concentrations, possibly due to excess carbohydrate accumulation damaging the chloroplast (Ainsworth and Long, 2004; Arp, 1991; Gunderson and Wullschleger, 1994; Perez-Lopez et al., 2015). Additional plant responses to long-term elevated CO_2 concentrations include changes in leaf number and leaf area (Arp, 1991; Drake et al., 1997; Gunderson and Wullschleger, 1994; Mortensen and Ulsaker, 1985). Common growth responses to elevated CO_2 seen in many species include increased lateral shoots and plant height (Gislerod and Nelson, 1989; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011). Elevated CO_2 often shows an increase in carbohydrate concentration in plant tissues, usually resulting in increased biomass (Drake et al., 1997; Erwin and Gesick, 2017; Frantz and Ling, 2011; Kirschbaum, 2011; Morgan et al., 2001). For example, Zhang et al. (2011) found that elevated CO_2 (760 $\mu mol \cdot mol^{-1}$) increased soluble sugar content by 77.81% and starch by 122.39% in New Guinea impatiens (*Impatiens hawkeri*) (Zhang et al., 2011). This increase in carbohydrate concentration in response to elevated CO_2 concentrations not only increases above ground biomass, but specifically tends to increase leaf mass area (LMA) (Frantz and Ling, 2011; Giri et al., 2016; Gislerod and Nelson, 1989; Mortensen, 1987; Mortensen and Moe, 1992; Mortensen and Ulsaker, 1985; Prior et al., 2011). Finally, elevated CO_2 has variable effects on flowering, such as number of flowers or time to flower (Frantz and Ling, 2011; Mortensen and Moe, 1992; Mortensen and Ulsaker, 1985; Niu et al., 2000; Prior et al., 2011).

Long-term exposure to elevated CO_2 concentrations has been correlated with a reduction in photosynthetic capacity and decreased biochemical processes of photosynthesis, showing diminishing positive return to this input (Ainsworth and Long, 2004; Arp, 1991; Drake et al., 1997; Dillon et al., 2018; Gunderson and Wullschleger, 1994; Kirschbaum and Lambie, 2015; Mortensen, 1987; Rogers and Humphries, 2000). One indicator of photosynthetic acclimation is decreased rate of Rubisco carboxylase (V_{cmax}) and rate of photosynthetic electron transport (J_{max}). For example, a survey of FACE studies across 109 species found that V_{cmax} was reduced on average by 13%, and J_{max} by 5% in response to enriched CO_2 concentrations, indicating a decrease in the efficacy of Rubisco dehydrogenase, with changes in J_{max} tending to mirror those in V_{cmax} (Ainsworth and Long, 2005; Azcón-Bieto, 1994; Bunce, 1994; Drake et al., 1997; Gonzàlez-Meler, 1997; Gunderson and Wullschleger, 1994; Kirschbaum, 2011; Wullschleger et al., 1994). Photosynthetic acclimation is accompanied by a decreased triose phosphate utilization (TPU) rate, indicating an excess of carbohydrates outpacing utilization (Dahal and Vanlerburghe, 2018; Lombardozzi et al., 2018; Yang et al., 2016). For example, eucalyptus (*Eucalyptus camaldulensis*) displayed a 38% decrease in TPU compared to ambient conditions after exposure to ten weeks at 800 $\mu mol \cdot mol^{-1}$ CO_2 (Dillon et al., 2018).

Intermittent CO_2 application has been suggested by early studies to possibly bypass the photosynthetic acclimation response to elevated CO_2 (Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011). These studies evaluated intermittent CO_2 elevation over a few hours rather than continuous daily elevation, which did not show benefit in African violet (*Saintpaulia ionantha* 'Nicole'), 'Lena', and 'Rosa Roccoco', soybean (*Glycine max* 'Fiskeby V'), and tomato (*Lycopersicum esculentum* 'Virosa') compared to plants grown at ambient (Mortensen, 1986; Mortensen, 1987). Meanwhile, Frantz

and Ling (2011) suggested that to bypass the detrimental effects of long-term exposure, elevated CO_2 can be applied short-term in the form of a few days or weeks at a time. A study by Jones et al. (1985) showed that photosynthetic rate could be returned to its original rate prior to acclimation with a five-day break from elevated CO_2 in soybean, suggesting a duration component to CO_2 application and possible success with oscillation. Many studies call for more research on responses to short-term CO_2 elevation at varying stages of plant development (Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011).

Not only have many studies concluded that plants respond to elevated CO_2 concentrations at the species level, but there is also evidence that responses could be cultivar-specific (Ainsworth and Long, 2004; Cotrufo and Gorissen, 1997; Erwin and Gesick, 2017; Frantz and Ling, 2011; Giri et al., 2016; Perez-Lopez et al., 2015). Changes such as biomass, leaf area, net photosynthetic rate and other photosynthetic biochemical processes, and magnitude of the acclimation responses have been seen to vary at the cultivar level (Anderson et al., 2001; Erwin and Gesick, 2017; Frantz and Ling, 2011; Giri et al., 2016; Perez-Lopez et al., 2015).

Horticulture species have received much less attention compared to agronomic and forest species when it comes to evaluating the effects of elevated CO_2 (Prior et al., 2011). Research is lacking to support the conjecture that horticulture species will benefit from long-term elevated CO_2 during production (Prior et al., 2011). In order to evaluate and better adapt management conditions to using elevated CO_2 as an input of production, more research is needed (Mortensen, 1987; Prior et al., 2011). By further evaluating these responses on a species basis, specific programs can be developed based on production stage by establishing best management practices for elevated CO_2 (Mortensen, 1987; Prior et al., 2011). Therefore, the objective of this study was

to evaluate species-specific morphological and physiological responses to long-term exposure of elevated CO_2 concentrations in controlled environments for petunia (*Petunia ×hybrida* 'Dreams Midnight') and pansy (*Viola ×wittrockiana* 'Matrix Blue Botch Improved') to determine the timing and extent of potential acclimation responses.

2.3 Materials and Methods

2.3.1 Plant Material and Germination Environment

Petunia 'Dreams Midnight' and pansy 'Matrix Blue Botch Improved' seeds were sown in a 128-cell tray (14-mL individual cell volume) filled with commercial germination mix comprised of (by volume) 80% fine sphagnum peat, 10% perlite, and 10% vermiculite (BM2 Germinating Mix; Berger, Saint-Modeste, Canada). Trays were immediately placed in a reach-in growth chamber (PG2500; Conviron, Winnipeg, Canada) with air temperature, relative humidity, and CO_2 concentration setpoints of 21 °C, 55%/65% day/night, and 400 $\mu mol \cdot mol^{-1}$, respectively. Light was provided by light-emitting diode (LED) fixtures (GreenPower LED DR/W production modules; Signify, Eindhoven, Netherlands) with a 16-h photoperiod (0800 to 0000 HR) and an average photosynthetic photon flux density (*PPFD*) at canopy height of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Seedlings were irrigated daily with water soluble fertilizer at a concentration of 150 $mg \cdot L^{-1}$ N (Jack's LX 13N–2P–13K Plug Formula for High Alkalinity Water; JR Peters Inc.; Allentown, Pennsylvania). Other macro- and micronutrients contained in the fertilizer in $mg \cdot L^{-1}$ were 22.5 P, 150 K, 69 Ca, 34.5 Mg, 0.15 B, 0.075 Cu, 0.75 Fe, 0.375 Mn, 0.075 Mo, and 0.375 Zn. Seedlings were thinned to one plant per cell 4 d after germination.

2.3.2 Growth Chamber Environment

Twenty-eight days after germination, 40 uniform seedlings were randomly selected and transplanted into 11.4-cm (550 ml) containers using commercial potting media comprised of (by

vol.) 85% sphagnum peat and 15% perlite (BM6 Growing Mix; Berger Horticultural Products Ltd., Berger, Saint-Modeste, Canada). Twenty plants were randomly assigned to one of two reach-in growth chambers (PG2500; Conviron), each maintaining a CO_2 concentration setpoint of either 400 (ambient) or 1000 $\mu mol \cdot mol^{-1}$ (elevated) during the established 16-h photoperiod with injection controlled using a CO_2 gas analyzer (LI-830; LI-COR Inc., Lincoln, NE). The CO_2 concentration in each chamber was measured over the 16-h photoperiod using a CO_2 probe (GMP252; Vaisala, Woburn, MA), with a mean ± SD over the 16-h photoperiod of 425. ± 55 and 1014 ± 84 $\mu mol \cdot mol^{-1}$, respectively, across 3 experimental replications. Carbon dioxide setpoints were switched between chambers between replications to randomize for chamber effects. Fixed mounted infrared thermocouples with ABS plastic housing (OS36-01-T-80F; Apogee Instruments Inc., Logan, Utah) were installed in each chamber to measure leaf temperature, with a mean ± SD of 21 ± 0.6 and 21 ± 0.4 °C, and precision thermistors (ST-100; Apogee Instruments, Inc.) were used to measure air temperature with a mean ± SD of 21 ± 0.1 and 21 ± 0.1 °C. Relative humidity probes (EE-08-SS; Apogee Instruments, Inc.) were installed in each chamber to measure relative humidity, with a mean ± SD of 62 ± 11 and 63 ± 9% during the day and 70 ± 6 and 70 ± 3% during the night. Radiation quality and intensity were measured at the beginning of each experimental replication by taking seventeen spectral scans per treatment using a spectrometer (LI-180; LI-COR Inc.) at canopy height averaging 250 ± 15 and 252 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ for the two chambers. Environmental setpoints were measured every 30 s and the average was logged every 15 min by a data logger (model CR1000X; Campbell Scientific, Logan, UT). Plants were irrigated as needed with water-soluble fertilizer at a concentration of 150 $mg \cdot L^{-1}$ N (Jack's LX 21N–5P–20K All Purpose Formula for High Alkalinity Water). Other

macro- and micronutrients contained in the fertilizer in $mg \cdot L^{-1}$ were 36 P, 142.5 K, 1.05 Mg, 0.15 B, 0.075 Cu, 0.75 Fe, 0.375 Mn, 0.075 Mo, and 0.375 Zn.

2.3.3 Gas Exchange Data Collection

Gas exchange measurements were collected using a portable photosynthesis meter (LI-6800; LI-COR Inc., Lincoln, NE). Photosynthetic responses to increasing CO_2 concentration were conducted using a combined 6 cm^2 leaf chamber and light source (Li-6800-01A Multiphase Flash Fluorometer; LI-COR Inc.). Measurements were collected beginning 7 d after transplant and continued every 7 d for a total of 28 d. For each day of data collection, the most recent fully expanded leaf of five plants from each treatment was selected for gas exchange measurements. For plants grown in the ambient treatment, the CO_2 concentration inside the leaf chamber was decreased from 400 to 50 $\mu mol \cdot mol^{-1}$, returned to 400 $\mu mol \cdot mol^{-1}$, and then increased to a maximum of 1000 $\mu mol \cdot mol^{-1}$ in steps of 100 $\mu mol \cdot mol^{-1}$ to prevent feedback inhibition during measurements. For plants grown in the elevated treatment, the CO_2 concentration inside the leaf chamber was decreased from the maximum level of 1000 to 50 $\mu mol \cdot mol^{-1}$ in steps of 100 $\mu mol \cdot mol^{-1}$. Two minutes of acclimation were allowed at each step before measuring. Cuvette leaf temperature and relative humidity matched the growth chamber environment. An LED light source provided a *PPFD* of 1000 $\mu mol \cdot m^{-2} \cdot s^{-1}$ to achieve light saturation. The Plantecophys package, an R package for analyzing and modelling leaf gas exchange data, was used to determine photosynthesis parameters by fitting individual A-C_i curves with the fitaci function (Duursma, 2015). The curves were analyzed at leaf temperature with triose phosphate utilization limitation (TPU) set at measured vapor-pressure deficit. TPU was used in the model to estimate differences more accurately for photosynthetic measurements (Lombardozzi et al. 2018; Yang et al., 2016). Estimates included maximum photosynthetic rate of Rubisco carboxylation (V_{cmax})

and maximum rate of photosynthetic electron transport (J_{max}). The photosyn function from the Plantecophys package was used to estimate stomatal conductance (G_s) (Duursma, 2015). Additional curves were collected on five test plants per treatment to determine the photosynthetic rate under operating conditions, specifically at a target *PPFD* of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$. For plants grown in the ambient treatment, the CO_2 concentration inside the leaf chamber was set to ambient then increased to a maximum of 1000 $\mu mol \cdot mol^{-1}$ in steps of 100 $\mu mol \cdot mol^{-1}$ to prevent feedback inhibition during measurements. For plants grown at the elevated treatment, the CO_2 concentration inside the leaf chamber was decreased from the maximum level of 1000 $\mu mol \cdot mol^{-1}$ to 400 $\mu mol \cdot mol^{-1}$ in steps of 100 $\mu mol \cdot mol^{-1}$ to prevent feedback inhibition during measurements. Two minutes of acclimation were allowed at each step before measuring. Cuvette leaf temperature and relative humidity matched the growth chamber environment. The measurement of interest was photosynthetic rate of both treatments at 400 and 1000 $\mu mol \cdot mol^{-1}$.

2.3.4 Morphological Data Collection

Immediately after collecting gas exchange measurements, plants were harvested. Relative chlorophyll content (RCC; SPAD-502 Chlorophyll Meter; Konica Minolta Inc., Tokyo, Japan) was collected on the most recent fully expanded leaf. Number of leaves was collected by removing the leaves at the axil and leaf area (LA; cm^2) was measured using a leaf area meter (LI-3100C; LI-COR Inc.). Stem caliper (mm) was measured just above the hypocotyl and at the junction of the first axillary bud. Stem length from apical bud to soil line was measured. Leaves and stems were then dried in a forced air oven maintained at 70 °C to determine leaf (LDM; mg) and shoot (stems + leaves) dry mass (SDM; mg). Leaf mass area (LMA; $mg \cdot cm^{-2}$) was calculated using the measured values for LDM and LA. Flowering data was collected on all test plants

including days to first visible bud, days to the first fully reflexed flower, and number of flowers and buds per plant on the harvest date.

2.3.5 Statistical Analysis

Analysis was done using R 4.0.0 and the lme4, lmerTest, and emmeans packages (Bates et al., 2015; Kuznetsova et al., 2017; Lenth et al., 2019). One observational unit was one measurement per plant with a total of n=60 observations. A mixed model was fit using morphological data collected (continuous) as the response. Fixed effects included CO_2 treatment (categorical: 400, 1000 $\mu mol \cdot mol^{-1}$). Repetition (categorical: 1, 2, 3) was included as a random effect to account for the split plot design. CO_2 treatments were compared using Tukey adjusted pairwise comparisons.

Model assumptions of linearity and equal scatter were both satisfied, checked using residual diagnostic plots. Week (harvest time) was not included in the model, but each week (1, 2, 3, 4) was treated as a discrete event. No trend over time was tested. This model was chosen with repetition as random to account for the effect of randomizing the chambers between repetitions. This allowed analysis to show the comparisons between the CO_2 treatments accounting for the effect of any possible differences between environments in the chambers. After the model was tested with the rand() function (Kuznetsova et al., 2017), the random effect was found to be statistically significant justifying its use in the model.

2.4 Results

2.4.1 Morphological Data

Stem caliper and length showed no difference between CO_2 treatments for petunia for all harvest days (Table 1). However, pansy stem caliper and stem length on day 21 were 10% and 6% greater under elevated compared to ambient conditions, respectively (Table 2).

Differences were not observed for pansy stem caliper and stem length for any other harvest days (Table 2). Leaf number was 13% greater under elevated conditions for petunia compared to ambient on day 7, with no differences observed for any other harvest day (Table 1). For pansy, leaf number was similar between CO_2 treatments for all harvest days (Table 2). Leaf area was only different between CO_2 treatments on day 14 for both species, with a 13% and 14% increase under elevated compared to ambient conditions for petunia and pansy, respectively (Tables 1 and 2). Petunia RCC only showed differences on day 21, with 14% greater RCC observed under elevated compared to ambient conditions (Table 1). No differences in RCC were observed for pansy on any harvest day (Table 2).

For petunia, differences in LDM were observed on days 7, 14, and 21, with the highest values observed under elevated conditions. For example, on day 21, LDM for plants grown under elevated conditions was 22% greater than those under ambient (Table 1). Similarly, LDM for pansy was greatest under elevated conditions on days 7, 14, and 21. Specifically, on day 21 LDM was 17% greater under elevated compared to ambient conditions (Table 2). However, on day 28 no difference in LDM between ambient and elevated conditions was observed (Tables 1 and 2). Differences in SDM for petunia and pansy were observed on all harvest dates, with greater biomass accumulation under elevated compared to ambient conditions. For example, petunia SDM on day 7, 14, 21, and 28 was 15.6%, 21.6%, 26%, and 19.2% greater under elevated compared to ambient conditions, respectively (Table 1). Similarly, pansy SDM on day 7, 14, 21, and 28 was 26%, 28.8%, 21.9%, and 14.9% greater under elevated compared to ambient conditions, respectively (Table 1).

For petunia, LMA differed between treatments on days 14, 21, and 28, with the highest values observed for plants grown under elevated conditions. For instance, on day 28, petunia

LMA under elevated conditions was 9% greater than ambient (Table 1). Comparably, pansy LMA was greater under elevated conditions compared to ambient on days 7, 14, and 28 (Table 2). Specifically, on day 28, pansy LMA was 17% greater under elevated compared to ambient conditions (Table 2).

There were no observable differences in flower initiation, time to first fully reflexed flower, or flower number for either species on any harvest day (Table 3).

2.4.2 Photosynthesis Data

Higher V_{cmax} values were observed for petunia under ambient conditions on all measurement days. For example, V_{cmax} was 27% greater under ambient compared to elevated conditions on day 28 (Table 4). Similarly, higher V_{cmax} values were observed for pansy under ambient conditions on days 14, 21, and 28. On day 28, V_{cmax} for plants grown under ambient conditions was 20% greater than elevated (Table 5). Petunia J_{max} differed on all measurement days between CO_2 treatments, with higher values observed for plants grown under ambient compared to elevated conditions. For instance, on day 28, J_{max} for petunia was 7% greater under ambient compared to elevated conditions (Table 4). Differences in J_{max} for pansy were observed on days 14, 21, and 28, with the highest values observed under ambient conditions. Specifically, J_{max} was 27% under ambient compared to elevated conditions for pansy on day 28 (Table 5).

A reduced TPU rate was observed for petunia plants grown under elevated conditions on all measurement days. For example, on day 28, petunia TPU was 15% greater under ambient compared to elevated conditions (Table 4). Similarly, pansy TPU was reduced under elevated conditions on days 14, 21, and 28. For instance, on day 28, TPU under ambient conditions was 15% greater compared to elevated (Table 5). For G_s measured at operating point (measured at the CO_2 concentration of respective treatment environment), values were greater under ambient

conditions for both species on all measurement days. For example, on day 28, G_s under ambient conditions was 103% and 71% greater than elevated for petunia and pansy, respectively (Table 4 and Table 5).

Net photosynthetic rate for petunia and pansy measured at 400 $\mu mol \cdot mol^{-1}$ CO_2 under operating conditions was greater for plants grown under ambient conditions for all measurement days (Table 6). For example, net photosynthetic rate of petunia at a CO_2 concentration of 400 $\mu mol \cdot mol^{-1}$ was 36%, 27%, 27%, and 23% greater for plants grown under ambient compared to elevated conditions on days 7, 14, 21, and 28, respectively (Table 6). For pansy, net photosynthetic rate at a CO_2 concentration of 400 $\mu mol \cdot mol^{-1}$ was 28%, 17%, 19%, and 14% greater under ambient compared to elevated conditions on days 7, 14, 21, and 28, respectively (Table 6). Net photosynthetic rate measured at 1000 $\mu mol \cdot mol^{-1}$ CO_2 under operating conditions was also significant for all measurement days for both species. Specifically, net photosynthetic rate of petunia at a CO_2 concentration of 1000 $\mu mol \cdot mol^{-1}$ was 21%, 17%, 16%, and 19% greater under ambient compared to elevated conditions on days 7, 14, 21, and 28, respectively (Table 6). Similarly, net photosynthetic rate at 1000 $\mu mol \cdot mol^{-1}$ CO_2 was 16%, 9%, 9%, and 11% greater for pansy grown under ambient compared to elevated conditions on days 7, 14, 21, and 28, respectively (Table 6).

2.5 Discussion

2.5.1 Morphological Data

Previous studies have shown potential for long-term carbon dioxide (CO_2) elevation to be beneficial for ornamental plant production during the finishing stage; however, research is still needed to evaluate the extent and timing of morphological and physiological responses in

horticultural species to elevated CO_2 application (Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011).

In the present study, elevated CO_2 had little effect on stem caliper and stem length for pansy or petunia. Similarly, leaf number and area showed a limited response to elevated CO_2 compared to ambient for both species, with differences only observable prior to day 14. This indicates that elevated CO_2 may increase growth rate in the first days or weeks of exposure for pansy and petunia, but does not greatly influence overall plant size after 28 days. Furthermore, this response indicates that pansy and petunia have acclimated to elevated CO_2, possibly adjusting their growth rate to show no difference in stem caliper, stem length, leaf number, or leaf area compared to plants grown at ambient by 28 days of exposure. An adjustment in shoot growth rate may be due to a sink limitation response, as the plant reaches maximum capacity for root growth in its container, shoot growth rate becomes restricted while carbohydrate concentration increases, indicated by an increase in biomass and LMA (Arp, 1991). This is consistent with previous studies with petunia 'Madness White' where five weeks at 800 $\mu mol \cdot mol^{-1}$ CO_2 had no significant influence on stem morphology compared to ambient (Frantz and Ling, 2011). Similarly, Frantz and Ling (2011) found no change in leaf number or area for petunia 'Madness White' after five weeks of exposure to 800 $\mu mol \cdot mol^{-1}$ CO_2 compared to ambient, showing that long-term exposure to CO_2 may reduce the benefit to plant growth that is expected when using short-term predictions (Mortensen, 1987; Mortensen and Ulsaker, 1985). In the same way, while short-term studies predict large differences in leaf area, long-term studies show no change in total leaf area in response to elevated CO_2 across many species, indicating that a response to elevated CO_2 may be acclimation of growth rate compared to initial increases in ontogeny (Drake et al., 1997; Gunderson and Wullschleger, 1994). There is also the possibility

of sink limitation combined with an inability to metabolize accumulated carbohydrates. A common response in long-term CO_2 studies is that as plants fill their container, their ability to utilize carbon for plant growth becomes limited. Carbon accumulates rather than being used for plant growth, and this inability to metabolize carbohydrates reduces the potential for plant growth stimulation and dampens photosynthesis (Arp, 1991; Frantz and Ling, 2011; Makino and Mae, 1999; Rogers et al., 1998). This sink limitation response may explain why some growth responses were apparent on days 7, 14, and 21, but when plants had mostly filled their containers by day 28, growth was no longer stimulated by elevated CO_2. While growth rate of the shoot may acclimate to elevated CO_2 long-term with no differences in stem caliper or length and leaf area or number, plants commonly respond with increased biomass and LMA (Frantz and Ling, 2011; Gislerod and Nelson, 1989; Mortensen and Ulsaker, 1985).

In the present study, LDM was greater for plants grown at an elevated CO_2 concentration on days 7, 14, and 21, but not day 28, for both species, possibly indicating acclimation of leaf mass to elevated CO_2. Meanwhile, SDM was different between treatments for petunia and pansy on all harvest dates, with plants grown at elevated having the highest values. Increased aboveground biomass in response to elevated CO_2 is a common response across species in long-term studies (Frantz and Ling, 2011; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011). However, previous studies have also concluded that long-term exposure to elevated CO_2 does not sustain increased biomass (Frantz and Ling, 2011). Specifically, Frantz and Ling (2011) found that LDM was not affected by elevated CO_2 compared to ambient conditions in petunia 'Madness White' after seven weeks of exposure to 800 $\mu mol \cdot mol^{-1}$ CO_2 from a 10% increase in LDM at five weeks. However, the present study observed plants grown at 1000 rather than 800 $\mu mol \cdot mol^{-1}$ CO_2, possibly hastening this response. Similar LDM between CO_2 treatments on day

28 can be attributed to multiple factors. Not only had the photosynthetic rate been reduced, possibly causing a decrease in leaf carbohydrate content, but flower initiation began after day 21, possibly opening up new sinks for carbohydrates to be utilized for plant growth (Frantz and Ling, 2011; Lewis et al., 2002). While LDM showed no difference after 28 days, SDM showing an increase in biomass at elevated CO_2 for every harvest date is consistent with previous studies. For example, elevated CO_2 increased dry weight by 10-30% in pansy 'Delta Yellow Blotch' and 'Delta Primrose Blotch' after 4 weeks at 1000 $\mu mol \cdot mol^{-1}$ (Niu et al., 2000). However, similar to LDM, the magnitude of difference between CO_2 treatments for SDM began to diminish after day 21 when plants started flowering, possibly indicating biomass being allocated to the new sink potential.

An increase in biomass with no significant increase in plant size insinuates accumulation of sugars, for example, hexose carbohydrates and starch (Arp, 1991; Giri et al., 2016; Gunderson and Wullschleger, 1994). This is reflected in LMA differences for both species, as long-term exposure to elevated CO_2 consistently results in increased LMA (Arp, 1991; Ainsworth and Long, 2004; Anderson et al., 2001; Drake et al., 1997; Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Mortensen and Ulsaker, 1985). This significant increase in sugars possibly helps explain physiological changes and responses of photosynthesis to an elevated CO_2 concentration due to damage to the chlorophyll and feedback inhibition to the photosynthetic mechanism (Arp, 1991; Cave et al., 1981; Drake et al., 1997; Kirschbaum and Lambie, 2015; Makino and Mae, 1999; Moore et al., 1999; Mortensen, 1987; Mortensen and Ulsaker, 1985; Wulff and Strain, 1982).

Few differences in RCC from CO_2 treatment were observed for both species. Chlorophyll responses to elevated CO_2 are species-specific, with the possibility of a slight increase in the

short-term but no change in the long-term (Ainsworth and Long, 2004; Giri et al., 2016; Gunderson and Wullschleger, 1994). Shifts in the ratio of chlorophyll-α and chlorophyll-β are common in response to elevated CO_2 concentrations but usually do not result in a change in total chlorophyll content (Arp, 1991). This may be attributed to a mutual shading response due to thicker leaves from increased LMA or additional palisade layers (Arp, 1991). For instance, while total chlorophyll content did not change, Perez-Lopez et al. (2015) found that elevated CO_2 increased chlorophyll-β concentration by 64% and 52% in green lettuce (*Lactuca sativa* 'Blonde of Paris Batavia')and red lettuce 'Oak Leaf', respectively. Similarly, starch accumulation due to long-term elevated CO_2 exposure can inhibit the function of chlorophyll in the leaves, causing reduction in photosynthetic rate and processes as a symptom of acclimation (Arp, 1991; Cave et al., 1981; Makino and Mae, 1999; Mortensen, 1987; Wulff and Strain, 1982).

There were no observable differences for any harvest date in either petunia or pansy for first visible flower bud, first fully reflexed flower, or number of flowers on the harvest date. This is consistent with previous studies evaluating petunia 'Madness White' which showed no difference in timing for the appearance of the first flower between 400 and 800 $\mu mol \cdot mol^{-1}$ CO_2 (Frantz and Ling, 2011). Likewise, an elevated CO_2 concentration (1000 $\mu mol \cdot mol^{-1}$) did not influence time to flower or development in pansy 'Delta Yellow Blotch' or 'Delta Primrose Blotch' (Niu et al., 2000). The widely observed response of no change to flowering may be due to a reduced photosynthetic capacity from acclimation to elevated CO_2, possibly showing little benefit to using long-term exposure for improved plant quality or quickened production. Specifically, while flowers may present the opportunity for new carbon sinks, the dry mass of individual flowers are shown to increase in response to elevated CO_2 rather than development or number (Cummings and Jones, 1918; Frantz and Ling, 2011; Mortensen, 1987; Niu et al., 2000;

Prior et al., 2011). More studies are needed to specifically observe flower timing in response to elevated CO_2, as there is little evidence of CO_2 causing flower induction alone; rather, previous studies observed earlier flower induction is often a response to an interaction between lighting treatment and elevated CO_2 (Arp, 1991; Cummings and Jones, 1918; Frantz and Ling, 2011; Kirschbaum, 2011; Mortensen, 1987; Mortensen and Moe, 1992; Mortensen and Ulsaker, 1985; Niu et al., 2000; Prior et al., 2011). For example, Niu et al. (2000) found that changes in DLI possibly interacted with elevated CO_2 to alter flower development in pansy 'Delta Yellow Blotch' and 'Delta Primrose Blotch'; however, no difference in flowering rate was observed when comparing solely 400 and 600 $\mu mol \cdot mol^{-1}$ CO_2.

2.5.2 Photosynthesis Data

In the present study, both species displayed increased V_{cmax} and J_{max} for plants grown under ambient compared to elevated conditions on all measurement days. Changes in J_{max} tended to mirror those associated with changes in V_{cmax}, which is consistent with other species (Gunderson and Wullschleger, 1994). For example, previous research across 109 species has shown average V_{cmax} reductions of 13% and J_{max} reductions of 5% in response to elevated CO_2 concentrations (Ainsworth and Long, 2005; Kirschbaum, 2011). In another study, eucalyptus showed a 39% decrease in J_{max} and 34% decrease in V_{cmax} compared to ambient after ten weeks of exposure at 800 $\mu mol \cdot mol^{-1}$ CO_2 (Dillon et al., 2018). A decrease in Rubisco dehydrogenase content or activity has been proposed to coincide with reductions in V_{cmax} (Azcón-Bieto, 1994; Bunce, 1994; Drake et al., 1997; Gonzàlez-Meler, 1997; Wullschleger et al., 1994). Similarly, TPU was greater for both species under ambient conditions across all measurement dates. This is consistent with prior studies, as decreased TPU is a common physiological response to elevated CO_2 concentrations and is associated with accumulated hexose sugars and starch, also indicated

by the increase in LMA (Dahal and Vanlerburghe, 2018; Lombardozzi et al., 2018; Yang et al., 2016). For example, eucalyptus displayed a decrease in TPU of 38% in response to ten weeks at 800 μmol·mol^{-1} compared to ambient conditions (Dillon et al., 2018). Similarly, tobacco (*Nicotiana tabacum*) was found to reduce TPU after 18 days of exposure to 1000 μmol·mol^{-1} CO_2 (Dahal and Vanlerburghe, 2018). With both pansy and petunia showing a decrease in V_{cmax}, J_{max}, and TPU in response to elevated CO_2 concentrations, this indicates photosynthetic acclimation as early as 7 days of exposure for petunia and 14 days for pansy. This difference in time for photosynthetic processes to acclimate may also emphasize the contention of species-specific responses. The difference in these rates mirror the results seen in growth measurements, as possible feedback inhibition and damage to the photosynthetic apparatus can come from the accumulated carbohydrates indicated in increased biomass and LMA (Arp, 1991; Cave et al., 1981; Makino and Mae, 1999; Mortensen, 1987; Wulff and Strain, 1982). The reduction in these rates also did not recover during the study, indicating the plants acclimated to an elevated CO_2 concentration in the form of various photosynthetic mechanisms.

Both species in the present study displayed decreased G_s measured at operating point in response to production under elevated conditions for all measurement days. Stomatal conductance has commonly been shown to decrease in response to elevated CO_2 (Arp, 1991). For example, G_s was found to decrease for multiple species after elevation of CO_2 in across many field chamber studies (Anderson et al., 2001). For example, in 41 observations covering 28 species, average reduction in G_s was found to be 20% (Drake et al., 1997). After just 3 weeks at an elevated CO_2 concentration, G_s of kale (*Brassica oleracea*), spinach (*Spinacea oleracea*), and lettuce was reduced by nearly 60%, 65%, and 65%, respectively, compared to an ambient concentration (Erwin and Gesick, 2017; Giri et al., 2016). Stomatal conductance is mediated by

changes in photosynthesis, so reduced photosynthetic capacity coinciding with lower G_s is expected (Drake et al., 1997). Reduced G_s could be a function of reduced stomatal aperture, effecting the ability for CO_2 to enter the stomata or diffuse into the leaf, further limiting photosynthesis rate (Drake et al., 1997; Gislerod and Nelson, 1989; Gunderson and Wullschleger, 1994).

Net photosynthetic rate measured at 400 $\mu mol \cdot mol^{-1}$ CO_2 operating point for petunia and pansy was significant for all measurement days. Long exposure to elevated CO_2 on a broad range of natural species shows a down regulation of net photosynthesis from survey measurements (Dillon et al., 2018). For example, eucalyptus showed an 11% reduction in net photosynthetic rate for plants grown at 800 $\mu mol \cdot mol^{-1}$ CO_2 for ten weeks compared to plants grown at an ambient concentration measured at the same concentration (Dillon et al., 2018). The same trend was detected when assessing overall net photosynthesis taken from A-C_i curves (Dillon et al., 2018). This coincided with an overall decrease in biochemical processes of photosynthesis, like V_{cmax}, J_{max}, and TPU. Acclimation to elevated CO_2 in many species is often measured between 1 to 6 days, with longer exposure showing a steady decrease in net photosynthetic rate (Arp, 1991; Drake et al., 1997; Gunderson and Wullschleger, 1994; Kirschbaum and Lambie, 2015; Mortensen, 1987; Mortensen and Ulsaker, 1985). This is consistent with the present study, as petunia and pansy showed photosynthetic acclimation in as early as seven days. Furthermore, once plants showed acclimation in photosynthetic processes, plants remained in this physiological state for the duration of the study with no indication of photosynthetic recovery. From a production standpoint, the immediate photosynthetic increase is often upwards of 40% compared to ambient, with a potential for exponential growth based off of early photosynthetic model predictions (Drake et al., 1997; Prior et al., 2011). However, the present study observed a

nearly 40% decrease in net photosynthetic capacity after even just seven days for plants grown at 1000 μmol·mol^{-1} CO_2 compared to ambient. This emphasizes the discrepancy between predicted benefits of elevated CO_2 compared to realized responses throughout production. Moreover, an increase in LMA for plants grown at elevated conditions did not lead to an expected increase in net photosynthetic rate based on leaf-level gas exchange, further demonstrating acclimation to elevated CO_2 (Poorter et al., 2009). However, there is evidence that plants grown under elevated CO_2 in the present study were still operating at an increased photosynthetic rate compared to ambient despite acclimation, resulting in the moderate increases of LDM and SDM compared to ambient. Thus, the utilization of long-term elevated CO_2 for controlled environment production is understandable due to the marginal benefit to biomass accumulation. However, it stands to reason that extending the present study an additional 28 days would likely show even fewer differences between treatments based on the incremental decline in biomass observed in previous studies (Frantz and Ling, 2011). However, further research is needed to evaluate if capitalizing on the potential increase in photosynthesis prior to acclimation is attainable and meaningful to enhance production.

2.6 Conclusion

While responses of horticultural species to long-term elevated CO_2 concentrations continue to be studied, more research is needed to identify the timing and extent of these species-specific responses to better understand CO_2 as an input of production. For the industry, the morphological and physiological responses to long-term application of elevated CO_2 concentrations during the finishing stage of petunia ‘Dreams Midnight’ and pansy ‘Matrix Blue Blotch Improved’ shows little benefit for improved plant quality or quickened production. Elevated CO_2 had no impact to plant size and flowering, however there was an increase in

biomass. There was also a significant reduction in photosynthetic rate and processes, translating into quick photosynthetic acclimation in petunia and pansy. However, the present study gives foundational research that can be used to further evaluate the effect of timing and possibly duration of elevated CO_2 application to develop and optimize best management practices for annual bedding plant species.

Table 1. Morphological data for petunia (*Petunia ×hybrida* 'Dreams Midnight) including stem caliper, stem length, leaf number, leaf area (LA), stem dry mass (StDM), leaf dry mass (LDM), shoot dry mass (SDM), leaf mass area (LMA), and relative chlorophyll content (RCC). Plants were grown in 11.4-cm containers (550 ml) using reach-in growth chambers with CO_2 concentration set points of 400 (ambient) and 1000 µmol·mol^{-1} (elevated) harvested at 7, 14, 21, and 28 days after experiment initiation.

CO_2 (µmol·mol^{-1})	Caliper (mm)	Length (mm)	Leaf Number	LA (cm^2)	LDM (g)	SDM (mg)	LMA (mg·cm^{-2})	RCC
				Day 7				
Ambient	5.03±0.18[z]	2.23±0.12	19.5±1.49 b[y]	71.33±5.66	0.22±0.01 b	0.32±0.03 b	3.23±0.12	42.5±1.09
Elevated	5.00±0.13	2.31±0.13	22.1±1.68 a	82.91±8.10	0.28±0.02 a	0.37±0.03 a	3.44±0.14	42.8±0.92
				Day 14				
Ambient	7.72±0.26	3.62±0.19	57.47±4.19	272.65±20.50 b	0.77±0.05 b	1.02±0.06 b	2.86±0.06 b	49.5±0.86
Elevated	8.08±0.12	3.46±0.16	61.07±3.31	309.05±17.33 a	0.94±0.05 a	1.24±0.04 a	3.06±0.05 a	51.4±1.02
				Day 21				
Ambient	9.38±0.27	4.73±0.27	103.33±4.99	630.12±46.93	1.83±0.08 b	2.65±0.12 b	2.99±0.12 b	52.9±0.68 b
Elevated	9.91±0.12	4.81±0.26	109.20±5.80	672.19±44.10	2.23±0.12 a	3.35±0.17 a	3.39±0.16 a	60.1±0.91 a
				Day 28				
Ambient	11.14±0.19	6.85±0.67	147.07±9.45	892.20±71.23	2.53±0.16	4.36±0.32 b	2.90±0.11 b	55.7±1.58
Elevated	11.99±0.28	6.73±0.63	149.67±7.07	921.48±47.09	2.87±0.11	5.20±0.26 a	3.17±0.13 a	57.9±1.76

[z]Mean values are based on five samples from each treatment across three experimental repetitions (n=15) [y]Means sharing a letter across CO_2 treatments are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant differences between treatments.

Table 2. Morphological data for pansy (*Viola ×wittrockiana* 'Matrix Blue Blotched Improved') including stem caliper, stem length, leaf number, leaf area (LA), stem dry mass (StDM), leaf dry mass (LDM), shoot dry mass (SDM), leaf mass area (LMA), and relative chlorophyll content (RCC). Plants were grown in 11.4-cm containers (550 ml) using reach-in growth chambers with CO_2 concentration set points of 400 (ambient) and 1000 µmol·mol^{-1} (elevated) harvested at 7, 14, 21, and 28 days after experiment initiation.

CO_2 (µmol·mol^{-1})	Caliper (mm)	Length (mm)	Leaf Number	LA (cm^2)	LDM (g)	SDM (mg)	LMA (mg·cm^{-2})	RCC
				Day 7				
Ambient	3.61±0.21[z]	1.93±0.09	11.13±0.82	36.09±2.06	0.17±0.01 b	0.23±0.02 b	4.73±0.26 b	47.0±1.33
Elevated	3.53±0.18	2.01±0.08	12.20±0.98	39.38±2.49	0.23±0.01 a	0.29±0.02 a	5.96±0.21 a	48.2±0.56
				Day 14				
Ambient	6.16±0.15	2.56±0.10	22.67±1.59	84.21±4.50 b	0.45±0.03 b	0.59±0.04 b	5.32±0.15 b	60.3±1.55
Elevated	6.11±0.14	2.66±0.10	22.87±1.56	95.66±4.85 a	0.59±0.04 a	0.76±0.05 a	6.27±0.30 a	58.9±0.73
				Day 21				
Ambient	7.97±0.14 b[y]	3.27±0.12 b	38.33±2.43	174.04±9.52	0.97±0.06 b	1.37±0.09 b	5.59±0.15	65.9±1.20
Elevated	8.79±0.18 a	3.47±0.13 a	41.40±2.49	189.07±11.68	1.13±0.08 a	1.67±0.11 a	5.91±0.13	64.5±1.14
				Day 28				
Ambient	9.24±0.26	3.85±0.16	59.20±3.03	270.12±13.85	1.45±0.08	2.22±0.11 b	5.40±0.19 b	66.3±1.27
Elevated	9.16±0.17	3.89±0.14	57.47±2.17	261.26±12.22	1.64±0.13	2.55±0.14 a	6.32±0.23 a	64.3±1.76

[z]Mean values are based on five samples from each treatment across three experimental repetitions (n=15) [y]Means sharing a letter across CO_2 treatments are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant differences between treatments.

Table 3. Flowering data for petunia (*Petunia ×hybrida* 'Dreams Midnight) and pansy (*Viola ×wittrockiana* 'Matrix Blue Blotched Improved') including days to first bud, days to flower, and number of flowers at harvest. Plants were grown in 11.4-cm containers (550 ml) using reach-in growth chambers with CO_2 concentration set points of 400 (ambient) and 1000 µmol·mol^{-1} (elevated) measured after flower initiation 21 and 28 days after experiment initiation.

	Petunia		Pansy	
CO_2 treatment	**Days to flower**	**Number of flowers**	**Days to flower**	**Number of flowers**
Ambient	21.0±0.76	9.56±1.15	19.8±0.46	2.51±0.24
Elevated	21.6±0.49	10.17±1.18	19.7±0.33	2.98±0.28

[z]Mean values are based on 15 samples from each treatment across three experimental repetitions (n=60). Means were found to have no significant differences between treatments.

Table 4. Photosynthesis data for petunia (*Petunia ×hybrida* 'Dreams Midnight) including maximum photosynthetic rate of Rubisco carboxylation (V_{cmax}), maximum rate of photosynthetic electron transport (J_{max}), triose phosphate utilization rate (TPU), and stomatal conductance measured at operating point (G_s; 400 µmol·mol^{-1} and 1000 µmol·mol^{-1} CO_2 for ambient and elevated, respectively). Plants were grown in 11.4-cm containers (550 ml) using reach-in growth chambers with CO_2 concentration set points of 400 (ambient) and 1000 µmol·mol^{-1} (elevated) harvested at 7, 14, 21, and 28 days after experiment initiation.

CO_2 (µmol·mol^{-1})	**V_{cmax} (µmol·m^{-2} s^{-1})**	**J_{max} (µmol·m^{-2} s^{-1})**	**TPU (µmol·m^{-2} s^{-1})**	**G_s at operating point**
		Day 7		
Ambient	95.31±1.43[z] a[y]	256.02±8.44 a	12.45±0.21 a	0.60±0.01 a
Elevated	90.82±1.18 b	235.35±5.96 b	11.94±0.19 b	0.45±0.02 b
		Day 14		
Ambient	99.87±1.73 a	295.37±13.95 a	13.08±0.16 a	0.61±0.02 a
Elevated	96.18±2.62 b	256.52±13.44 b	12.35±0.34 b	0.43±0.02 b
		Day 21		
Ambient	91.52±2.31 a	250.70±12.66 a	12.43±0.20 a	0.54±0.04 a
Elevated	83.02±3.07 b	218.23±15.40 b	11.18±0.26 b	0.34±0.05 b
		Day 28		
Ambient	77.69±5.24 a	174.30±12.04 a	11.89±0.17 a	0.55±0.05 a
Elevated	61.04±5.78 b	162.29±17.18 b	10.31±0.31 b	0.27±0.04 b

[z]Mean values are based on five samples from each treatment across three experimental repetitions (n=15) [y]Means sharing a letter across CO_2 treatments are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant differences between treatments.

Table 5. Photosynthesis data for pansy (*Viola ×wittrockiana* 'Matrix Blue Blotched Improved') including maximum photosynthetic rate of Rubisco carboxylation (V_{cmax}), maximum rate of photosynthetic electron transport (J_{max}), triose phosphate utilization rate (TPU), and stomatal conductance measured at operating point (G_s; 400 µmol·mol^{-1} and 1000 µmol·mol^{-1} CO_2 for ambient and elevated, respectively). Plants were grown in 11.4-cm containers (550 ml) using reach-in growth chambers with CO_2 concentration set points of 400 (ambient) and 1000 µmol·mol^{-1} (elevated) harvested at 7, 14, 21, and 28 days after experiment initiation.

CO_2 (µmol·mol^{-1})	**V_{cmax} (µmol·m^{-2} s^{-1})**	**J_{max} (µmol·m^{-2} s^{-1})**	**TPU (µmol·m^{-2} s^{-1})**	**G_s at operating point**
		Day 7		
Ambient	76.96±1.54[z]	208.88±7.17	11.94±0.13	0.33±0.02 a
Elevated	78.20±1.59	206.89±8.32	11.10±0.28	0.21±0.02 b
		Day 14		
Ambient	90.54±1.15 a[y]	282.98±12.98 a	13.15±0.29 a	0.32±0.02 a
Elevated	83.02±1.86 b	245.76±11.41 b	11.91±0.31 b	0.19±0.01 b
		Day 21		
Ambient	89.85±0.88 a	292.42±6.18 a	13.49±0.09 a	0.42±0.02 a
Elevated	82.04±1.92 b	232.40±13.24 b	12.21±0.15 b	0.30±0.02 b
		Day 28		
Ambient	88.74±1.09 a	290.98±10.18 a	13.21±0.14 a	0.41±0.02 a
Elevated	73.83±2.81 b	228.48±11.93 b	11.46±0.05 b	0.24±0.02 b

[z]Mean values are based on five samples from each treatment across three experimental repetitions (n=15) [y]Means sharing a letter across CO_2 treatments are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant differences between treatments.

Table 6. Net photosynthetic rate (A) measured at 400 and 1000 µmol·mol^{-1} CO_2 for petunia (*Petunia ×hybrida* 'Dreams Midnight) and pansy (*Viola ×wittrockiana* 'Matrix Blue Blotched Improved'). Measurements were taken with cuvette conditions matching the production environment, specifically leaf temperature, relative humidity, and photosynthetic photo flux density (PPFD) were 21 ºC, 55%, and 250 µmol·m^{-2}·s^{-1}, respectively. Plants were grown in 11.4-cm containers (550 ml) using reach-in growth chambers with CO_2 concentration set points of 400 (ambient) and 1000 µmol·mol^{-1} (elevated) harvested at 7, 14, 21, and 28 days after experiment initiation.

	Petunia		Pansy	
CO_2 treatment	**CO_2 400 (µmol·mol^{-1})**	**CO_2 1000 (µmol·mol^{-1})**	**CO_2 400 (µmol·mol^{-1})**	**CO_2 1000 (µmol·mol^{-1})**
	Day 7			
Ambient	10.97±0.30[z] a[y]	15.59±0.42 a	11.74±0.16 a	16.86±0.35 a
Elevated	8.06±0.41 b	12.94±0.53 b	9.17±0.31 b	14.59±0.33 b
	Day 14			
Ambient	11.85±0.14 a	16.82±0.31 a	12.18±0.22 a	17.06±0.49 a
Elevated	9.32±0.33 b	14.33±0.35 b	10.41±0.33 b	15.72±0.22 b
	Day 21			
Ambient	11.86±0.44 a	17.11±0.34 a	12.71±0.12 a	17.82±0.26 a
Elevated	9.33±0.46 b	14.73±0.48 b	10.71±0.52 b	16.39±0.24 b
	Day 28			
Ambient	10.53±0.37 a	16.45±0.50 a	12.38±0.15 a	17.61±0.29 a
Elevated	8.57±0.62 b	13.88±0.59 b	10.84±0.15 a	15.94±0.44 b

[z]Mean values are based on five samples from each treatment across three experimental repetitions (n=15) [y]Means sharing a letter across CO_2 treatments are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant differences between treatments.

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CHAPTER 3. THE EFFECT OF CO_2 ENRICHMENT TIMING AND DURATION ON PANSY AND PETUNIA SEEDLING QUALITY FOR INDOOR PRODUCTION

3.1 Summary

While crops often immediately respond to elevated carbon dioxide (CO_2) concentrations beneficially (e.g., increased photosynthesis), the initial responses are often not carried on through production, possibly mitigating the gain of using this input. The timing and extent of these acclimation responses in horticulture species remains mostly unknown. Therefore, the objective of this research was to determine species-specific responses to enriched CO_2 concentrations for pansy (*Viola ×wittrockiana* 'Matrix Blue Blotched Improved') and petunia (*Petunia ×hybrida* 'Dreams Midnight) during propagation. To investigate the impact on propagation of CO_2 enrichment at varying times and duration, pansy and petunia seeds were sown in 128-cell trays and placed in growth chambers with air temperature, relative humidity, and radiation intensity setpoints of 21 °C, 55%, and 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively. Carbon dioxide treatments were established using the two growth chambers with setpoints of either 400 (ambient) or 1000 $\mu mol \cdot mol^{-1}$ (enriched) maintained during a 16-h photoperiod. Treatments consisted of seedlings grown for 28 days at ambient (Amb_{28}), 28 days at elevated (Elv_{28}), 14 days at ambient then 14 days at elevated (Amb_{14}:Elv_{14}), and 14 days at elevated then 14 days at ambient CO_2 concentration (Elv_{14}:Amb_{14}). Harvest data was collected weekly, and four weeks after germination seedlings were transplanted into the greenhouse to determine impacts on finishing quality and flowering. After 4 weeks, both species produced greater total dry mass (roots + leaves + stem) under Elv_{28} and Amb_{14}:Elv_{14} compared to Amb_{28} , with no difference in leaf area. Similarly, plants in both species produced higher leaf mass area grown under Elv_{28} and

Amb_{14}:Elv_{14} than Amb_{28} and Elv_{14}:Amb_{14}. Pansy showed no difference in biomass or size after transplant into the greenhouse but possible decreased days to flower under Elv_{28}. Therefore, days to flower may be influenced by elevated CO_2 during seedling production but there may be little contribution to growth rate long term after transplant. These results provide useful information regarding the timing and extent of response to enriched CO_2 concentrations for pansy and petunia. However, due to physiological acclimation potentially occurring within one week of treatment initiation, additional research is needed to best understand how this input can be further optimized for controlled environment production.

3.2 Introduction

The 2018 wholesale value for floriculture crops in the US is estimated at $4.77 billion. Approximately one-third of this total is represented by annual bedding plants, with over 423 million square feet of greenhouses used for floriculture production (U.S. Dept. Agr., 2019). Typically, production of plugs from seed for the ornamental annual bedding plant market occurs in the winter and early spring (Styer, 2003). While the average ambient CO_2 concentration is currently 409.8 $\mu mol \cdot mol^{-1}$, it is common for concentrations to drop as low as 200 $\mu mol \cdot mol^{-1}$ in a greenhouse environment (Both et al., 2017; Erwin and Gesick, 2017; Lan et al., 2020; Mortensen, 1987). This generally happens on sunny, cold days when the greenhouse is full of plants, but the ventilation is too low to replenish the CO_2. Crop demand for CO_2 becomes greater than supply, limiting photosynthesis and similarly, plant growth (Both et al., 2017; Erwin and Gesick, 2017). Even with proper ventilation, greenhouse concentrations can still commonly fall to 250-300 $\mu mol \cdot mol^{-1}$ (Mortensen, 1987).

Current atmospheric CO_2 concentrations are too low for maximum photosynthetic capacity, mainly due to competition between CO_2 and O_2 fixed by the enzyme ribulose

biphosphate carboxylase (Rubisco) which results in possible photorespiration and a loss in carbon (Mortensen, 1987). Carbon dioxide concentrations near 900 μmol·mol^{-1} nearly eliminate O_2 inhibition of photosynthesis due to an increased CO_2/O_2 ratio for many species (Mortensen, 1987). Numerous studies have shown that CO_2 concentrations between 800 to 1200 μmol·mol^{-1} have the potential to increase plant growth, while further increases beyond 1200 μmol·mol^{-1} often provide little benefit (Both et al., 2017; Erwin and Gesick, 2017). Carbon dioxide concentrations of 600 to 1000 μmol·mol^{-1} are often the most practical and within the optimal range for most species (Mortensen, 1987). Increasing CO_2 concentrations have the greatest effect on increasing net photosynthetic rate from the first 100 μmol·mol^{-1} above ambient (400 to 500) and incrementally decrease positive benefits as CO_2 increases (Both et al. 2017; Erwin and Gesick, 2017).

Early short-term studies concluded that increasing the CO_2 concentration in greenhouses was an economically efficient way to enhance growth in ornamental and vegetable crops (Mortensen, 1987; Prior et al., 2011). Enriching greenhouse environments with CO_2 is a well-known method of stimulating photosynthesis and leading to increased carbon uptake and assimilation, increasing plant growth (Mortensen and Moe, 1992; Mortensen, 1987; Prior et al., 2011). This can be attributed to competitive inhibition of photorespiration by CO_2 and increased leaf internal CO_2 concentrations (Drake et al., 1997; Gunderson and Wullschleger, 1994; Kirschbaum and Lambie, 2015; Mortensen, 1987; Prior et al., 2011). In a survey across 60 experiments, overwhelming evidence concludes that immediate responses to enriched CO_2 concentrations include increased net photosynthetic rate by approximately 58% compared to plants in ambient concentrations (Drake et al., 1997). For example, the net photosynthetic rate was doubled by increasing the CO_2 concentration to 900 compared to 330 μmol·mol^{-1} with

measurements taken at experiment initiation in begonia (*Begonia ×hiemalis* 'Schwabenland') (Mortensen and Ulsaker, 1985). Similarly, a study conducted by Mortensen and Moe (1983) concluded that elevated CO_2 (900 μmol·mol^{-1}) increases photosynthetic rate by 50% compared to 350 μmol·mol^{-1} at 5 days of exposure in chrysanthemum (*Chrysanthemum morifolium* 'Horim'). However, experiments in the past have often been designed to convince growers about the benefits of CO_2 based solely on short-term benefits without fully considering possible long-term impacts (Both et al. 2017; Mortensen, 1987; Prior et al., 2011).

The short-term positive impact of elevated CO_2 concentration and predictions based on those measurements are often not sustained in instances where plants are exposed to CO_2 enrichment long-term (Drake et al., 1997; Mortensen, 1987; Prior et al., 2011). The duration of exposure to elevated CO_2 concentration is correlated to reduction in photosynthetic capacity, showing diminishing positive response long-term (Arp, 1991; Drake et al., 1997; Kirschbaum and Lambie, 2015; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011). The long-term response of plants to elevated CO_2 is partially related to sink size and the limited ability for the plant to metabolize fixed carbon (Arp, 1991; Both et al., 2017; Frantz and Ling, 2011; Makino and Mae, 1999; Rogers et al., 1998). Plant responses to elevated CO_2 are often thought to be impacted by container size (Arp, 1991). Plants grown in small containers, like much of the floriculture industry, may become sink limited from these root zone restrictions. For example, in a review of field and container studies for responses to elevated CO_2 concentrations conducted by Arp (1991), a highly significant correlation was found between smaller container size and increased root/shoot ratio. This indicated that plants in elevated CO_2 concentrations allocate excess carbohydrates to increased root growth, eventually filling the root zone, limiting capacity for further carbohydrate storage (Arp, 1991).

However, additional long-term responses to elevated CO_2 concentrations may prove beneficial for production (Prior et al., 2011). Above ground biomass generally increases with exposure to elevated CO_2 (Ainsworth and Long, 2004; Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Prior et al., 2011). Species including cut flowers, vegetables, and bedding plants show positive responses to elevated CO_2 like increased dry weight, height, number of leaves, leaf area, and lateral branching (Gislerod and Nelson, 1989; Mortensen, 1987). For example, Frantz and Ling (2011) found that 800 $\mu mol \cdot mol^{-1}$ CO_2 increased biomass by 10% compared to plants grown at an ambient concentration in petunia 'Madness White' after 5 weeks. Similarly, Zhang et al. (2012) found that 760 $\mu mol \cdot mol^{-1}$ CO_2 increased leaf area by 10% and had a significant impact on lateral branch development in New Guinea impatiens (*Impatiens hawkeri*). Elevated CO_2 has also been shown to result in shorter time to flower as well as increased flower number for many species (Drake et al., 1997; Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Mortensen and Moe, 1992; Mortensen and Ulsaker, 1985; Prior et al., 2011).

The influence of CO_2 is largely dependent on species (Frantz and Ling, 2011). Species have unique responses to elevated CO_2 concentrations, presenting the possibility for species-specific CO_2 protocols (Mortensen, 1987). Thus, by evaluating these species-specific responses, best management practices for CO_2 usage in production can be developed for different growth stages (Mortensen, 1987; Prior et al., 2011).

Early studies suggest possible benefit and bypass of the acclimation of photosynthesis in response to elevated CO_2 with intermittent CO_2 application (Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011). The earliest studies on intermittent CO_2 application evaluated cutting elevated time to a several hours a day, rather than continuous application (Mortensen, 1987). These studies found that cutting

application time to a few hours a day did not show benefit compared to continuous daily application in three cultivars of African violet (*Saintpaulia ionantha* 'Nicole', 'Lena', and 'Rosa Roccoco'), soybean (*Glycine max* 'Fiskeby V'), and tomato (*Lycopersicum esculentum* 'Virosa') (Mortensen, 1986; Mortensen, 1987). Frantz and Ling (2011) suggest that possible short-term exposure (several days to a few weeks) to elevated CO_2 may keep the benefits with a bypass of the detrimental effects of long-term exposure. While there is little research on short-term usage of elevated CO_2 with oscillation between days or weeks, studies show that a five-day break from elevated CO_2 returns the photosynthetic rate to 75% compared to an ambient concentration in soybeans, suggesting a possible timing component to CO_2 acclimation (Jones et al., 1985). Many studies call for more research on short-term experiments and evaluation of responses to CO_2 during various stages of development (Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011).

While the effects of elevated CO_2 on plants is well known, horticulture species have received much less attention than agronomic and forest species likely as a result of complex experiment design (Frantz and Ling, 2011; Mortensen, 1987; Prior et al., 2011). While it is conjectured that horticulture species will benefit from elevated CO_2 in production, research is lacking to support this contention (Prior et al., 2011). Seedlings require special attention due to the potential for sink limitations which may prevent full utilization of CO_2 as an input for the entire seedling production cycle (Arp, 1991; Both et al., 2017; Erwin and Gesick, 2017; Mortensen, 1987). However, enriched CO_2 environments have potential to increase seedling quality by producing a compact habit and high root and shoot biomass, which facilitates processing, shipping, and transplanting (Craver, 2018; Oh et al., 2010; Pramuk and Runkle, 2005; Randall and Lopez, 2014). While studies have shown long-term elevated carbon dioxide

(CO_2) concentrations may prove beneficial for production during propagation, more research is needed on short-term experiments and to evaluate responses to strategic timing of CO_2 application (Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Mortensen, 1987; Mortensen and Moe, 1992; Prior et al., 2011). Therefore, the objective of this study was to evaluate species-specific morphological responses to timing and duration of elevated CO_2 concentrations in controlled environments for petunia (*Petunia ×hybrida* 'Dreams Midnight') and pansy (*Viola ×wittrockiana* 'Matrix Blue Botch Improved') seedlings.

3.3 Materials and Methods

3.3.1 Plant Material and Germination Environment

Seeds of pansy 'Matrix Blue Botch Improved' and petunia 'Dreams Midnight' were sown in 128-cell trays (14-mL individual cell volume) filled with commercial germination mix comprised of (by volume) 80% fine sphagnum peat, 10% perlite, and 10% vermiculite (BM2 Germinating Mix; Berger, Saint-Modeste, Canada). Trays were divided into 64-cell sections to facilitate data collection. Trays were immediately placed in a reach-in growth chamber (PG2500; Conviron, Winnipeg, Canada) with air temperature and relative humidity setpoints of 21 °C and 55%/65% day/night, respectively. Light was provided by light-emitting diode (LED) fixtures (GreenPower LED DR/W production modules; Signify, Eindhoven, Netherlands) with a 16-h photoperiod (0800 to 0000 HR) and an average photosynthetic photon flux density (*PPFD*) at canopy height of 250 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Trays were placed under treatment conditions immediately upon hypocotyl emergence. Seedlings were irrigated daily with water soluble fertilizer at a concentration of 150 $mg \cdot L^{-1}$ Nitrogen (Jack's LX 13N–2P–13K Plug Formula for High Alkalinity Water, JR Peters Inc.; Allentown, Pennsylvania). Other macro and micronutrients

contained in the fertilizer in $mg \cdot L^{-1}$ were 22.5 P, 150 K, 69 Ca, 34.5 Mg, 0.15 B, 0.075 Cu, 0.75 Fe, 0.375 Mn, 0.075 Mo, and 0.375 Zn.

3.3.2 Growth Chamber Environment

Carbon dioxide treatments were established in two separate reach-in growth chambers (PG2500; Conviron) with setpoints of either 400 or 1000 $\mu mol \cdot mol^{-1}$ during the established 16-h photoperiod with injection controlled using a CO_2 gas analyzer (LI-830; LI-COR Inc., Lincoln, NE). Static CO_2 concentration treatments consisted of seedlings grown under either ambient (400 $\mu mol \cdot mol^{-1}$; Amb_{28}) or elevated (1000 $\mu mol \cdot mol^{-1}$; Elv_{28}) CO_2 concentrations for 28 d after hypocotyl emergence. Dynamic CO_2 concentration treatments were also implemented, with seedlings grown for 14 d under ambient then 14 d under elevated (Amb_{14}:Elv_{14}) or 14 d under elevated then 14 d under ambient CO_2 concentrations (Elv_{14}:Amb_{14}). CO_2 concentrations were measured in each growth chamber using a CO_2 probe (GMP252; Vaisala, Woburn, MA) with a mean ± SD CO_2 concentration over the 16-h photoperiod of 425 ± 55 and 1014 ± 84 $\mu mol \cdot mol^{-1}$, respectively, across 4 experimental replications. Carbon dioxide setpoints alternated chambers each replication to randomize for chamber effects. Fixed mounted infrared thermocouples with ABS plastic housing (OS36-01-T-80F; Apogee Instruments Inc., Logan, Utah) were installed in each chamber to measure leaf temperature, with a mean ± SD of 21 ± 0.6 and 21 ± 0.4 °C, and precision thermistors (ST-100; Apogee Instruments, Inc.) were used to measure air temperature with a mean ± SD of 21 ± 0.1 and 21 ± 0.1 °C for chamber 1 and chamber 2, respectively. Relative humidity probes (EE-08-SS; Apogee Instruments, Inc.) were installed in each chamber to measure relative humidity, with a mean ± SD of 62 ± 11 and 63 ± 9% during the day and 70 ± 6 and 70 ± 3% during the night for chamber 1 and chamber 2, respectively. Radiation quality and intensity were measured at the beginning of each experimental replication by taking seventeen

spectral scans per treatment using a spectrometer at canopy height averaging at 250 ± 15 and 252 ± 15 $\mu mol \cdot m^{-2} \cdot s^{-1}$ (LI-180; LICOR Inc.) for chamber 1 and chamber 2, respectively. Environmental setpoints were measured every 30 s and the average was logged every 15 min by a data logger (model CR1000X; Campbell Scientific, Logan, UT).

3.3.3 Seedling Data Collection

At 14, 21, and 28 d after hypocotyl emergence, five seedlings from each tray were randomly sampled for data collection. A leaf area meter (LI-3100C; LI-COR, Lincoln, Nebraska) was used to collect individual seedling leaf area (LA; cm^2) by removing the leaves at the axil, leaves were then counted for seedling leaf number. Stem length (mm) from apical bud to soil line and stem caliper (mm) at the hypocotyl were measured. Relative chlorophyll content (RCC) was measured (SPAD-502 Chlorophyll Meter; Konica Minolta Inc., Tokyo, Japan) on the most recently fully expanded leaf. At harvest, seedling roots, leaves, and stems were washed and dried in a forced air oven maintained at 70 °C to determine root (RDM), leaf (LDM), and stem dry mass (SDM). Leaf mass area (LMA = LDM/LA; $mg \cdot cm^{-2}$) was calculated based on LA and dry mass measurements. Total dry mass was calculated from the sum of root, leaf, and stem dry mass (TDM = RDM + LDM + SDM).

3.3.4 Finishing Environment

At 28 d after hypocotyl emergence, five uniform seedlings from each tray were randomly selected and transplanted into 11.4-cm (550 ml) containers using commercial potting media comprised of (by volume) 85% sphagnum peat and 15% perlite (BM6 Growing Mix; Berger Horticultural Products Ltd., Sulphur Springs, Texas). The first repetition plants were left to grow in the greenhouse from March 20, 2020 to April 17, 2020, the second from July 7, 2020 to August 4, 2020, the third from July 28, 2020 to August 25, 2020, and the fourth from August 25,

2020 to September 22, 2020. The greenhouse environment had a mean daily light integral of 12.21 $\mu mol \cdot m^{-2} \cdot d^{-1}$ with light intensity measured and collected every 5 seconds and logged on a data logger over an average 16-h photoperiod (LI-1500 datalogger; LICOR Inc.). Average daily air temperature was measured using precision thermistors (ST-100; Apogee Instruments, Inc.) with a mean ± SD of 21.9 ± 1.7 °C measured every 15 and the average logged every 30 minutes to a datalogger (model CR-1000x; Campbell Scientific, Logan, UT).

3.3.5 Finishing Environment Data Collection

Plants were irrigated as needed with water-soluble fertilizer at a concentration of 150 $mg \cdot L^{-1}$ N (Jack's LX 21N–5P–20K All Purpose Formula for High Alkalinity Water, JR Peters Inc., Pennsylvania) Other macro- and micronutrients contained in the fertilizer in $mg \cdot L^{-1}$ were 36 P, 142.5 K, 1.05 Mg, 0.15 B, 0.075 Cu, 0.75 Fe, 0.375 Mn, 0.075 Mo, and 0.375 Zn. Data was collected on time of flower when the initial flower was fully reflexed. Additional destructive data collected at this time included plant height, width, and number of nodes. Vegetative material was then harvested and dried in a forced air oven maintained at 70°C to determine shoot dry mass.

3.3.6 Statistical Analysis

Analysis was done using R 4.0.3 and the lme4, lmerTest, and emmeans packages (Bates et al., 2015; Kuznetsova et al., 2017; Lenth et al., 2019). One observational unit was one measurement per plant with a total of n=80 observations. A mixed model was fit using morphological data collected (continuous) as the response. Fixed effects included CO_2 treatment (categorical: Amb_{28}, Elv_{28}, Amb_{14}:Elv_{14}, Elv_{14}:Amb_{14}). Repetition (categorical: 1, 2, 3, 4) was included as a random effect to account for the split plot design. CO_2 treatments were compared using Tukey adjusted pairwise comparisons. Model assumptions of linearity and equal scatter were both satisfied, checked using residual diagnostic plots. Week (harvest time) was not

included in the model, but each week (2, 3, 4) was treated as a discrete event. No trend over time was tested. This model was chosen with repetition as random to account for the effect of randomizing the chambers between repetitions. This allowed analysis to show the comparisons between the CO_2 treatments accounting for the effect of any possible differences between environments in the chambers. After the model was tested with the rand() function (Knuth, 1981), the random effect was found to be statistically significant justifying its use in the model.

3.4 Results

3.4.1 Seedling Growth and Morphology

Carbon dioxide treatment had no effect on stem caliper for both petunia and pansy for all harvest dates (Table 1, Table 2). Additionally, CO_2 treatment had no effect on stem length for petunia for all harvest dates. However, stem length for pansy was greater under an elevated CO_2 concentration on day 28 (Table 2). Specifically, seedlings grown under Elv_{28} had 42%, 29%, and 25% greater stem length compared to Amb_{28}, Amb_{14}:Elv_{14}, and Elv_{14}:Amb_{14}, respectively (Table 2).

For leaf area, CO_2 treatment had no effect on petunia or pansy for all harvest dates (Table 1, Table 2). Similarly, leaf number for petunia showed no difference between treatments for all harvest dates (Table 1). However, leaf number for pansy differed on day 28, with an increase of 20%, 15%, and 15% under Elv_{28} compared to Elv_{14}:Amb_{14}, Amb_{14}:Elv_{14} and Amb_{28}, respectively (Table 2).

For petunia, the impact of CO_2 treatment was only apparent on day 28 (Table 1). Specifically, RDM was 39% greater for Amb_{14}:Elv_{14} compared to Amb_{28} (Table 1). For pansy RDM, CO_2 treatment was significant on days 14, 21, and 28, with the highest values observed under Elv_{28} (Table 2). For example, on day 28 RDM was 75% and 43% greater under Elv_{28}

compared to Amb_{28} and Elv_{14}:Amb_{14}, respectively (Table 2). CO_2 treatment was also significant for petunia LDM on days 14, 21 and 28, with the highest values observed under Amb_{14}:Elv_{14} and Elv_{28} (Table 1). For example, on day 28, LDM for Amb_{14}:Elv_{14} was 33% and 25% greater than Amb_{28} and Elv_{14}:Amb_{14}, respectively (Table 1). Similarly, LDM was 32% and 24% greater under Elv_{28} compared to Amb_{28} and Elv_{14}:Amb_{14}, respectively (Table 1). Comparable results were observed for pansy LDM, with the highest LDM values observed for Elv_{28} on days 14 and 28 (Table 2). For example, on day 28, LDM for Elv_{28} was 37% greater compared to Amb_{28}. For TDM, CO_2 treatment was significant on days 21 and 28 for petunia and days 14, 21, and 28 for pansy, with the greatest values observed for Elv_{28} and Amb_{14}:Elv_{14} (Table 1, Table 2). For example, on day 28, TDM for petunia was 35% and 26% greater under Amb_{14}:Elv_{14} compared to Amb_{28} and Elv_{14}:Amb_{14}, respectively (Table 1). Additionally, TDM for petunia was 32% greater under Elv_{28} compared to Amb_{28} (Table 1). Similarly, TDM for pansy was 48% and 27% greater for Elv28 compared to Amb_{28} and Elv_{14}:Amb_{14}, and 35% greater for Amb_{14}:Elv_{14} compared to Elv_{14}:Amb_{14}, respectively, on day 28 (Table 2).

RCC for both petunia and pansy was greatest on day 28 for plants subjected to elevated CO_2 at some point during production (Table 1 and Table 2). For example, on day 28 RCC for petunia was 17% and 12% greater under Amb_{14}:Elv_{14} compared to Amb_{28} and Elv_{28}, respectively (Table 1). In pansy, RCC was 10% greater under Elv_{28} compared to Amb_{28} and 13% greater under Elv_{14}:Amb_{14} compared to Amb_{28} (Table 2).

For LMA, CO_2 treatment was significant on all harvest dates for both species, with Elv_{28} and Amb_{14}:Elv_{14} having the greatest values on day 28 (Figure 1). For example, on day 14 Elv_{28} was 13% and 30% greater than Amb_{28} for petunia and pansy, respectively (data not shown). Additionally, on day 28, LMA of petunia under Elv_{28} was 26% and 22% greater than Amb_{28} and

Elv_{14}:Amb_{14}, respectively, and 21% and 17% greater under Amb_{14}:Elv_{14} compared to Amb_{28} and Elv_{14}:Amb_{14}, respectively (Figure 1). For pansy LMA, on day 28 Amb_{14}:Elv_{14} was 28% and 23% greater compared to Elv_{14}:Amb_{14} and Amb_{28}, respectively, and Elv_{28} was 22% and 17% greater compared to Elv_{14}:Amb_{14} and Amb_{14}, respectively (Figure 1).

3.4.2 Finishing

Upon transplant into the greenhouse, CO_2 treatment had no effect on plant width, height, or SDM at time of flowering for either species (Table 3). Additionally, CO_2 treatment had no effect on days to flower for petunia (Table 3). However, pansy flowered significantly earlier when seedlings were produced under Elv_{28} (Table 3). Specifically, Elv_{28} flowered 20% and 16% (average ~3 d) sooner than Amb_{28} and Amb_{14}:Elv_{14}, respectively (Table 3).

3.5 Discussion

3.5.1 Morphology and Growth

In the present study, CO_2 treatment had little to no effect on stem length and caliper, with pansy showing potential for increased stem length on day 28 with long-term exposure (Elv_{28}). Similarly, long-term exposure to elevated CO_2 increased pansy leaf number, indicating larger seedlings. However, leaf area was not significantly affected by CO_2 treatment in pansy or petunia, implying that seedlings subjected to long-term CO_2 enrichment were less compact and had a higher number of small leaves. Compared to plants grown at an ambient concentration or even plants grown under short-term CO_2 enrichment (Elv_{14}:Amb_{14} and Amb_{14}:Elv_{14}), seedlings from Elv_{28} show unchanged or fewer desirable characteristics such as compact habit and sturdiness for easier shipping and transplant (Craver, 2018; Oh et al., 2010; Pramuk and Runkle, 2005; Randall and Lopez, 2014). However, no significant differences in leaf area for treatment groups shows that there is no benefit on leaf area whether CO_2 is applied early, late, or long-

term. These results are similar to a previous study evaluating pansy where smaller containers, for example 12.5 cm^3 cell trays, resulted in fewer growth differences between 800 $\mu mol \cdot mol^{-1}$ and 400 $\mu mol \cdot mol^{-1}$ compared 1200 cm^3 containers (Both et al., 2017). Similarly, Frantz and Ling (2011) found no significant difference in petunia 'Madness White' leaf area when comparing plants grown for five weeks at 800 $\mu mol \cdot mol^{-1}$ CO_2 versus ambient even in 10-cm pots. However, elevated CO_2 has commonly been found to increase leaf number across species, especially in the juvenile stage of development (Ainsworth and Long, 2004; Mortensen, 1987; Mortensen and Moe, 1983; Mortensen and Moe, 1992; Mortensen and Ulsaker, 1985). The influence of CO_2 on leaf responses depends largely on the species, which further explains the discrepancy in responses observed for petunia and pansy (Frantz and Ling, 2011). For example, an elevated CO_2 concentration (940 $\mu mol \cdot mol^{-1}$) had no significant effect on chrysanthemum 'Fiesta' leaf number after six weeks compared to ambient conditions (Gislerod and Nelson, 1989).

In terms of biomass accumulation, there was little difference observed between long-term (Elv_{28}) and late short-term enrichment (Amb_{14}:Elv_{14}). Specifically, by day 28, RDM, SDM, and TDM all showed a similar trend, with seedlings grown under Elv_{28} and Amb_{14}:Elv_{14} having the greatest biomass. This trend indicates there may be little benefit to exposing seedlings to elevated CO_2 early during propagation, specifically prior to two weeks in the current study. Furthermore, between 21 and 28 d, dry mass measurements nearly tripled in size, with the magnitude of growth strongly indicating the benefit of potential enrichment late during seedling production. Early exposure to elevated CO_2 concentrations showed little lasting benefit to plant growth and quality upon returning to ambient after 14 d (Elv_{14}:Amb_{14}). Meanwhile, exposing plants to CO_2 enrichment late during propagation may be just as effective at enhancing plant

mass as long-term exposure. The similar responses observed between Elv_{28} and Amb_{14}:Elv_{14} may be due to sink limitations. Specifically, RDM and SDM can only increase in response to elevated CO_2 up to a certain threshold before biomass increases are limited by plug's capacity to store accumulating carbohydrates (Arp, 1991). However, there are few studies that compare short-term and long-term biomass accumulation. The observed increase in RDM is consistent with previous findings, suggesting that plants grown at an elevated CO_2 concentration show increased rooting in the form of higher RDM (Arp, 1991; Contrufo and Gorissen, 1997; Gislerod and Nelson, 1989; Mauney et al., 1994; Morgan et al., 2001; Mortensen, 1987; Prior et al., 2011). For example, Gislerod and Nelson (1989) found that elevated CO_2 (940 $\mu mol \cdot mol^{-1}$) increased RDM by 55% after six weeks compared to ambient for chrysanthemum 'Fiesta'. Likewise, 700 elevated from 350 $\mu mol \cdot mol^{-1}$ CO_2 increased RDM by 44% in soybean 'Stonewall' grown in field chambers (Prior et al., 2003). Similar to RDM, above ground biomass generally increases with exposure to elevated CO_2 (Ainsworth and Long, 2004; Frantz and Ling, 2011; Kirschbaum and Lambie, 2015; Prior et al., 2011). These results are similar to previous research that found elevated CO_2 (600 $\mu mol \cdot mol^{-1}$) increased dry weight by 10-30% in pansy 'Delta Yellow Blotch' and 'Delta Primrose Blotch' compared to ambient (Niu et al., 2000). Consistent with the previously mentioned studies, begonia 'Schwabenland' displayed an increase in SDM after 5 weeks at an elevated CO_2 concentration of 1500 $\mu mol \cdot mol^{-1}$ compared to ambient (Mortensen and Ulsaker, 1985). Kirschbaum (2011) concluded that herbaceous plants generally tend to increase biomass by 45% from exposure to elevated CO_2 concentrations, including short-term studies (Kirschbaum, 2011).

Relative chlorophyll content was impacted by CO_2 treatment late during production (day 28) in the present study. Plants grown at elevated CO_2 concentrations often invest fewer

resources into the production of chlorophyll, depending on species (Ainsworth and Long, 2004; Gunderson and Wullschleger, 1994). However, plant responses in the form of changes to chlorophyll content are extremely species-specific, with plants sometimes showing a slight increase in response to elevated CO_2 concentrations, often in the short term (Ainsworth and Long, 2004; Arp, 1991; Giri et. al., 2016; Gunderson and Wullschleger, 1994). Long-term exposure to elevated CO_2 can cause starch accumulation which can inhibit or even breakdown chlorophyll in the leaves (Arp, 1991; Cave et al., 1981; Makino and Mae, 1999; Mortensen, 1987; Wulff and Strain, 1982). Thus, while the two-week exposure to elevated CO_2 under Amb_{14}:Elv_{14} in the present study increased RCC for petunia, the four-week exposure under Elv_{28} likely resulted in chlorophyll breakdown. For pansy, Elv_{28} and Elv_{14}:Amb_{14} had the highest RCC which is consistent with studies evaluating similar enriched CO_2 concentrations (Gunderson and Wullschleger, 1994). For example, Perez-Lopez et al. (2015) found that the concentration of chlorophyll-b increased by 64% in lettuce (*Lactuca sativa* 'Blonde of Paris Batavia') and 52% in lettuce 'Oak Leaf' after exposure to 700 $\mu mol \cdot mol^{-1}$ CO_2 compared to 400 $\mu mol \cdot mol^{-1}$. Meanwhile, Zhang et al. (2012) found that New Guinea impatiens decreased chlorophyll content by 18% after exposure to 760 $\mu mol \cdot mol^{-1}$ CO_2 for ten weeks compared to ambient. Again, the discrepancy in these studies further indicates the response of RCC to CO_2 concentration is highly species-specific.

Similar to biomass, LMA was highest under long-term and late exposure to elevated CO_2. As early as day 14 in the present study, seedlings grown under elevated CO_2 displayed greater LMA with no impact to LA, possibly indicating accumulation of carbohydrates. Additionally, there was little difference between seedlings under Elv_{28} and Amb_{14}:Elv_{14} on day 28, indicating 14 days of late CO_2 enrichment was equally effective at manipulating leaf morphology in terms

of LMA. Therefore, increasing CO_2 concentration late in propagation could increase LMA, resulting in thicker, sturdier seedlings better suited for shipping and transplant (Craver, 2018; Oh et al., 2010; Pramuk and Runkle, 2005; Randall and Lopez, 2014). These results are consistent with previous research on various ornamental, agronomic, and natural species where an increase in LMA is commonly observed under elevated CO_2, often reflecting the accumulation of non-structural carbohydrates (Arp, 1991; Frantz and Ling, 2011; Giri et al., 2016; Gunderson and Wullschleger, 1994). For example, 760 $\mu mol \cdot mol^{-1}$ CO_2 increased soluble sugar content by 77.81% and starch by 122.39% in New Guinea impatiens compared to 380 $\mu mol \cdot mol^{-1}$ CO_2 (Zhang et al., 2011). Similarly, plants grown in sink-limiting containers have been shown to exhibit greater LMA when grown at elevated CO_2 concentrations compared to field- or ambient-grown plants (Anderson et al., 2001; Arp, 1991). The potentially sink-limiting plug tray volume may explain why seedlings grown at an elevated CO_2 concentration in the present study exhibited greater LMA than those under ambient for the same duration. Sink-limiting conditions may also explain the similar LMA responses in Elv_{28} and Amb_{14}:Elv_{14}, as seedlings likely possessed insufficient plug cell volume to store additional carbohydrates as RDM from long-term exposure (Arp, 1991; Both et al., 2017).

3.5.2 Finishing

In the present study, the only impact from CO_2 concentration during propagation on finishing quality was slightly earlier flowering for pansy. Frantz and Ling (2011) found that petunia ‘Madness White’ showed no difference in timing for the appearance of the first flower among CO_2 treatments (400 $\mu mol \cdot mol^{-1}$ compared to 800 $\mu mol \cdot mol^{-1}$), although the study was conducted using CO_2 enrichment during finishing. Similarly, CO_2 enrichment during finishing did not influence time to flower or development for pansy ‘Delta Yellow Blotch’ or ‘Delta

Primrose Blotch' (Niu et al., 2000). Previous studies have shown that loss of photosynthetic capacity from plants at elevated CO_2 is rapidly reversible upon exposure to ambient CO_2 (Moore et al., 1999). For example, when plants were transferred to normal ambient CO_2 after long term exposure to elevated CO_2, accumulated carbohydrates in the leaves have been found to disappear after 3 days and the photosynthetic rate recovered to the level of plants grown in ambient CO_2 (Arp, 1991). However, morphological changes such as increased leaf thickness due to accumulated carbohydrates is not reversible and may continue to affect photosynthetic capacity after termination of CO_2 enrichment (Arp, 1991; Gunderson and Wullschleger, 1994). This confirms the results in the present study, as only time to flower was significant in pansy. This indicates that differences due to CO_2 treatment in photosynthetic rate and carbohydrate storage likely recovered in a short time span, limiting differences in growth after transplant into ambient CO_2 conditions. Thus, while biomass does increase during the seedling stage as a result of CO_2 enrichment, there is little lasting benefit upon transplant into the greenhouse.

3.6 Conclusion

The responses of plants to elevated CO_2 in agronomic and environmental situations is well-known; however, species-specific guidelines for horticultural crop production in controlled environments are limited. In the present study, elevated CO_2 was found to increase biomass without increasing plant size, producing sturdier and more robust pansy and petunia plugs. Additionally, similar plug quality was attained regardless of whether the environment was enriched with CO_2 for the entire production period or short-term at the end of the propagation stage, limiting the benefit of long-term CO_2 injection. Therefore, based on the present study, CO_2 enrichment is most beneficial during the final two weeks of propagation for the production of high-quality pansy and petunia seedlings. However, further research is required to fully evaluate

the effect of timing and duration for CO_2 enrichment and to develop species-specific best management practices to assist growers in managing this production input effectively.

Table 7. Morphological data for petunia (*Petunia ×hybrida* 'Dreams Midnight') seedlings including stem caliper, stem length, leaf number, leaf area (LA), root dry mass (RDM), leaf dry mass (LDM), shoot dry mass (TDM), and relative chlorophyll content (RCC) harvested 14, 21, and 28 d after germination.. Seedlings were grown in 128-cell plug trays using reach-in growth chambers with CO_2 concentration set points where Amb_{28} = grown 28 days at ambient CO_2 (400 µmol·mol^{-1}), Elv_{28} = grown 28 days at elevated CO_2 (1000 µmol·mol^{-1}), Amb_{14}:Elv_{14} = grown 14 days at ambient CO_2 then 14 days at elevated CO_2, Elv_{14}:Amb_{14} = grown 14 days at elevated CO_2 then 14 days at ambient CO_2.

CO_2 (µmol·mol^{-1})	**Caliper (mm)**	**Length (mm)**	**Leaf Number**	**LA (cm^2)**	**RDM (mg)**	**LDM (mg)**	**TDM (mg)**	**RCC**
				Day 14				
Amb_{28}	0.92±0.03[z]	5.63±0.20	4.9±0.15	2.66±0.23	1.60±0.16	7.30±0.60 b	9.69±0.77	30.6±0.61
Elv_{28}	0.96±0.03	5.43±0.22	4.7±0.14	2.52±0.16	1.50±0.17	8.24±0.60 a	10.57±0.80	31.9±0.69
				Day 21				
Amb_{28}	1.24±0.04	8.74±0.50	7.8±0.24	10.23±1.14	9.46±1.25	36.29±3.57 bc	48.85±5.18 ab	32.0±0.82
Elv_{28}	1.26±0.02	9.20±0.39	7.7±0.25	10.65±1.32	10.44±1.78	43.49±5.04 a	57.57±7.35 a	33.0±1.24
Amb_{14}:Elv_{14}	1.28±0.03	8.90±0.33	7.6±0.17	9.77±1.02	9.30±1.22	40.67±3.79 ab	52.98±5.33 ab	33.2±1.47
Elv_{14}:Amb_{14}	1.24±0.03	9.10±0.42	7.4±0.19	9.62±0.92	8.70±1.30	32.67±3.05 c	44.77±4.72 b	33.1±1.06
				Day 28				
Amb_{28}	1.51±0.05	13.9±0.49	10.0±0.33	18.63±1.61	24.37±2.59 b[y]	88.99±9.20 b	123.50±12.82 c	30.7±1.01 b
Elv_{28}	1.61±0.04	15.2±0.60	9.3±0.28	20.59±1.82	31.27±3.65 ab	117.52±9.51 a	163.44±14.94 ab	32.1±1.76 b
Amb_{14}:Elv_{14}	1.62±0.07	13.8±0.40	9.4±0.31	20.39±1.47	33.94±5.03 a	118.39±11.79 a	166.17±18.67 a	36.0±1.12 a
Elv_{14}:Amb_{14}	1.60±0.05	14.2±0.53	9.75±0.26	19.51±1.39	26.62±3.12 ab	94.71±8.09 b	132.11±12.14 bc	33.8±1.06 ab

[z]Mean values are based on five samples from each treatment across three experimental repetitions (n=20) [y]Means sharing a letter across CO_2 treatments are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant differences between treatments.

Table 8. Morphological data for pansy (*Viola ×wittrockiana* 'Matrix Blue Blotch Improved') seedlings including stem caliper, stem length, leaf number, leaf area (LA), root dry mass (RDM), leaf dry mass (LDM), shoot dry mass (TDM), and relative chlorophyll content (RCC) harvested 14, 21, and 28 d after germination.. Seedlings were grown in 128-cell plug trays using reach-in growth chambers with CO_2 concentration set points where Amb_{28} = grown 28 days at ambient CO_2 (400 µmol·mol^{-1}), Elv_{28} = grown 28 days at elevated CO_2 (1000 µmol·mol^{-1}), Amb_{14}:Elv_{14} = grown 14 days at ambient CO_2 then 14 days at elevated CO_2, Elv_{14}:Amb_{14} = grown 14 days at elevated CO_2 then 14 days at ambient CO_2.

CO_2 (µmol·mol^{-1})	**Caliper (mm)**	**Length (mm)**	**Leaf Number**	**LA (cm^2)**	**RDM (mg)**	**LDM (mg)**	**TDM (mg)**	**RCC**
				Day 14				
Amb_{28}	0.74±0.03[z]	8.6±0.34	3.8±0.09	3.36±0.28	2.87±0.21 b	11.1±0.85 b	15.2±1.05 b	30.9±0.57
Elv_{28}	0.79±0.02	8.0±0.37	3.7±0.08	3.48±0.28	4.13±0.29 a	14.8±1.03 a	20.4±1.32 a	31.4±0.38
				Day 21				
Amb_{28}	1.06±0.04	13.1±0.56	5.3±0.15	9.84±0.35	8.91±0.58 b	43.0±2.18	56.0±2.75 b	38.2±0.75
Elv_{28}	1.15±0.04	14.3±0.73	5.3±0.19	9.74±0.82	13.62±1.58 a	51.6±3.83	70.5±6.28 a	41.1±1.75
Amb_{14}:Elv_{14}	1.09±0.03	13.4±0.64	5.1±0.21	8.31±0.38	9.77±1.38 b	45.1±3.57	58.7±4.74 ab	38.1±1.12
Elv_{14}:Amb_{14}	1.07±0.04	13.9±0.50	5.1±0.19	9.34±0.63	10.51±1.30 ab	41.6±3.56	56.5±5.13 ab	37.8±1.31
				Day 28				
Amb_{28}	1.21±0.03	15.9±1.03 b[y]	7.3±0.26 b	14.91±0.77	25.0±1.47 c	92.5±4.65 b	129.89±6.43 c	36.1±1.31 b
Elv_{28}	1.37±0.05	22.6±0.90 a	8.4±0.26 a	16.63±0.58	43.8±2.19 a	126.8±8.34 a	192.66±14.90 a	39.8±1.22 a
Amb_{14}:Elv_{14}	1.25±0.04	17.5±0.87 b	7.3±0.36 b	14.42±0.94	41.7±2.25 ab	114.7±9.07 ab	174.84±13.71 ab	38.8±1.07 ab
Elv_{14}:Amb_{14}	1.19±0.04	18.1±1.21 b	7.0±0.46 b	16.0±0.92	30.7±4.88 bc	103.9±3.27 ab	152.28±4.81 bc	40.6±0.79 a

[z]Mean values are based on five samples from each treatment across three experimental repetitions (n=20) [y]Means sharing a letter across CO_2 treatments are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant differences between treatments.

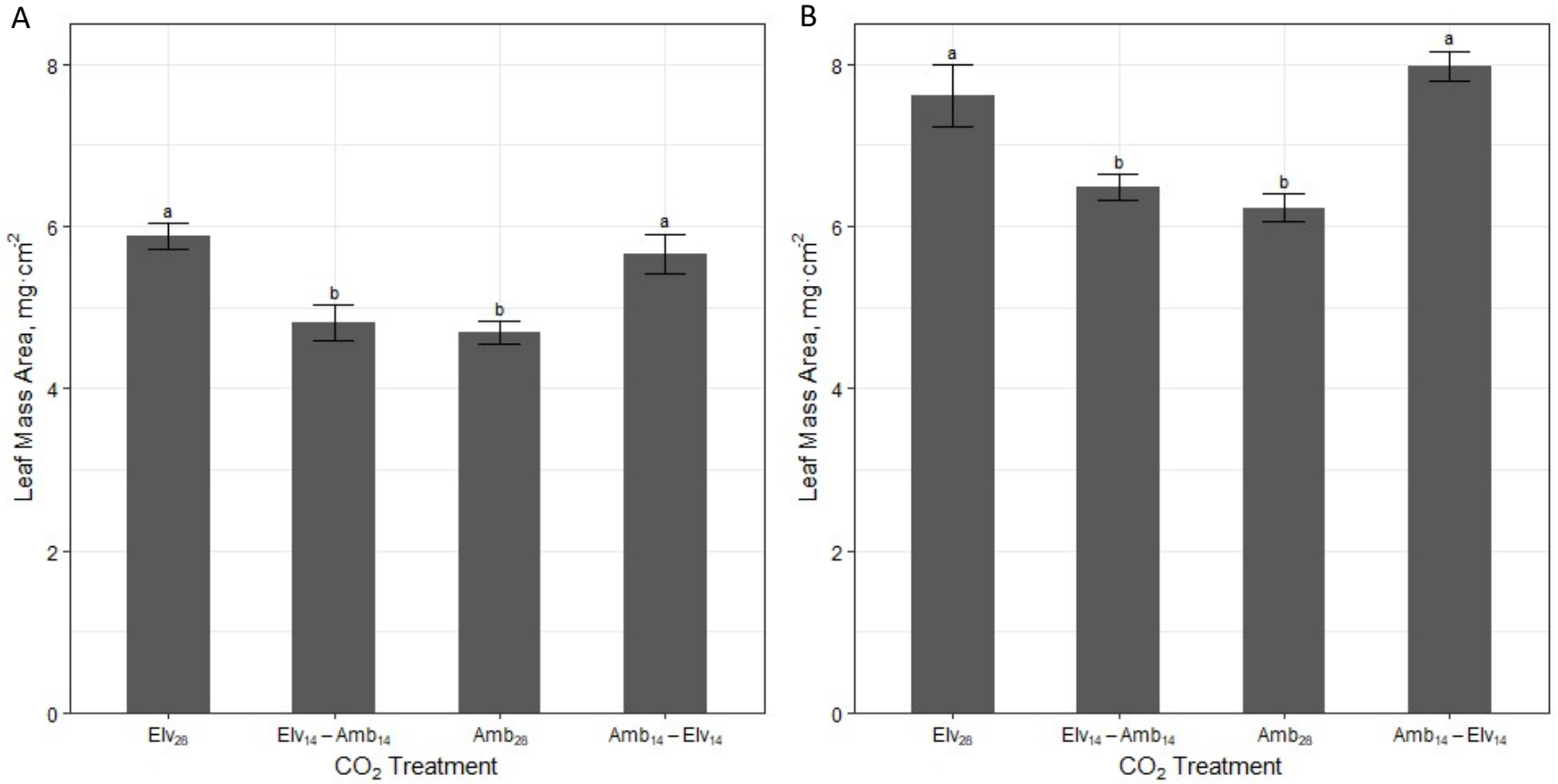

Figure 1. Leaf mass area (LMA) for petunia (**A**, Petunia ×hybrida 'Dreams Midnight') and pansy (**B**, Viola ×wittrockiana 'Matrix Blue Blotch Improved') collected 28 d after germination Seedlings were grown in 128-cell plug trays using reach-in growth chambers with CO_2 concentration set points where Amb_{28} = grown 28 days at ambient CO_2(400 µmol·mol^{-1}), Elv_{28} = grown 28 days at elevated CO_2(1000 µmol·mol^{-1}), Amb_{14}:Elv_{14} = grown 14 days at ambient CO_2 then 14 days at elevated CO_2, Elv_{14}:Amb_{14} = grown 14 days at elevated CO_2 then 14 days at ambient CO_2. Mean values are based on five samples from each treatment across three experimental repetitions (n=15). Means sharing a letter across CO_2 treatments are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$.

Table 9. Morphological data including days to flower (DTF), average plant width, average plant height, and shoot dry mass for Petunia (*Petunia ×hybrida* 'Dreams Midnight') and Pansy (*Viola ×wittrockiana* 'Matrix Blue Blotch Improved') collected at time of individual initial fully reflexed flower. Seedlings were grown in 128-cell plug trays using reach-in growth chambers with CO_2 concentration set points where Amb_{28} = grown 28 days at ambient CO_2 (400 µmol·mol^{-1}), Elv_{28} = grown 28 days at elevated CO_2 (1000 µmol·mol^{-1}), Amb_{14}:Elv_{14} = grown 14 days at ambient CO_2 then 14 days at elevated CO_2, Elv_{14}:Amb_{14} = grown 14 days at elevated CO_2 then 14 days at ambient CO_2. Plants were then transplanted into 11.4 cm (550 ml) containers to continue growth in a common greenhouse environment until initial flower.

CO_2 (µmol·mol^{-1})	**DTF**	**Width (cm)**	**Height (cm)**	**SDM (g)**
		Petunia		
Amb_{28}	17.05±3.52[z]	19.8±5.47	11.25±1.25	1.95±1.08
Elv_{28}	17.70±3.91	20.98±5.53	11.60±2.19	2.24±1.18
Amb_{14}:Elv_{14}	16.40±3.66	19.95±3.97	11.00±2.20	1.89±0.91
Elv_{14}:Amb_{14}	17.25±3.48	20.88±5.66	11.15±2.06	2.14±1.11
		Pansy		
Amb_{28}	20.40±3.76 a[y]	16.00±2.95	9.55±1.47	1.41±0.64
Elv_{28}	17.05±2.26 b	15.15±2.50	9.50±1.28	1.10±0.39
Amb_{14}:Elv_{14}	19.75±2.88 a	15.53±2.55	9.65±1.18	1.24±0.47
Elv_{14}:Amb_{14}	18.70±2.52 ab	15.63±2.81	8.70±1.46	1.21±0.56

[z]Mean values are based on five samples from each treatment across three experimental repetitions (n=20) [y]Means sharing a letter across CO_2 treatments are not statistically different by Tukey's honest significant difference (HSD) test at $P \leq 0.05$. Means with no lettering were found to have no significant differences between treatments.

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