

EFFECTS OF ELEVATED CARBON DIOXIDE ON
ORNAMENTALS AND LEAFY GREENS GROWN IN A
GREENHOUSE

By

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Title of Study: EFFECTS OF ELEVATED CARBON DIOXIDE ON ORNAMENTALS AND LEAFY GREENS GROWN IN A GREENHOUSE

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Abstract: Carbon dioxide, being a major input for photosynthesis, plays an important role in plant production and propagation. To understand how elevated CO₂ affects horticultural crops under greenhouse production, three studies were initiated: 1) pot production of ornamentals under varying irrigation and fertilizer levels, 2) propagation of an ornamental shrub, and 3) production of leafy greens in a hydroponics system. Each of the studies was conducted in a split-plot design in which one of the greenhouses was supplemented from morning to mid-day with CO₂ to achieve an average concentration of 800 ppm and the other greenhouse was maintained under ambient conditions (400 ppm). The studies were conducted in spring and summer of 2016 and 2017.

Container grown geranium and fountain grass showed greater growth rate under elevated CO₂ in 10 or 15 cb irrigation than 5 cb when greater rate of fertilizer (6 or 9 g) was used. Elevated CO₂ delayed flowering in geraniums but showed no effect in fountain grass. Since, species-specific response has been reported, grower needs to be cautious before CO₂ supplementation if early flowering is desired. Propagation of *Euonymus* 'Moonshadow' through cutting in elevated CO₂ had no effect in rooting percentage, reduced root number, and promoted early leaf senescence. However, 5000 ppm concentration of rooting hormone containing 1.5% IBA and 0.5% NAA showed greater root numbers compared to other treatment in ambient condition. Thus, elevated CO₂ might not be effective in promoting rooting of *Euonymus* cuttings.

Basil, lettuce, and Swiss chard produced in a hydroponics system under elevated CO₂ were larger and achieved a marketable size earlier compared to similar plants grown under ambient conditions. However, elevated CO₂ also resulted in leaf tipburn for lettuce, which might affect the market quality. On the other hand, leafy greens in elevated CO₂ showed lower nitrogen (N) and phosphorous content compared to ambient condition. Since N is an essential component of many proteins and amino acid, this might affect the nutritional quality of crop. Thus, there is a necessity for more extensive studies with greater treatment combinations to produce nutritious crops in hydroponic at elevated CO₂.

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CHAPTER I

REVIEW OF LITERATURE

Introduction

The rapid increase in the atmospheric concentration of greenhouse gases, produced through anthropogenic activities, has received a lot of attention lately. These gases have the ability to trap solar radiation and increase temperature on earth. Among all greenhouse gases, lately carbon dioxide (CO₂) has received an increased interest because of its importance in global warming and role in photosynthetic activity in plants (Tyree and Alexander, 1993). Up to the 1960s, the earth's atmosphere was believed to be composed of 0.03% (300 PPM) CO₂ (Wittwer and Robb, 1963). However, with the industrial development and burning of fossil fuels, 1-2 ppm CO₂ is being added each year and the current CO₂ level of 400 ppm will be doubled within the end of this century (Ruiz-vera et al., 2017).

Photosynthesis is a complex bio-chemical process of biomass addition in plants (Sestak et al.1985). Kadam et al. (2012) describes CO₂ as an essential component of photosynthesis. Under optimal growth conditions, photosynthesis is generally limited by unavailability of CO₂ in C₃ plants (Arp, 1991). Increasing ambient CO₂ level can enhance

photosynthesis in most plants but may depend on the type of photosynthetic pathway, as different pathways have different mechanisms for photosynthetic response (Ehleringer and Monson, 1993). Light level and temperature changes with changing weather and climate. For example, light level and temperature goes down in winter resulting in reduced photosynthesis and limiting plant growth. However, the temperature as well as light level is higher in summer and this value changes year to year and season to season (Hegseth and Sakshaug, 1983). Thus, plants have acclimatized to different levels of light and temperature over time by modifying their morphology and by balancing the source-sink relation (Bjorkman, 1981). However, CO₂ level in the atmosphere had remained fairly constant for a long period of time. Because of the constant CO₂ level in the atmosphere, it is really hard to predict how plants will acclimatize to the elevated CO₂ concentration (Arp, 1991). Direct quantification of physiological and phenological responses of plants to elevated CO₂ is difficult (Wolfe and Erickson, 1993). Quantification involves many assumptions and is affected by factors like light, nutrient, temperature, growing environment, water availability, and insect and disease presence.

CO₂ supplementation for greenhouse ornamentals production

For over 200 years, the benefits of supplemental CO₂ in plant growth have been known (Mortensen, 1987). However, the interest and need for research on supplemental CO₂ studies has risen and fallen over time. In the initial years of CO₂ application, CO₂ was supplemented through decomposition of soil rich in organic matter (Nederhoff, 1994a). Later CO₂ was supplemented as pure forms in cylinders, or produced through combustion of fossil fuel (Hand, 1982; Slack and Hand, 1986). According to Nederhoff (1994a), either a small CO₂ burner in the middle of a greenhouse or big flu gas generator,

which can maintain heat as well as CO₂, can be used in greenhouses. But incomplete combustion and impurities in hydrocarbon compounds (kerosene or propane) might produce harmful gases like ethylene, carbon monoxide, sulfur dioxide, and nitrogen compound, which could be toxic to plants. However, after 1970s, significant improvement in technology has resulted in production of CO₂ without impurities and even if produced, monitoring of harmful gases has become easy with development of different sensors (Moe and Mortensen, 1986). After the commercial release of infra-red CO₂ sensors in 1979, application of supplemental CO₂ in European greenhouse increased extensively (Nederhoff, 1994b). Mortensen and Ulsaker (1985) recommended 900 ppm of CO₂ for optimal plant growth in many species and any level beyond this showed a negative effect in crops. For many species, most of the experiments have been conducted at either 335, 500, or 1500 ppm of CO₂. Thus, it is impossible to recommend an exact level for optimum growth (Mortensen, 1987).

Generally, the beneficial effect of supplemental CO₂ has been observed in plants when supplied at a constant rate from sunrise to sunset in non-ventilated greenhouse (Mortensen, 1987). Since solar radiation, and thereby photosynthesis, increases from dawn to solar-noon before gradually decreasing, some have reported supplementation of CO₂ up to mid-day (Nederhoff, 1994; Sanchez-Guerro et. al., 2005) In some cases, it is not feasible to supplement CO₂ for an entire day. That is, greenhouse ventilation is often required to moderate temperature. Supplementing CO₂ in such an open system would result in tremendous inefficiency and the potential for diminished economic benefit of the practice (Sanchez-Guerro et. al., 2005; Mortensen, 1987). However, Nederhoff (1994a) recommended CO₂ supplementation even during ventilation, so that CO₂ level in the

greenhouse remains above the outside level when the greenhouse is closed mostly in the morning and late afternoon.

According to Croonenborghs et al. (2009), CO₂ studies in horticultural plants have focused mainly on field grown shrubs, trees, and greenhouse grown vegetables and only a few of the studies have reported effects in greenhouse grown ornamentals. Studies in impatiens (*Impatiens hawakeri* Bull) (Zhang et. al., 2012), pansy (*Viola × wittrockiana* DC.) (Niu et. al., 2000), chrysanthemum (*Chrysanthemum morifolium* Ramet.) (Kuehny et al., 1991), begonia (*Begonia x hiemalis* Fotsch.) (Mortensen and Ulsaker, 1985), ornamental bromeliads (*Aechmea fasciata* Baker) (Croonenborghs et al., 2009) and many other ornamental species have shown a positive response to elevated CO₂. Springer and Ward (2007) reviewed how CO₂ affects flowering in ornamental plants and concluded that there is a species-specific response to elevated CO₂. Doubling the ambient CO₂ concentration has been reported to increase photosynthesis in geranium (*Pelargonium hortorum* Bailey) (Kelly et al., 1990) and gladiolus (*Gladiolus* L. ‘American Beauty’) (Kadam et al., 2012), reduced stomatal conductance and transpiration in parsley (*Petroselinum crispum* (Mill) Fuss), (Petropoulos et al., 2008), accelerated flowering in petunia (*Petunia hybrid* Juss.) and enhanced plant quality and flower in gerbera (*Gerbera jamesonii* L.) (Bakrel, 1982). However, negative effects like delayed flowering in short-days plant species (Reekie and Hicklenton, 1994), and pale green leaf color and reduced flowering quality in bromeliads (Croonenborghs et al., 2009) were reported under elevated CO₂. Since the response to elevated CO₂ is species-specific, generalization across species, genus, or family might result in an incorrect assumption (Croonenborghs et. al., 2009).

Carbon dioxide enrichment and effect on plant growth

The concept of CO₂ supplementation for plant growth is not a new topic. A study of history reveals that the effect of CO₂ enrichment on plant growth has been studied since the early 19th century (Enoch and Olesen, 1993). Before 1930, many studies claimed CO₂ in soil and atmosphere play an equal role in plant growth. However, after the 1930s most CO₂ enrichment studies have been based on atmospheric supplementation, as plants obtain 95-99% CO₂ from the air (Enoch and Olesen, 1993).

Theoretically, a rise in CO₂ level in the atmosphere can enhance photosynthesis in plants. Doubling of the current CO₂ level generally enhances C₃ photosynthesis by 40-45% and 10-20% in C₄ plants (Ghannoum et al., 2000). In ambient CO₂, rubisco is substrate limited in C₃ plants and shows greater response to elevated CO₂ resulting in a higher rate of photosynthesis (Long et al., 2004). However, C₄ plants can be substrate saturated even at a low level of CO₂. Like the enzyme rubisco in C₃ plants, PEP-carboxylase in C₄ plants is responsible for CO₂ fixation. Since this enzyme can saturate at low CO₂ levels, increasing the CO₂:O₂ ratio in the atmosphere has a reduced effect on photosynthesis (Lara and Andreo, 2011). Photorespiration (preference of O₂ rather than CO₂ by rubisco in light) is generally higher in C₃ plants than C₄ plants and increases with increasing temperature. Supplemental CO₂ increases the CO₂:O₂ ratio and reduces photorespiration, thus enhancing photosynthesis and net carbon assimilation (Lara and Andreo, 2011; Sage, 1998).

Effect of elevated CO₂ and temperature

Studies have shown positive, negative, or no interaction between increasing temperature and CO₂ conditions (Lara and Andreo, 2011). According to Reddy et al. (2005), photosynthesis in cotton (*Gossypium hirsutum* L.) decreased when moving from 31 °C to 36 °C at ambient CO₂ levels. However, when exposed to 700 ppm, photosynthesis increased linearly by 22% at 26 °C and 54% at 36 °C. Thus, elevated CO₂ might ameliorate the effect of higher temperature and increase optimum temperature requirement of plant. Similarly, doubling ambient CO₂ concentration in lamb's quarters (*Chenopodium album* L.) increased photosynthesis at 23 °C and 34 °C by 31 and 51%, respectively (Sage and Grise, 1996). In contrast, Sage et al. (1995) and Long (1991) reported that the phenomenon of increased photosynthesis with increased temperature at elevated CO₂ condition is not universal. Increase in temperature at elevated CO₂ condition enhanced photo-inhibition in two species of eucalyptus (*Eucalyptus macrorhyncha* Muell and *E. rossii* Baker and Sm) (Roden and Ball, 1996). Different species show different photosynthetic response to an increase in temperature at elevated CO₂. A study in rice (*Oryza sativa* L.) and soybean (*Glycine max* (L.) Merr) reported a decrease in photosynthesis in rice and an increase for soybean when temperature was raised from 32 °C to 40 °C at double the ambient CO₂ condition (Vu et al., 1997).

Photosynthesis is a thermos-sensitive chemical reaction (Lara and Andreo, 2011). The difference in photosynthetic response of different species at higher temperature may be due to different optimal temperature requirements of crops and an interactive effect of high temperature and elevated CO₂ (Bunce, 1998). Many studies suggest downregulation of rubisco and decreased photosynthesis at elevated temperature (Kim and Portis, 2005;

Sage et al., 1989; Salvucci and Crafts-Brandner, 2004; Salvucci and Crafts-Brandner, 2004). Rubisco is temperature sensitive and elevated temperature inhibits functioning of rubisco activase. This is the enzyme responsible for binding CO₂ in C₃ plants, thus down-regulation significantly reduce the rate of photosynthesis (Parry et al., 2008). The elevated CO₂ compensates for the downregulation of rubisco and makes a consistent ratio of ribulose-1,5-bisphosphate (RuBP) regeneration and RuBP consumption even at elevated temperature (Cen and Sage, 2005). As a result, increasing CO₂ could ameliorate the downregulation of rubisco by increasing optimum growing temperature of a plant (Reddy et al., 1998).

Elevated CO₂ × water interaction in plants

Water availability plays a significant role in plant performance. Exposure of plants to an extensive period of drought affects photosynthesis, transpiration, stomatal conductance, and production of biomass (Win et al., 2015; Zhao et al., 2013). Extensive drought in smooth cordgrass (*Spartina alterniflora* Loisel.) showed a breakdown and inhibition of chlorophyll activities in cells (Brown and Pezeshki, 2007). Exposure to a double ambient CO₂ concentration has been reported to decrease stomatal conductance in C₃ and C₄ plants (Ainsworth and Rogers, 2007; Reddy et al., 2005). C₃ and C₄ plants exhibited 20-35% reduction in stomatal conductance because of partial stomatal closure at elevated CO₂ (Ainsworth and Long, 2005; Ainsworth and Rogers, 2007; Wand et al., 1999). A meta-analytic review of Ainsworth and Long (2005) reported a 15% reduction in stomatal conductance in no stress condition in 40 plant species and even higher, up to 29% in low soil N and water stressed conditions.

Mott (1988) stated that guard cells are more sensitive to the intercellular CO₂ concentration rather than external CO₂ concentration. Elevated CO₂ affects the ion and solute concentration, which is mediated for turgor pressure inside the guard cells of the stomata. This turgor pressure is solely responsible for closing and opening of stomatal aperture. The turgidity of guard cells increases with increasing CO₂ concentration, which enforces stomatal closure and reduces stomatal conductance (Ainsworth and Rogers, 2007). Decrease in stomatal conductance at elevated CO₂ is because of partial stomatal closure, which leads to a decrease in rate of transpiration too. Since transpiration is the mechanism for adjusting leaf temperature, reduction in stomatal conductance and transpiration increases leaf temperature. However, increased leaf area and raise in leaf temperature might counter the effect. Increased leaf temperature causes an increase in vapor pressure gradient between air and leaf surface, which further enhance transpiration (Kimball, 2010; Reddy et al., 2005; Serraj et al., 1999).

Water stress is a major problem in ornamental crops causing loss of aesthetic value and crop failure. Crop loss is due to a reduced number of tillers, spikes, flowers, and quality (Farooq et al., 2009). Plants show a long term and short term response to water stress. Short term responses of plants g include reduced stomatal conductance along with reduced transpiration (Osakabe et al., 2014). The most prominent effect of water stress is on photosynthesis. Stomatal closure, stimulated via water stress, affects the rate of photosynthesis because of a decreased internal CO₂ concentration (Chaves et al., 2009; Flexas et al., 2006). Reduced cell growth and biomass was reported in potato (*Solanum tuberosum* L.) and parsley (*Petroselinum crispum* (Mill.) Fuss) (Heuer and Nadler, 1995; Petropoulos et al., 2008) under water stressed condition. Reduced cell

growth leads to reduced leaf area resulting in a lower rate of photosynthesis (Ren et al., 2007; Wilkinson and Davies, 2010).

Thus, growth limited by low water availability could be compensated with increased photosynthesis under an elevated CO₂ concentration in water-limited condition. Increase in leaf area, dry weight, and carbohydrate level under elevated CO₂ have been reported under water stressed condition (Kimball, 1983; Reddy et al., 1995). Reddy et al. (2005) reported increased water use efficiency (WUE) in cotton at double ambient CO₂. However, Wolfe and Erickson (1993) stated that WUE of water-stressed plants at double ambient CO₂ may seem higher initially. Increase in leaf area and root volume because of elevated CO₂ might lead to greater water uptake. Because of increased growth rate under elevated CO₂, plants grown under elevated CO₂ concentration may face severe water stress at later growth stages than the plants grown under ambient CO₂ concentration. Similar responses were reported in other studies (Prior et al., 1997; Rogers et al., 1992).

Elevated CO₂ and plants nutrient requirement

Many studies have shown that higher nutrient levels increase plant productivity for both field and greenhouse grown plants. With the increase in CO₂ concentration, rate of photosynthesis increases, which leads to increase in root and shoot growth promoting more water uptake. The demand for nutrients increases with respect to plant growth (Stitt and Krapp, 1999). Among all nutrients, nitrogen (N) will be limited over time and limits growth under elevated CO₂ condition (Del Pozo et al., 2007; Newton, 1991). Indirectly, enzymes present in thylakoids and the Calvin cycle represent almost all protein content in plants and N is an essential component of these proteins (Taiz and Fizeiger, 2002). In

elevated CO₂ and N limited conditions, plants become unable to utilize all the photo-assimilates due to downregulation of these proteins (Ainsworth et al., 2003).

Although interaction studies of supplemental CO₂ and different nutrient concentrations are available, most of the studies are conducted in food crops. Studies in rice (*Oryza sativa* L.) (Bannayan et al., 2005; Makino et al., 1997; Nakano et al., 1997), safflower (Mohamed et al., 2013), and lentil (Nasser et al., 2008) reported low leaf N in plants under elevated CO₂ condition. Different hypotheses have been developed to explain the causes of lower leaf N levels. According to Kant et al. (2012), excess carbohydrate accumulation dilutes N in plant tissue at elevated CO₂ condition, thus reduces N level. The authors also stated that, low transpiration pulls results in reduced nutrient uptake. Because of reduced nutrient uptake, plants show N deficiency in their leaves. Further, low N availability in the soil and reduced demand of N because of down regulation of rubisco may affect tissue N level (Kant et al., 2012). Arp et al. (1998) found that the difference in leaf N content at ambient and elevated CO₂ condition depends on the level of N in the soil. Lower soil N leads to lower tissue N, and the available tissue N cannot redistribute further for increasing rate of photosynthesis. However, at maximum or suboptimal N level in the soil, redistribution of leaf N occurs which still causes the leaf N levels to remain low but leads to an increase in photosynthesis (Arp et al., 1998). The difference in leaf N content between ambient and elevated CO₂ condition may range from 10-15%. Although leaf N content is reduced at an elevated CO₂ condition, a 31% increase in photosynthetic N use efficiency (PNUE) was seen across 15 species (Leakey et al., 2009) and 50% increase in sunflower (*Helianthus annuus* L.) (Zerihun et al., 2000) has been reported. Nitrogen deficiency might lead to a lower sink capacity to store excess

photoassimilates in ambient CO₂ resulting in reduced plant growth. Thus, elevated CO₂ helps in ameliorating the negative effect of N deficiency by improving PNUE and strengthening the sink capacity (Leakey et al., 2009; Zerihun et al., 2000).

CO₂ application in hydroponics

From the early 19th century, soilless culture has been in practice either in the form of research trials or small scale production. Overtime, different methods of soilless culture like aeroponics, hydroponics, rockwool culture, vermiculite culture, and sand/gravel culture were developed (Maxwell, 1986). Bradley and Marulanda (2000) described hydroponics as a soilless technique of growing plants in water. Today hydroponics is becoming popular because of year round production in a controlled environment (Both et. al., 1997) and 10 times greater growth rate compared to conventional field systems (Resh, 2012).

Both (1995) stated that year round production of leafy greens requires control of environmental factors, like supplemental light in the winter and shade in summer are crucial for greater plant growth. Supplemental CO₂ can be as beneficial as supplemental lighting to increase yield of crops (Mortenesen, 1987). In autumn, spring, and winter, when the vent system is closed and ambient CO₂ concentration goes down, supplemental CO₂ could have a beneficial effect (Willits and Peet, 1989). Many authors have reported an increase in growth of lettuce by 30% with supplemental CO₂ in hydroponics (Both et. al., 1997; Willits & Peet, 1989).

Similarly, fresh weight of spinach (*Spinacea oleracea* L.) and basil (*Ocimum basilicum* L.) were significantly greater at 1500 ppm CO₂ than at ambient conditions.

Increased photosynthesis and decreased transpiration has been reported in hydroponic peanut (*Arachis hypogea* L.) (Stanciel et. al., 2000). Thus, this reduced transpiration may further reduce total water use during entire crop production. Generally, 1000-1500 ppm of CO₂ has shown to be beneficial in leafy greens (Chalabi et. a., 1997); however, interveinal chlorosis in basil, due to accumulation of large size starch grain, has been observed at 1500 ppm CO₂.

Carbon dioxide and root growth

Carbohydrates are considered as energy for adventitious root development. Thus, research has attempted to promote rooting through the treatments associated with increasing carbohydrate supply in plants, like supplemental CO₂ and light (Davis and Potter, 1983; French, 1990). According to Obrist and Arnone Iii (2003), an increase in atmospheric CO₂ generally increases root biomass, shoot biomass, and leaf area. Apparently, raising CO₂ often leads to an increase in root growth, regardless of plant species (Rogers et al., 1992; Wolfe and Erickson, 1993). Supplemental CO₂ in greenhouse raised the percentage of cuttings in many horticultural crops (French, 1989; Prokaj et al., 2006). Supplemental CO₂ increased root number and length in propagation of sweet potato (*Ipomea batatas* (L.) Lam.) (Bhattacharya et al., 1985), cuttings of blueberry (*Vaccinium corymbosum* L.) (Prokaj et al., 2006), and cuttings of *Rhododendron* ‘Anna Rose Whitney’ (French, 1990). Similarly, cuttings of leafy pea (*Pisum sativum* L.) and soybean (*Glycine max* (L.) Merr.) showed an increased carbohydrate content and root length but not the root number in the CO₂ enriched treatment (Davis and Potter, 1989).

Although many studies claim better root initiation because of accumulated carbohydrates in CO₂ elevated condition, several study claimed no relation of stem carbohydrate levels in rooting. According to Davis and Potter (1987), carbohydrate levels in some cultivars of *Rhododendron* L. before and during propagation might affect rooting. However, the effect of carbohydrate level in stems of *R.* 'Roseum Elegans' on rooting was very little. Similarly, propagation of two Australian ornamental plants, geraldton wax (*Chamelaucium uncinatum* Schaver. × *Chamelaucium floriferum* Ms.) and correa (*Correa schlechtendalii* Behr.) showed increased carbohydrate levels under CO₂ enriched condition (Grant et al., 1992). Rooting in different species showed no relation of accumulated carbohydrates in rooting, thus CO₂ supplementation could inhibit rooting or have no significant effect. French (1989) reported a negative effect of supplemental CO₂ in rooting of rhododendron when propagated in fall. The variation in effect of supplemental CO₂ in rooting might be because of a difference in species and environmental condition. Beside these, rooting hormone, concentration, and media temperature also played an important role in rooting of pecan (*Carya illinoensis* (Wangenh.) K. Koch.) cuttings (Zhang et al., 2015).

Research goal and objectives

The general objective of this research is to understand the effect of CO₂ supplementation through burning of natural gas in greenhouse production systems.

The specific objectives of this research are:

1. To determine suitable irrigation and fertilization regimes for pot grown geranium and fountain grass under supplemental CO₂ condition.
2. To understand the effect of supplemental CO₂ on greenhouse grown leafy greens in hydroponics system.
3. To understand the effect of supplemental CO₂ and commercial rooting hormone product in rooting of *Euonymus* cuttings.

Hypothesis

1. Well watered geranium and fountain grass will require higher rate of fertilizer to proportionate greater growth rate under elevated CO₂.
2. Leafy greens grown in hydroponics will perform better in supplemental CO₂ condition.
3. Rooting of ornamental cuttings will improve through application of supplemental CO₂ alone, rooting hormone alone, or combine effect of CO₂ and rooting hormone.

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CHAPTER II

IRRIGATION AND FERTILIZATION MANAGEMENT IN GREENHOUSE ORNAMENTALS UNDER ELEVATED CO₂

Abstract

Multiple environmental factors affect the response of plants to supplemental carbon dioxide (CO₂) concentration. To investigate how irrigation and fertilization requirements of ornamental crops change under elevated CO₂, a greenhouse study was conducted. One greenhouse was supplemented with CO₂ to maintain a daily average of 800 ppm during the supplemental period, and another greenhouse was kept at ambient conditions. Irrigation treatments of 5, 10 and 15 cb were maintained in each greenhouse using a tensiometer and had 0, 3, 6, or 9 g fertilizer treatments in each irrigation regime. Plugs of geranium and fountain grass obtained from commercial nursery were grown for 12 and 16 weeks, respectively. The results showed that elevated CO₂ increased the dry weight of geranium and fountain grass by 35% and 39%, respectively. Under the two driest irrigation regimes (10 and 15 cb), photosynthesis of geranium increased with elevated CO₂ compared to the ambient condition. Similarly, moderately watered (10 cb) fountain grass with greater fertilizer rate (6 or 9 g) had greater rate of photosynthesis and plants of both species were larger. Although elevated CO₂ resulted in a greater inflorescence count in both species, flowering was delayed in geranium. Water use efficiency of both species increased, but rate of transpiration was lower only in fountain increased, but rate of

transpiration was lower only in fountain grass at elevated CO₂. Either 6 or 9 g fertilizer treatment had greater values for most of the parameter in both species. Larger and compact plants could be grown under elevated CO₂ with 10 or 15 cb irrigation at greater fertilizer rate. However, this data may not be sufficient to generalize ornamental plant responses to elevated CO₂, so future work should emphasize a wider range of irrigation, fertilizer, and CO₂ levels on different species.

Introduction

In the terrestrial ecosystem, carbon is added to green plants through fixing of atmospheric CO₂ by the process of photosynthesis. The amount of CO₂ present inside a plant leaf (internal CO₂) plays a significant role in the rate of photosynthetic assimilation, which depends upon available CO₂ surrounding the plant (Nederhoff, 1994a). Each year the level of atmospheric greenhouse gases increases globally. Among the greenhouse gases, CO₂ is getting a lot attention, because of its direct impact on global food production (Tyree and Alexander, 1993). Every year one to two parts per million (ppm) of CO₂ is being added in the atmosphere through human and anthropogenic activities and scientists believe that the current CO₂ level of 400 ppm might double by the end of this century (Frantz, 2011; Hughes and Bazzaz, 2001). Although the global CO₂ level is increasing, sealed or unventilated greenhouse in winter show a relatively lower CO₂ concentration. Slack and Hand (1985) reported that 200 ppm CO₂ concentration in unventilated condition significantly reduced growth and yield of cucumber (*Cucumis sativus* L.). Thus, to provide the optimum growing condition for vegetables and ornamental plants, Mortensen (1987) and Nederhoff (1994a) suggested the use of supplemental CO₂ under sealed and non-ventilated greenhouses. Lara and Andreo (2011)

stated that carboxylation enzyme (rubisco in C₃ plants) have an equal affinity to bind with either CO₂ or O₂ in their binding sites. If carboxylation enzyme binds with O₂, photorespiration occurs resulting in reduced net photosynthesis. However, the enzyme PEP-carboxylase in C₄ plants binds with HCO₃⁻ instead of CO₂. Thus, the authors reported that the response of C₃ species to elevated CO₂ is greater compared to C₄ species. Thus, to provide the optimum growing condition for vegetables and ornamental plants, Mortensen (1987) and Nederhoff (1994) suggested the use of supplemental CO₂ under sealed and non-ventilated greenhouses.

Carbon dioxide supplementation or CO₂ fertigation is a process of adding CO₂ into the plant growing environment for the purpose of increasing plant photosynthetic efficiency and growth. The source of CO₂ in greenhouse supplementation systems is typically a hydrocarbon compound that when burned releases CO₂ and into the environment (Laumb et al., 2013). For example, common sources can include natural gas or other petroleum products. Ghannoum et al. (2000) reported that doubling the ambient CO₂ level could enhance photosynthesis by 40-45% and 10-20% in C₃ and C₄ plants, respectively. Generally, CO₂ level of 600-900 ppm is desirable for greenhouse crop production (Arp et al., 2000; Sanchez-Guerro et al., 2009). Since photosynthesis peaks early in the morning to late afternoon, many European commercial greenhouses use supplemental CO₂ during this period of time only (Sanchez-Guerro et al., 2005). Papenhagen (1983) reported that height, dry weight, and number of flowers were increased in geranium under elevated CO₂. Similarly, in a review of the effect of elevated CO₂ in greenhouse grown ornamentals and vegetables, Mortensen (1987) reported an increase in growth, decrease in maturity period, and early flowering of ornamental crops.

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

Water stress is a major problem affecting plant growth in the ornamental industry, which significantly affects plant size, flower number, and plant quality (Farooq et al., 2009). Under drought stress, plants stomatal aperture close down to regulate moisture loss resulting in a lower rate of transpiration. Thus, prolonged stomatal closure under drought stress may result in a lower internal CO₂ concentration and rate of photosynthesis might decrease resulting in decreased plant growth (Chaves et al., 2009; Flexas et al., 2006). The negative impact of drought stress could be ameliorated to some extent through supplemental CO₂. Morison and Gifford (1983) reported that the stomatal conductance of plants decreases under elevated CO₂. However, the level of internal CO₂ increases and net assimilation rate is either not affected or increased under elevated CO₂ (Mott, 1988). Along with increased photosynthetic rate under elevated CO₂, decrease in stomatal conductance results in a lower transpiration rate (Reddy et al., 2005). Because of this

increased photosynthesis and decrease in transpiration, water use efficiency increases (Kimball, 2010).

Similar to drought stress, soil nutrients also showed significant interaction effect with elevated CO₂ (Penuelas et al., 1995). A higher production of any crop under elevated CO₂ implies a higher nutrient demand by the crop (Sancez-Guerro et al., 2009). A positive effect of elevated CO₂ can be seen in nutrient stressed plants if leaching of nutrients is reduced, nutrient uptake is increased, or efficiency of the absorbed nutrient element is increased (Arp et al., 1998). It has been reported that growth rate of maize (*Zea mays* L.) was increased even in lower nitrogen level under elevated CO₂ (Hocking and Meyer, 1991). In nutrient stressed plants, nitrogen use efficiency of the plant increases under elevated CO₂, and growth of the plant is not affected in spite of lower nutrient level (Bowes, 1993). To proportionate the increased plant growth under elevated CO₂, growers usually overwater and put excess fertilizer to ensure a stress-free growing condition. As a result, more nutrients leach out from the media resulting in economic loss. According to Stanghellini et al. (2003), a greenhouse grower in Spain losses more than 50% of the applied fertilizer using open loop irrigation. Thus, application of adequate irrigation is crucial to conserve water as well as control nutrient leaching. Shock and Wang (2011) reported in a review that scheduling irrigation based on soil water tension is a powerful method to enhance plant production. The author also reported that a tensiometer is a cheap and an efficient tool for scheduling irrigation with greater efficiency and control of leaching. According to Lieth et al. (1990), 70-90% of irrigation water could be conserved through use of a tensiometer in greenhouse-potted plants. To our knowledge, no studies have been made in understanding the interaction effect of

elevated CO₂ with irrigation and fertilizer requirements of greenhouse grown ornamental plants. Thus, to understand how the interaction of elevated CO₂ with other environmental factors affects morphology and physiology of plants, a greenhouse study was conducted. The objective of the study was to evaluate the effect of elevated CO₂ on the fertilizer and irrigation requirement of geranium (*Pelargonium hortorum* L.H. Bailey) and fountain grass (*Pennisetum alopecuroides* L.).

Materials and Methods

Experimental Setup and Treatments:

The study was carried out at the Oklahoma State University horticulture research greenhouses in Stillwater, OK, in spring of 2016 and repeated in spring of 2017. The experiment was arranged as a split-plot design with 10 replications each year. Two identical greenhouses (whole main plots) were used in the experimental setup with one greenhouse fitted with a natural gas-burning CO₂ generator (Johnson Gas Appliance Company, Cedar Rapids, IA) in the middle of the house (Fig. 2.1 and appendix I). The generator was set to turn on from 6:00 a.m. to 2:00 p.m. for the first three months starting from 24 February in 2016 (first year experiment) and 20 February in 2017 (second year experiment) and then reduced to 6:00 a.m. to 12:00 p.m. at the end of growth cycle as temperatures increased causing the ventilation to come on at 8:00 a.m. instead of 10:00 a.m. Extech CO210 CO₂ Monitor (FLIR Commercial System Inc., Nashua, NH) monitored the CO₂ concentration along with temperature and humidity in both greenhouses.

Two more factorial treatments were applied in each CO₂ treatments. Three irrigation levels (sub-plots) of 5, 10, and 15 centibars (cb) were setup on three different benches using an automatic gauge on the tensiometer (IRROMETER, Riverside, CA). The volumetric water content in 5, 10 and 15 cb irrigation treatments were approximately 50, 40, and 30%, respectively (Fig. 2.2). The tensiometer was connected to a solenoid valve, which turned on the irrigation when the tensiometer reached the respective set point. The tensiometer was inserted at a depth of 10 cm in between the edge and center of one container for each irrigation level. The ‘tensiometer pot’ was located in the center of the bench. Irrigation was applied using drip emitters. Osmocote plus 15-9-12 (Everris NA Inc., Dublin, OH) fertilizer was applied at the rates of either 0, 3, 6, or 9 g per plant and nested within each irrigation treatment. The fertilizer was polymer coated 3-4 months slow release formula, and N was available in the form of ammonium nitrate and ammonium phosphate. Half of the fertilizer was top dressed at the time of transplanting and the remaining half was applied 40 days after treatment (DAT). Each fertilizer treatments had 10 single plant replications and arranged randomly within each water stress treatment.

Plant Materials and Growth Condition:

On 26 February 2016 and 20 February 2017, plug trays of geranium (*Pelargonium hortorum* L.H. Bailey ‘Pinto Premium Rose Bicolor’) and fountain grass (*Pennisetum alopecuroides* L. sp. Fountain Grass) were obtained from Park Seed (Greenwood, SC). These trays were kept on a mist bench until pots and media were ready for transplanting. The plants were manually misted twice a day. Plugs were transplanted into azalea pots having 15.24 cm diameter and 1.33 L maximum liquid volume capacity

(Landmark plastic corporation, Akron, OH) containing about 0.45 kg of 902 Metro-mix media (Sun-Gro Horticulture, Bellevue, WA).

Pots were moved to their respective benches and the greenhouse set for a 21 °C/18 °C (day/night) regime in both greenhouses. Mid-day (1200 HR) photosynthetic photon flux density (PPFD) ranged from 600-1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and plants were grown under natural photoperiods.

Growth Analysis:

Growth index (average of height and width), number of flowered umbels, number of flowers in an umbel, and days to first flowering were measured in geranium before harvesting. For fountain grass, growth index, numbers of tillers, days to first flowering and spike numbers were recorded. Geranium and fountain grass had growth cycles of 12 and 16 weeks, respectively. Shoots, stem cut at the media level, were harvested in both species at the end of the growing cycle and oven dried at 60 °C for 72 hours to determine shoot dry weight.

Data collection with Li-cor 6400 started in geranium from 24 May in 2016 and 16 May in 2017 during flowering state. Similarly, fountain grass data were collected from 16 June 2016 and 11 June 2017. All plant samples were used for data collection in geranium, however, only half of the samples in fountain grass were used for Li-cor measurement. For both species, rate of photosynthesis, transpiration, stomatal conductance, and water use efficiency (defined as the ratio of net photosynthesis to transpiration) were measured using a Li-6400 (LI-COR, NE). The Li-cor with 6400-02B LED light source chamber was used from 9 am to 3 pm during flowering stage to measure physiological parameters.

The data was taken only once at flowering stage of growth cycle. During these measurements (hereafter referred to as Li-cor measurements), the instrument reference CO₂ was kept at 400 ppm at ambient condition and at 800 ppm at an elevated CO₂ condition. The light level was fixed at 1200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and block temperature was set at 28 °C. Since youngest leaves shows greater assimilation rate, one of the first five young leaves was used as a nondestructive sample for Li-cor measurement from each plant in geranium. However, the leaves of fountain grass were small, three leaves were combined without breaking for a single Li-cor measurement.

Statistical Analysis:

All data were subjected to analysis of variance (ANOVA) using SAS (Statistical Analysis System) version 9.4 (SAS Institute, Cary, NC). The analysis was made considering three factorial treatments; CO₂ concentration, irrigation, and fertilizer treatments in split-plot design. The second year study was the replication of first year study. PROC MIXED procedure was used to compute means at 5% level of significance. When treatments showed a significant difference, a macro program, pdmix800, was used to separate means using Tukey-Kramer test.

Results

Growth index (GI)

The main effect of fertilizer and the interaction effect of CO₂ × Irrigation was significant in geranium (Table 2.1). Since no interactions involving fertilizer treatment were significant, data were pooled across CO₂ and irrigation treatments to compare the main effect of fertilizer. The greatest value was observed under the greatest fertilizer

level of 9 g. Growth index (GI) significantly decreased with decreasing levels of fertilizers having the lowest value with the control treatment (Table 2.2). The interaction of CO₂ × irrigation had the greatest GI with elevated CO₂ at 10 cb, but the value was statistically similar to 15 cb irrigation treatments in both CO₂ treatments and 5 cb treatment in ambient CO₂ condition (Table 2.3).

In fountain grass, the two-way interactions of CO₂ × Irrigation, CO₂ × Fertilizer, and Irrigation × Fertilizer were significant; however, no significant three-way interaction was observed (Table 2.1). In CO₂ × Irrigation, greatest values were observed at 10 cb at both ambient and elevated CO₂ conditions (Table 2.4). In CO₂ × Fertilizer, GI was greatest in 6 g and 9 g fertilizer treatments in elevated CO₂ and 9 g fertilizer treatment in ambient CO₂ (Table 2.5). For Irrigation × Fertilizer, GI was greatest in 6 g and 9 g fertilizer treatments at 10 cb irrigation, but the 6 g treatment was not different from 6 and 9 g treatment in 5 cb, 3 g treatment in 10 cb, and 9 g treatment in 15 cb irrigation (Table 2.6).

Dry weight

There was no significant effect of irrigation or any other treatment interactions in both geranium and fountain grass (Table 2.1). Thus, data were pooled to estimate the main effects of CO₂ and fertilizer treatments in both species. Among fertilizer treatments, 3, 6 and 9 g fertilizer treatments were similar and were greater than the control treatment (Table 2.2). Geraniums grown under elevated CO₂ had greater dry weight by 34.7% compared to plants grown under ambient condition (Table 2.7). Fountain grass under elevated CO₂ showed increased dry weight (38.5%) than in ambient condition (Table 2.8). Similarly, fountain grass grown with 6 or 9 g fertilizer had the greatest dry weight but 6 g

was not different from 3 g treatment. Dry weight for the 9 g treatment was greater by 79.6% when compared with the control treatment (Table 2.8).

Number of inflorescences

In fountain grass, only CO₂ × Fertilizer and Irrigation × Fertilizer interactions were significant for spike number and no three-way interaction was observed (Table 2.1). The CO₂ × Fertilizer interaction showed that fountain grass with 6 or 9 g fertilizer at elevated CO₂ had a greater number of flowers (Table 2.5). Irrigation × Fertilizer interaction for spike number showed that fountain grass grown under 6 or 9 g fertilizer treatment had significantly higher spike number compared to any other treatment combinations (Table 2.6).

Only CO₂ × Fertilizer interaction was significant for the number of umbels in geranium and number of flowers per umbel were significant only for main effects of irrigation and fertilizer treatment (Table 2.1). Increasing fertilizer rate resulted in an increased number of flowers with the 9 g having the most number of flowers (Table 2.2). With decreasing rate of fertilizer, the number of umbels also decreased in both elevated and ambient condition. Geraniums grown under 10 cb irrigation treatment had greater number of flowers per umbel compared to other irrigation treatments (Table 2.7). Geraniums in 6 and 9 g fertilizer treatments under both elevated and ambient CO₂ had the greatest umbel numbers, though 6 and 9 g treatments in ambient CO₂ were not different from the 3 g treatment in both CO₂ treatments (Table 2.9).

Days to first flowering

There was no interaction for days to first flower in geranium, but main effect for fertilizer and CO₂ were observed (Table 2.1). The results showed that the flowering days in elevated CO₂ was significantly delayed compared to ambient condition. Geraniums under 6 or 9 g fertilizer treatments flowered earlier compared to other fertilizer treatments (Table 2.2). The mean number of days to first flowering in elevated CO₂ was 57.7 days, which was more than the 53.2 days in ambient condition (Table 2.7). In fountain grass, none of the treatments showed a significant difference in days to first flowering (data not shown).

Tiller numbers

No three-way interactions of CO₂, irrigation, and fertilizer treatments were observed for tiller numbers in fountain grass. However, the interactions of CO₂ × Irrigation and CO₂ × Fertilizer were significant (Table 2.1). The CO₂ × Irrigation interaction showed that fountain grass under 10 cb irrigation in elevated CO₂ had the greatest number of tillers and was similar to plants grown at 5 cb irrigation in elevated CO₂ (Table 2.4). For CO₂ × Fertilizer interaction, both 6 and 9 g fertilizer treatments showed greatest tiller numbers under elevated CO₂ (Table 2.5). The data also showed that the number of tillers decreased with decreasing fertilizer rate in both ambient and elevated CO₂ condition.

Photosynthesis

No three-way interaction was observed in geranium for photosynthesis. However, CO₂ × Irrigation and CO₂ × Fertilizer interactions were significant (Table 2.1). In CO₂ ×

Irrigation interaction, photosynthesis was significantly greater in 10 and 15 cb irrigation treatment under elevated CO₂ (Table 2.3). Similarly, 6 and 9 g fertilizer treatments in elevated CO₂ had greater photosynthesis among CO₂ × Fertilizer interaction treatments (Table 2.9). In fountain grass, three-way interaction was observed for photosynthesis. The result showed that 3, 6, or 9 g treatment in 10 cb irrigation at elevated CO₂ and 6 and 9 g fertilizer in 10 cb at ambient CO₂ had the greatest photosynthesis (Table 2.11).

Transpiration

Interaction of CO₂ × Irrigation and Irrigation × Fertilizer was observed for transpiration in geranium (Table 2.1). Geranium at 15 cb treatment had the greatest rate of transpiration in both ambient and elevated CO₂ (Table 2.3). Irrigation × Fertilizer interaction showed greatest rate of transpiration in the 0, 6, and 9 g fertilizer in 15 cb irrigation, but, 6 and 9 g fertilizer treatments were not different from 10 or 15 cb irrigation treatment in 3 g fertilizer (Table 2.10). In fountain grass, three-way interaction of treatments was observed. The result showed that transpiration was greatest in the 9 g fertilizer treatment in 15 cb irrigation when grown at ambient CO₂ condition, but not different from 3 g fertilizer in 5 cb irrigation, 6 g fertilizer in 15 cb irrigation and 9 g fertilizer in 10 cb irrigation. Fountain grass under elevated CO₂ had lower transpiration compared to ambient condition (Table 2.11).

Stomatal conductance

Only fertilizer showed a significant difference between treatments as a main effect in fountain grass (Table 2.1). The data showed that fountain grass under 9 g treatment had greater stomatal conductance and this value was statistically similar with

the stomatal conductance of 3 or the 6 g fertilizer treatments (Table 2.8). In geranium, $\text{CO}_2 \times \text{Fertilizer}$ and $\text{Irrigation} \times \text{Fertilizer}$ interaction was significant (Table 2.1). For $\text{CO}_2 \times \text{Fertilizer}$ interaction, stomatal conductance was greatest for 6 and 9 g fertilizer treatments in ambient CO_2 and control fertilizer treatment at elevated CO_2 . This value was statistically similar with the stomatal conductance at 0 and 3 g fertilizer treatments at ambient condition and 3 and 9 g fertilizer treatments at elevated CO_2 condition (Table 2.9). In $\text{Irrigation} \times \text{Fertilizer}$ interaction, stomatal conductance was significantly greater in the 15 cb irrigation treatment in all fertilizer treatment and the 10 cb irrigation treatment with no fertilizer (Table 2.10).

Water use efficiency (WUE)

The WUE of geranium was significant for $\text{CO}_2 \times \text{Fertilizer}$ interaction and did not show a significant effect of irrigation in any interaction, but had a significant main effect (Table 2.1). The results showed that WUE of geranium under 5 and 10 cb irrigation was greatest, but 5 cb irrigation was not different from 15 cb treatment (Table 2.7). For $\text{CO}_2 \times \text{Fertilizer}$ interaction, WUE was significantly greater with 6 and 9 g of fertilizer at elevated CO_2 condition. With decreasing fertilizer rate, WUE also decreased in both ambient and elevated CO_2 (Table 2.9). In fountain grass, WUE showed three way interactions. All irrigation and fertilizer treatment combinations under elevated CO_2 were greater to all other treatment combinations in ambient CO_2 except 6 and 9 g fertilizer treatments in 10 cb irrigation (Table 2.11).

Discussion

Effect on plant growth

Plants were compact and had greater dry weight under elevated CO₂. Similar results were reported in which C₃ plants had more compact plant size with more branches (Enoch and Honour, 1993). All possible two-way interaction combinations of CO₂, irrigation, and fertilizer were observed for GI in fountain grass. According to Ghannoum (2008), the growth response of C₄ plants under elevated CO₂ is highly influenced by environmental factor and each species responds differently. Both geranium and fountain grass showed increased dry weight under both elevated CO₂ and increasing fertilizer rate. A study in red clover (*Trifolium pretense* L.) (Meier and Fuhrer, 1997) and upland creeping bentgrass (*Agrostis capillaris* L.) (Bowler and Press, 1993) reported increased dry matter under elevated CO₂ and a higher nitrogen (N) rate. Previous studies with elevated CO₂ reported an increase in dry weight of C₃ plants up to 40-45% and by 10-20% in C₄ plants (Ghannoum et al., 2000). However, the results in our study showed an increase in shoot dry weight of geranium and fountain grass by 34.7% and 38.5%, respectively. The increased shoot dry weight of geranium under elevated CO₂ is within range of other studies, but increased dry weight of fountain grass is greater than other studies. Leakey et al. (2007) reported higher dry weight in the study of maize, also a C₄ plant. The authors stated that the a C₄ plants grown in pots generally have limited root volume. In such case, even the well-watered plants may not have enough root volume to absorb an adequate amount of water for potential shoot growth. However, this water stress could be somehow ameliorated under elevated CO₂ and may give a false impression of carbon gain directly by elevated CO₂ concentration only. Thus, future work

in C₄ plants should consider the depth of containers without root restrictions (Leakey et al., 2007).

Effect on flowering

Effects of environmental factors like light, nutrient, and temperature on flowering time are large and predictable (Simpson et al., 1999). However, the mechanism behind the effect of elevated CO₂ on flowering is still unclear and a wide range of results have been documented among species and even within cultivar (Springer and Ward, 2007). Date of flowering was significantly delayed under elevated CO₂ and also by decreasing fertilizer rate. Two C₃ ornamental plants, gerbera (*Gerbera jamesonii* L.) (Berkel, 1984) and impatiens (*Impatiens repens* L.) (Reimherr, 1984), also showed similar results with our study under elevated CO₂. Similarly, decrease in days to flower in chrysanthemum (*Dendranthema grandiflora* L.) has been reported under higher rate of application of slow release osmocote fertilizer (Asrar et al., 2014). In contrast to our studies, several studies have reported accelerated flowering of ornamental plants by 2 to 15 days under elevated CO₂ (Cleland et al., 2006; Mortensen and Ulsaker, 1985; Reekie et al., 1997). However, similar to our study, Leaky et al. (2006) reported no effect of CO₂ and water in flowering time of a C₄ maize plant. Similarly, fertilizer treatments in a C₄ plant, sugarcane (*Saccharum officinarum* L.), had no significant effect in days to panicle initiation (Brunkhorst, 2003). However, the number of inflorescences (umbel in geranium and spike in fountain grass) in this study were greatly influenced by CO₂ × Fertilizer interaction in both species and also by Irrigation × Fertilizer interaction in fountain grass. In many species, increased inflorescence number has been directly related to increased assimilation under elevated CO₂ (Joblonski et al., 2002). Due to availability of greater

assimilates, plant may continue to remain in vegetative stage delaying the flower primordia formation (Reekie and Bazzaz, 1991). However, because of surplus food in plants, some plant species attain minimum vegetative size required for flowering earlier in elevated CO₂ compared to ambient condition. Thus, elevated CO₂, higher fertilizer rate, and adequate water might initiate an earlier reproductive phase resulting in a greater number of flowering primordia and flower number (He et al., 2005; Teng et al., 2006). Both accelerated flowering or delayed flowering with bigger plant size have their own importance in the ornamental industry. Thus, it is crucial to identify the response of different commercial ornamental plant to elevated CO₂ and mechanics behind accelerated or delayed flowering in ornamental C₃ and C₄ species.

Effects in physiological parameters

Either slightly dry or moderately-watered irrigation treatments showed greater photosynthesis under elevated CO₂. Likewise, photosynthesis increased in elevated CO₂ when fertilizer rate was greater. Arp et al. (1998) also reported increased photosynthesis in sorrel (*Rumex obtusifolius* L.) when grown under well-watered and greater N condition. Among all treatment combinations, fountain grass also exhibited greater photosynthesis in elevated CO₂ under greater fertilizer and 10 cb irrigation treatment. Although the mechanism for stimulation of photosynthesis is clear in C₃ plants, stimulation C₄ species is still not clear (Leakey et al., 2009). The increased CO₂ level increases activity of rubisco enzyme for carboxylation reactions and suppresses competition for binding of oxygen (photorespiration), resulting in increased photosynthesis in C₃ (Lara and Andreo, 2011). In a review, Kimball (2016) reported that

some C₄ species might not respond well to elevated CO₂ under ample moisture and N status.

Stomatal conductance was influenced by CO₂ × Fertilizer as well as Irrigation × Fertilizer interactions in geranium. The inconsistent pattern in stomatal conductance in CO₂ × Fertilizer could be because of counter effects of each other. Usually, elevated CO₂ leads to reduced stomatal conductance and transpiration (Nackley et al., 2014), but also increases plant leaf area (Li et al., 2004). Thus, reduced stomatal conductance in elevated CO₂ and increased conductance due to increased leaf area might have counter acted each other (Li et al., 2004). Irrigation × Fertilizer showed greater transpiration and stomatal conductance in the 15 cb irrigation regimes irrespective of fertilizer treatment in geranium. The possible explanation for lower stomatal conductance and transpiration in geranium under higher irrigation could be restricted root respiration. Continuous irrigation level might have affected root respiration, causing stomatal closure resulting in reduced stomatal conductance (Else et al., 2001; Gravat and Kirby, 1998). Another possible explanation for higher stomatal conductance under reduced irrigation could be due to the drought tolerance nature of geranium (Chylinski et al., 2007) and the irrigation level imposed might not have resulted in stress. Unlike in geranium, stomatal conductance in fountain grass was influenced by fertilizer treatments only. The increased stomatal conductance under higher fertilizer rate might be because of increased leaf area (Li et al., 2004). However, transpiration in fountain grass was influenced by all three factors: CO₂, irrigation, and fertilizer. Transpiration was significantly greater in ambient condition at 10 and 15 cb irrigation under greater fertilizer levels. Xu et al. (2016) reported a significant decrease in the stomatal index under elevated CO₂ in many species,

which might result in lower transpiration in elevated CO₂ condition. Thus, increased leaf area, increased stomatal index under ample fertilizer, and irrigation in ambient CO₂ might have resulted in greater stomatal conductance in fountain grass.

Because of greater photosynthesis in elevated CO₂, water use efficiency (WUE) was significantly greater in fountain grass for all fertilizer and irrigation treatment combinations. The increased WUE could be the result of lower transpiration too in elevated CO₂. Reddy et. al. (2005) reported an increase in WUE through increased photosynthesis and decreased transpiration in cotton (*Gossypium hirsutum* L.). But, geranium showed greater WUE in both ambient and elevated CO₂ at slightly dry irrigation regime (15 cb) in spite of higher transpiration in those treatments.

Conclusion

Container production of geranium 'Pinto Premium Rose bicolor' and fountain grass (sp. Fountain Grass) showed greater growth rate and bigger sized plant under elevated CO₂ in 10 or 15 cb irrigation with greater rate of fertilizer. No significant response in fountain grass and delayed flowering was observed in geranium had delayed flowering. Thus, species-specific response could be observed in flowering at elevated CO₂ concentration. Growers need to be cautious before CO₂ supplementation if early flowering is desired. However, number of flowers and compactness of ornamental plant increases under elevated CO₂, which is one of the desired character of ornamental species. Most of the parameters had a greater value with supplemental CO₂. More fertilizer may be required to proportionate the rate of growth. Thus, future research

should focus on a wider range of fertilizer and irrigation regimes in elevated CO₂ with multiple C₃ and C₄ species to understand the effects on plant physiology and morphology.

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Table 2.1. Analysis of variance (ANOVA) showing effect of CO₂ (ambient at 400 ppm and elevated at an average of 800 ppm), irrigation (5, 10, and 15 cb tensiometer settings), and fertilizer (0, 3, 6, and 9 g of 15-9-12 Osmocote plus) treatments on geranium 'Pinto Premium Rose Bicolor' and fountain grass.

Effects	Grow th index	Dry weight (g)	No. of inflore- scences	Days to first flower	No. of flowers per umbel	No. of tillers	A _n ^z ($\mu\text{mol}\cdot\text{C}$ $\text{O}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	E ^z ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	g _s ^z ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	WUE ^z ($\mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ of H ₂ O)
Geranium										
CO ₂	ns ^y	*	ns ^x	**	ns	-	ns	ns	ns	***
Irrigation	ns	ns	ns	ns	*	-	*	***	***	*
Fertilizer	***	***	***	***	***	-	***	ns	ns	***
CO ₂ ×Irrigation	*	ns	ns	ns	ns	-	*	*	ns	ns
CO ₂ ×Fertilizer	ns	ns	*	ns	ns	-	*	ns	*	***
Irrigation×Fertilizer	ns	ns	ns	ns	ns	-	ns	*	*	ns
CO ₂ ×Irrigation×Fertilizer	ns	ns	ns	ns	ns	-	ns	ns	ns	ns
Fountain grass										
CO ₂	ns	**	***	ns	-	*	***	**	ns	*
Irrigation	**	ns	**	ns	-	*	***	ns	ns	ns
Fertilizer	***	***	***	ns	-	***	***	***	*	**
CO ₂ ×Irrigation	*	ns	ns	ns	-	*	***	ns	ns	ns
CO ₂ ×Fertilizer	***	ns	**	ns	-	**	ns	**	ns	ns
Irrigation×Fertilizer	***	ns	*	ns	-	ns	ns	ns	ns	ns
CO ₂ ×Irrigation×Fertilizer	ns	ns	ns	ns	-	ns	*	**	ns	*

^zIndicates abbreviation of photosynthetic rate (A_n), stomatal conductance (g_s), transpiration (E), and water use efficiency (WUE).

^yNs, *, **, *** indicate not significant and the level of significance at $P < 0.05$, $P < 0.001$, and $P < 0.001$, respectively

^xNo. of inflorescence indicates no. of umbels in geranium and no. of spikes in fountain grass.

Table 2.2. Response of geranium ‘Pinto Premium Rose Bicolor’ to different fertilizer treatments of Osmocote plus 15-9-12 supplied as a top dress.

Fertilizer (g)	Growth index	Dry weight (g)	Days to flower	Flowers per umbel
0	26.3d ^z	20.3b	58.1a	47.8d
3	29.8c	38.1a	56.3b	61.1c
6	31.5b	41.6a	53.8c	69.6b
9	32.7a	44.5a	53.8c	76.8a

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

Table 2.3. Effect of CO₂ (ambient at 400 ppm and elevated at an average of 800 ppm) and irrigation treatments using a tensiometer in growth of geranium ‘Pinto Premium Rose Bicolor’.

Irrigation (cb)	Growth index		Photosynthesis ($\mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		Transpiration ($\text{mmol}\cdot\text{H}_2\text{O}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
5	30.4ab ^z	29.1b	19.5b	22.8b	7.3b	6.3c
10	29.1b	31.6a	19.2b	29.6a	7.4b	7.5b
15	30.1ab	30.1ab	21.4b	28.5a	8.4a	8.5a

^zMeans (n=80) within a parameter followed by same letters are not significantly different at $P \leq 0.05$.

Table 2.4. Effect of CO₂ (ambient at 400 ppm and elevated at an average of 800 ppm) and irrigation treatments using a tensiometer in growth of fountain grass.

Irrigation (cb)	Growth index		No. of tillers	
	Ambient	Elevated	Ambient	Elevated
5	40.5b ^z	42.3b	25.7c	38.1ab
10	44.0ab	46.8a	30.9bc	42.6a
15	42.6b	41.2b	30.0bc	34.4bc

^zMeans (n=80) within a parameter followed by same letter are not significantly different at $P \leq 0.05$.

Table 2.5. Effect of CO₂ (ambient at 400 ppm and elevated at an average of 800 ppm) and fertilizer treatments (0, 3, 6, and 9 g Osmocote plus 15-9-12 supplied as a top dress) in growth of fountain grass.

Fertilizer (g)	Growth index		No. of spikes		No. of tillers	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
0	39.1c ^z	39.1c	2.2d	3.1cd	18.1e	23.8de
3	43.1b	42.9b	4.4bc	5.6b	28.5cd	36.4b
6	41.6b	46.1a	4.4bc	7.9a	32.6bc	44.6a
9	45.6a	46.3a	5.9b	9.3a	36.3b	48.6a

^zMeans (n=60) within a parameter followed by same letter are not significantly different at $P \leq 0.05$.

Table 2.6. Interaction effect of irrigation treatments (5, 10, and 15 cb using a tensiometer) and fertilizer treatments (0, 3, 6, and 9 g Osmocote plus 15-9-12 supplied as a top dress) in growth of fountain grass.

Fertilizer (g)	Growth index			Number of spikes		
	5 cb	10 cb	15 cb	5 cb	10 cb	15 cb
0	2.4f ^z	2.8ef	2.8ef	2.4f	2.8ef	2.8ef
3	4.5de	6.3bcd	4.3def	4.5de	6.3bcd	4.3def
6	6.5bcd	7.9ab	5.1cd	6.5bcd	7.9ab	5.1cd
9	6.8bc	9.2a	6.9bc	6.8bc	9.2a	6.9bc

^zMeans (n=40) within a parameter followed by same letter are not significantly different at $P \leq 0.05$.

Table 2.7 Response of geranium ‘Pinto Premium Rose Bicolor’ to CO₂ treatments (ambient at 400 ppm and elevated at an average of 800 ppm) and different irrigation treatments using a tensiometer.

Irrigation (cb)	Flowers per umbel	Water use efficiency ($\mu\text{mol}\cdot\text{CO}_2\cdot\text{mol}^{-1}\cdot\text{H}_2\text{O}$)	CO ₂ levels	Dry weight (g)	Days to flower
5	61.4b ^z	3.3ab	Ambient	30.8b ^y	53.2b
10	67.7a	3.4a	Elevated	41.5a	57.7a
15	62.3b	3.1b			

^zMeans (n=160) within a column followed by same letters are not significantly different at $P \leq 0.05$.

^yMeans (n=240) within a column followed by same letters are not significantly different at $P \leq 0.05$.

Table 2.8. Response of fountain grass to different fertilizer treatments (0, 3, 6, and 9 g of Osmocote plus 15-9-12 supplied as a top dress) and CO₂ treatments (ambient at 400 ppm and elevated at an average of 800 ppm).

Fertilizer (g)	Dry weight (g)	Stomatal conductance (mmol·m ⁻² ·s ⁻¹)	CO ₂ levels (ppm)	Dry weight (g)
0	41.7c ^z	0.2b ^y	Ambient	51.7b ^x
3	61.9b	0.22ab	Elevated	71.6a
6	68.2ab	0.23ab		
9	74.9a	0.26a		

^zMeans (n=120) within a column followed by same letter are not significantly different at $P \leq 0.05$.

^yMeans (n=60) within a column followed by same letter are not significantly different at $P \leq 0.05$.

^xMeans (n=240) within a column followed by same letter are not significantly different at $P \leq 0.05$.

Table 2.9. Effect of CO₂ (ambient at 400 ppm and elevated at an average of 800 ppm) and fertilizer treatments of Osmocote plus 15-9-12 in growth of geranium ‘Pinto Premium Rose Bicolor’.

Fertilizer (g)	No. of umbels		Photosynthesis ($\mu\text{mol}\cdot\text{CO}_2\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		Stomatal conductance ($\text{mmol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$)		Water use efficiency ($\mu\text{mol}\cdot\text{CO}_2\cdot\text{mol}^{-1}\cdot\text{H}_2\text{O}$)	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
0	5.1cd ^z	4.3d	15.7e	19.7d	0.55ab	0.58a	2.2d	2.6cd
3	6.3bc	6.3bc	19.2d	25.8b	0.56ab	0.54ab	2.6cd	3.6b
6	7.7ab	8.5a	21.7cd	29.8a	0.60a	0.47b	2.9c	4.5a
9	7.5ab	8.9a	23.7bc	32.6a	0.63a	0.51ab	3.1c	4.6a

^zMeans (n=60) within a parameter followed by same letters are not significantly different at $P \leq 0.05$.

Table 2.10. Effect of irrigation treatments using a tensiometer and fertilizer treatments of Osmocote plus 15-9-12 supplied as a top dress in stomatal conductance of geranium 'Pinto Premium Rose Bicolor'.

Fertilizer (g)	Irrigation (cb)	Transpiration (mmol·H ₂ O·m ⁻² ·s ⁻¹)	Stomatal conductance (mmol·m ⁻² ·s ⁻¹)
	5	6.4d ^z	0.43c
0	10	7.3cd	0.57ab
	15	9.3a	0.71a
	5	6.8cd	0.52bc
3	10	7.8bc	0.54bc
	15	7.9bc	0.58ab
	5	7.1cd	0.50bc
6	10	7.2cd	0.49bc
	15	8.1abc	0.61ab
	5	7.1cd	0.52bc
9	10	7.5cd	0.51bc
	15	8.7ab	0.68a

^zMeans (n=40) within a parameter followed by same letter are not significantly different at $P \leq 0.05$.

Table 2.11. Interaction effect of CO₂ (ambient at 400 ppm and elevated at an average of 800 ppm), irrigation treatments (5, 10, 15 cb using a tensiometer) and fertilizer treatments (0, 3, 6, and 9 g Osmocote plus 15-9-12 supplied as a top dress) in growth of fountain grass.

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

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

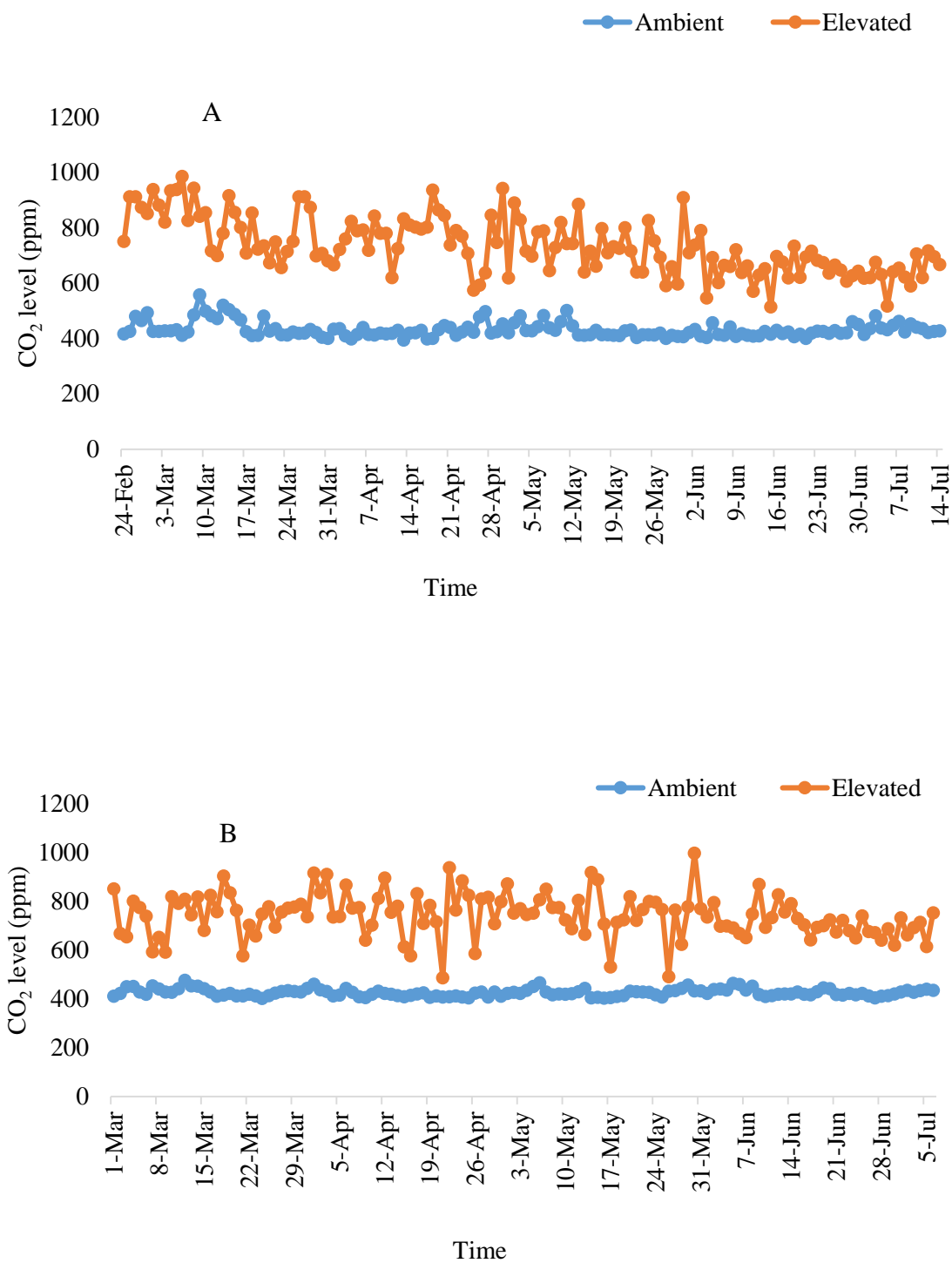


Figure 2.1. Average daily value of ambient and supplemental CO₂ concentration measured in greenhouses during the year 2016 (A) and 2017 (B) in Stillwater, OK.

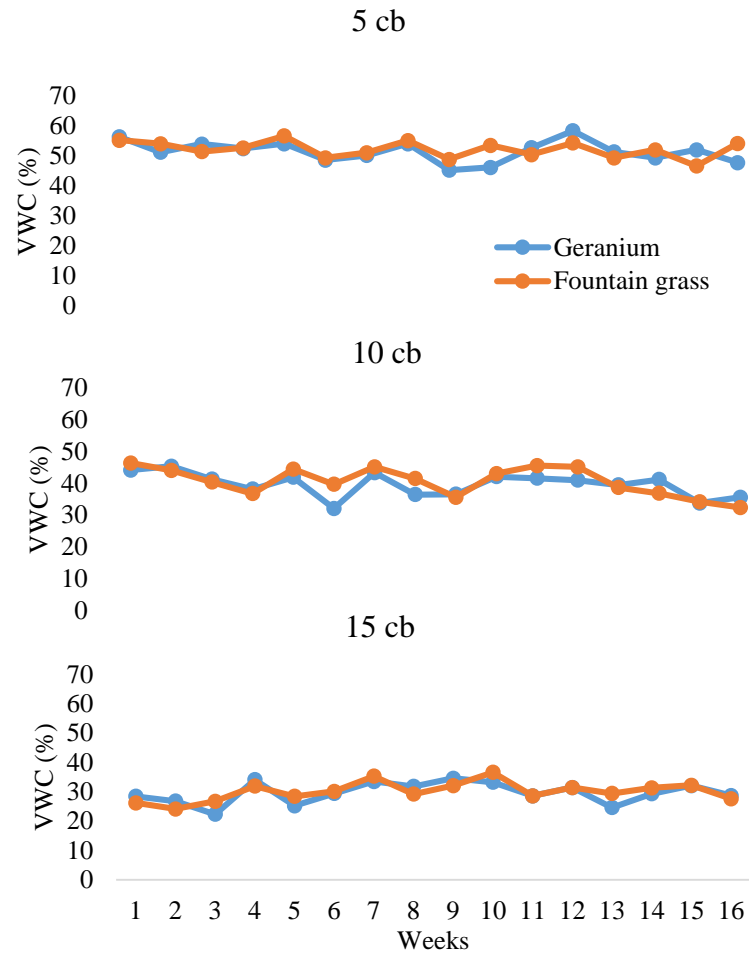


Fig. 2.2 Volumetric water content (VWC) of geranium and fountain grass measured by Field Scout hand held TDR 100 sensor (Spectrum Technologies, Inc., Aurora, IL) when plants were grown under 5, 10, and 15 cb irrigation regimes in soilless 902 metro mix media (Sun Gro Horticulture, Bellevue, WA).

CHAPTER III

EFFECTS OF ELEVATED CARBON DIOXIDE IN GREENHOUSE LEAFY GREENS GROWN IN HYDROPONICS

Abstract

Carbon dioxide concentration in a greenhouse plays an important role in plant production. The objective of this study was to understand how elevated CO₂ in a greenhouse affects growth of hydroponically grown basil, Swiss chard, and lettuce. A greenhouse was supplemented with an average of 800 ppm of CO₂ in the morning to mid-afternoon and another similar greenhouse was at ambient condition (400 ppm). Nutrient film Technique (NFT) was used in which all three species were replicated three times with 15 samples in each replication. The plants were grown for 46 days. The study showed that elevated CO₂ could significantly increase the height and width of hydroponically grown leafy greens. Basil, lettuce and Swiss chard showed an increased fresh weight in elevated CO₂ by 29%, 24.7%, and 39.5%, respectively. Basil, lettuce, and Swiss chard dry weight was also greater in elevated CO₂ treatment by 34.4%, 21.4%, and 40.1%, respectively. A significant reduction in SPAD and atLEAF values was observed under elevated CO₂. Along with chlorophyll, nitrogen (N), phosphorus (P), and magnesium (Mg) levels were also lower in plants grown in elevated CO₂, but the result

was not consistent in all three species. For N, basil and lettuce had significantly lower tissue N concentration in elevated CO₂. Species-specific response was observed for P content. Magnesium content was higher in ambient condition in Swiss chard only. However, Fe was significantly higher in elevated CO₂, but only for basil.

Introduction

Growing plants in soilless media is not a new technique. From the early 19th century, people have adopted methods of aeroponics, hydroponics, rockwool culture, vermiculite culture, and sand/gravel culture (Maxwell, 1986). However, those methods were in the form of research or nutrient trials. In recent years, hydroponics has been adopted worldwide for food production (Bradley and Marulanda, 2000). With an increasing human population, the demand for food is also increasing and farmland is being lost. Thus, the concept of vertical farming could be a popular method to solve the problem of decreasing usable land. In the horticulture industry, leafy greens and herbs are commonly produced in hydroponics (Both, 1998). Greens are produced in hydroponics because of year-round production and interest in local fresh products. Hydroponically grown crops are cleaner and reported to have greater postharvest life (Both, 1998; Succop and Newman, 2004).

There are different types of hydroponic systems. Growers can use a wick system, water culture, drip system, nutrient film technique (NFT), ebb & flow system, aeroponics, and vertical farming depending upon the necessity and types of crop grown. The NFT system was developed in 1960 by Allen Cooper and his colleagues (Resh, 2012) in which, a shallow film of nutrient solution runs continuously and plants

are grown in a trough (Both, 1998). Recycling of nutrient solution makes hydroponics a water and nutrient efficient method of production (Christie, 2014).

Both et al. (1997) stated that the production system could be profitable if the grower can maintain year-round production. However, year-round production of green leafy vegetables may require supplemental lighting in the winter and shade in the summer (Both, 1995). Mortensen, (1987) reported CO₂ enrichment can be equally beneficial as supplemental lighting. Carbon dioxide enrichment is used in many commercial greenhouses to increase the yield of cultivated crops. Generally, the concentration of CO₂ maintained is around 1000-1500 ppm (Chalabi et al., 2002; Holbrook et al., 1993; Mortensen, 1987). However, CO₂ enrichment was found to be beneficial only in autumn, spring, and winter when the vent system was closed (Willits and Peet, 1989). A study in cucumber (*Cucumis sativus* L.) showed that the CO₂ concentration inside a sealed greenhouse can be as low as 150 ppm during the day and CO₂ supplementation is the only way to maintain the desired concentration of CO₂ in winter for optimum production (Klaring et al., 2007). Supplemental CO₂ resulted in increased photosynthesis assimilation and increased growth of lettuce by 30% (Both et al., 1997; Willits and Peet, 1989). Another beneficial response is reduced transpiration under elevated CO₂, which may help to prevent water loss. However, reduced transpiration due to CO₂ enrichment has been known to cause tip-burn in lettuce (*Lactuca sativa* L.) (Both et al., 1997). Similarly, when basil (*Ocimum basilicum* L.) was grown under 1500 ppm CO₂, interveinal chlorosis was observed due to the accumulation of large grains of starch (Holbrook et. al, 1993).

Humans have been consuming herbs for thousands of years. In the past as well as today, herbs have been used for cosmetic, medicinal, and culinary purposes (Stapleton and Hochmuth, 2001). Tyson et al. (2001) reported that herbs cover more than 18% of greenhouse acreage in the United States and were the third most grown crop in greenhouses. Similarly, year-round production of leafy greens under protected environment is gaining in popularity in the United States (Parkell et. al., 2015). Although having a huge coverage of herbs and leafy greens in greenhouse production, very few studies have been conducted to understand how these plants respond to elevated CO₂. Thus, the objective of this study was to determine the effect of supplemental CO₂ on growth of lettuce, basil, and Swiss chard (*Beta vulgaris* L.) grown hydroponically with supplemental CO₂ being applied on part of the day.

Materials and Methods

Plant materials and Experimental setup:

Seeds of lettuce ‘Auvona’, Swiss chard ‘Magenta Sunset’ and basil ‘Cardinal’ were obtained from Johnny’s selected seeds (Winslow, ME) and were planted on 20 January at Oklahoma State University Research Greenhouse, Stillwater, OK. Seeds were sown on rockwool starter cube (Grodan Roxul Inc., Ontario, Canada) of 1.5 cm × 1.5 cm × 1.5 cm size. On 25 February (35 days after seeds were sown), each set of seedlings were transplanted into Hydrocycle 10 cm Pro NFT series (Growers Supply, Dyersville, IA) hydroponic tables. Peter’s 5-11-26 (J.R. Peters Inc., Allentown, PA) and calcium nitrate (American Plant Products, Oklahoma City, OK) was used with tap water to prepare the nutrient solution containing 250 ppm nitrogen (N). In the tank, 147.41 g of

Peter's and 197.5 g of calcium nitrate was added to make 250 ppm of N. In 2-week intervals, the tanks were flushed and refilled to remove the excess nutrient buildup. During the growing period, pH was maintained at 5.5-6.5 and EC level was around 3-3.5 mS·cm⁻¹.

Experimental design and growing condition:

The study was conducted in a split-plot design. Two identical greenhouses were used and one of the greenhouse was fitted with a natural gas-burning CO₂ generator (Johnson Gas Appliances, Cedar Rapids, IA) in the middle of the greenhouse. The CO₂ generator was set to produce a daily average of 800 ppm of CO₂ by burning natural gas (Fig 3.1 and appendix I) during supplementation period. The generator was automatic and turned on from 6:00 a.m. to 2:00 p.m. A CO₂ monitor (FLIR Commercial System Inc., Nashua, NH) monitored the CO₂ concentration in both greenhouses. Both greenhouses were set at 21/18 °C day/night temperature and exposed to natural photoperiod. All three species were replicated three times having 15 samples in each replication. The experiment was replicated by starting another set by seeding on 28 February 2017 and similar methods listed above were followed.

Data collection and statistics:

Data were collected 46 days after planting of plugs in the NFT system. Plant height (from top of the table to plant tip), width (average of diagonal width), specific leaf area (SLA), total leaf area (TLA), fresh weight, dry weight, and plant nutrient content were measured. Total leaf area was measured using a LI-3000C area meter (LI-COR,

Inc., Lincoln, NE). Specific leaf area was calculated through the ratio of leaf area of one side of an individual leaf to the dry weight of the same leaf. Plants were also scanned with a SPAD-502 chlorophyll meter (Konica Minolta, Japan) and atLEAF chlorophyll meter (FT Green LLC, Wilmington, DE) at the time of harvest. The SPAD and atLEAF readings for each sample were the average of single readings of tip, base, and blade of three different leaves. From each species, three samples were taken for leaf area measurement and the same samples were used for nutrient analysis. The samples were sent to the Soil, Water and Forage Analytical Laboratory (SWFAL) at Oklahoma State University for analysis of leaf nutrient content. The samples were kept in the oven at 60 °C for 72 hours to measure dry weight. Statistical analysis was performed at the 5% level of significance using SAS (Statistical Analysis System), version 9.4. PROC MIXED procedure for split-plot design was used to compute means and pdmix800, a macro program, was used to separate the means using Tukey-Kramer test.

Results

Both height and width of basil and Swiss chard were significantly greater under the elevated CO₂ condition (Table 3.1). There was no significant difference in height and width of lettuce between CO₂ treatments (Table 3.1). A 39.5% difference in fresh weight under ambient (296.8 g) and elevated CO₂ (414.1 g) was observed in Swiss chard. Similarly, fresh weight of basil and lettuce were greater in elevated CO₂ by 29% and 24.7%, respectively over ambient condition (Table 3.1). The dry weight of all three species was significantly greater under the elevated CO₂ condition. Basil and Swiss chard grown under elevated CO₂ were greater in size based on leaf number and compactness (data not shown). However, lettuce plants were compact and weighed more but were of

equal size in visual appearance (Fig 3.2). Lettuce plants showed tipburn in inner leaves at a later growth stage under elevated CO₂, but the plants under ambient condition were healthy. All three species had significantly higher total leaf area under the elevated CO₂ condition (Table 3.2). Total leaf area of basil, lettuce, and Swiss chard under elevated CO₂ increased by 41.9%, 22.6%, and 34%, respectively over ambient CO₂. However, the response of all three species was different for specific leaf area (SLA) (Table 3.2). Basil had an SLA of 260.7 and 160 cm²·g⁻¹ under ambient and elevated CO₂, respectively. However, SLA of lettuce was significantly greater in elevated CO₂ and was 271.1 and 321.6 cm²·g⁻¹ in ambient and elevated CO₂, respectively. In contrast to lettuce and basil, there was no significant difference of SLA in Swiss chard.

SPAD values were greater in ambient condition compared to elevated CO₂ condition for all three species. The SPAD values in ambient condition were greater by 5.1%, 6.4% and 6.8% in basil, lettuce, and Swiss chard, respectively. Similarly, atLEAF values were greater in ambient CO₂ condition for basil and Swiss chard by 6.2% and 7.3%, respectively. However, lettuce plants did not show any significant difference in atLEAF values under ambient and elevated CO₂. In leaf nutrient analysis for mineral content, only N, P, Mg, and Fe were different in plants grown in ambient and elevated CO₂ and the differences were inconsistent between species (Table 3.3). For N, basil and lettuce had a lower tissue N concentration in elevated CO₂. Similarly, P content was also significantly greater in lettuce and Swiss chard in ambient condition, but showed no difference in P content in basil when grown in ambient and elevated CO₂ condition. The Mg content was also greater in ambient condition only for Swiss chard. Whereas, only basil showed a greater Fe concentration when grown in elevated CO₂.

Discussion

In the present study, basil and Swiss chard showed a significant increase in height and all three species had a significant increase in width under elevated CO₂. The significant increase in height and width could be the result of an increase in the rate of photosynthetic assimilation. Upreti and Mahalaxmi (2000) reported similar findings with a 30% increment in height of mustard (*Brassica juncea* (L.) Czern.) when grown under 600 ppm of CO₂. Similarly, the increase in leaf number might have resulted in greater width (Upreti and Mahalaxmi, 2000). Along with growth, most of the studies on elevated CO₂ were focused on understanding the effects of CO₂ on biomass and dry matter production. As expected, all three species under the elevated CO₂ showed a significant increase in fresh and dry matter production. Studies in cat grass (*Dactylis glomerata* L.) (Hermenis et al., 2000), potato (*Solanum tuberosum* L.) (Mackowiak and Wheeler, 1995), and peanuts (*Arachis hypogea* L.) (Stanciel et al., 2000) had increased fresh weight and dry weight when doubling the ambient CO₂. Similarly, studies of hydroponically grown lettuce (Both et al., 1998; He et al., 2001), basil (Gabriel et al., 1993) and greenhouse grown Swiss chard 'Fordhook Giant' (Ziska et al., 1995) reported a significant increase in dry weight under elevated CO₂ than ambient condition.

Stanciel et al. (2000) reported that an increase in internal CO₂ concentration, because of elevated CO₂, reduces photorespiration and delays senescence. Along with this, elevated CO₂ resulted in increased branching and leaf number (Enoch and Honour, 1993). Because of this increase in light interception area, net photosynthesis increases. On the other hand, elevated CO₂ increases sink size, which results in increased photosynthetic accumulation and vegetative growth. Shoot biomass tends to increase

under elevated CO₂ until the commencement of seed set (Boote et al., 1992). Leafy greens are harvested before plants become reproductive, thus elevated CO₂ may result in greater production. However, the response of C₃ plants in terms of photosynthetic acclimation is specific (Gabriel et al., 1993) and shows a positive response up to a certain concentration of CO₂ only. Above 800-1000 ppm, some plants may reach a saturation point and net photosynthesis does not increase with increasing CO₂ (Stancel et al., 2000).

The increase in total leaves area is likely associated with an increase in photosynthetic assimilation. Enoch and Honour (1993) and Vessey et al. (1988) in soybean (*Glycine max* L. Merr.) reported an increase in leaf area of plants under elevated CO₂. However, Harmens et al. (2000) reported no significant difference in leaf area of the leaves in main stem in cat grass under ambient and elevated CO₂. Species specific response of plants for SLA was observed. However, most of the CO₂ related studies reported a decrease in SLA of a plant. Gabriel et al. (2000) reported a decrease in SLA with increasing CO₂ when basil and spinach (*Spinacea oleracea* L. 'Caramble') were grown hydroponically under ambient and 1500 ppm of CO₂. Because of storage of starch in leaves, the leaves of peanuts had greater dry weight at 800 and 1200 ppm as compared to ambient (400 ppm) and resulted in higher SLA (Stancel et al., 2000). However, Harmens et al. (2000) explained that a decrease in SLA simply cannot be explained through increased photosynthesis and accelerated growth of plant under elevated CO₂. Rather, SLA depends on how assimilates are distributed in shoots and roots during various growth stages. Thus, considering both root and shoot parameters in future studies will help in understanding species specific nature of partitioning of assimilates in the roots and shoots.

All three species grown under elevated CO₂ were lighter green compared to an ambient condition and had lower SPAD values and atLEAF except in lettuce for atLEAF. Araki (2006) reported a lower chlorophyll index in soybean when measured with SPAD under elevated CO₂ concentration. Similar to our study, chlorophyll level in hydroponically grown basil was significantly lower in the later growth stage when grown at 400 and 1500 ppm CO₂ (Gabriel et al., 1993). In contrast, chlorophyll content in lemon balm (*Melissa officinalis* L.) (Shoor et al., 2012), spinach (Gabriel et al., 1993), and SPAD value of cucumber seedlings (Li et al., 2008) showed no significant difference when grown under elevated and ambient CO₂. No literature was found using an atLEAF sensor for non-destructive measurement of chlorophyll in CO₂ studies. Since SPAD and atLEAF values have been positively correlated in many studies (Basyouni and Dunn, 2013), the lower sensor values under elevated CO₂ can be explained by lower chlorophyll level because of movement of N to other sink like roots in elevated CO₂. Although no significant difference was observed in Swiss chard leaf N content, basil and lettuce had significantly lower leaf N content in elevated CO₂. In a review of plant tissue N under elevated CO₂ in many species, tissue N content of above-ground tissue was lower by 10-15% (Cotrufo et al., 1998; Taub and Wang, 2008). Studies of leaf nutrients content in cotton (*Gossypium hirsutum* L.) (Huluka et al. 1994), chrysanthemum (*Chrysanthemum x morifolium* Ramat. 'Fiesta') (Kuenhy et al., 1991), and hydroponically grown lettuce (Chagvardieff et al., 1994) have reported a decrease in leaf N and P content and increase in leaf Fe content, which is similar to our study but the response was inconsistent among species. Reduced N content in crops due to degradation of chlorophyll and lower protein level has been reported in many crops when grown in elevated CO₂ (Kumari et al., 2013;

Taub et al., 2008). Although plants will be benefitted through increased biomass with supplemental CO₂, increased C:N may lead to lower nutritional quality of leafy greens (Kumari et al., 2013). However, increase Fe content in leafy greens when grown in elevated CO₂ has beneficial effect in human nutrition (Chiplonkar et al., 1999). Benjamin et al. (2012) reported that the effect of elevated CO₂ on plant nutrient content depends on available N, tissue type, species, and nutrient ion. Some nutrients responded well and some are not affected by elevated CO₂. A solid single mechanism for lower nutrient content under elevated CO₂ has not been developed yet. The hypothesis related to N dilution by increased carbohydrate, decreased N uptake, and decreased N demand of crops has been described (Benjamin et al., 2010; Taub and Wang, 2008). However, increased nutrient use efficiency compensates for the effects associated with lower tissue nutrient and increased overall growth of plants under elevated CO₂.

Tipburn in lettuce is generally associated with distribution of Calcium (Ca) ion within plant leaves (Assimakopoulou et al., 2013). Gilliam et al. (2011) reported that translocation of Ca ion in plant is predominated by apoplastic pathway (through cell wall) and rate of transpiration determines the Ca concentration in plant tissue. The distribution of Ca in plant tissue is heterogeneous and the concentration of Ca might differ between inner and outer leaves depending upon growing environment (Lee et al., 2013). Plants grown with supplemental CO₂ shows lower rate of transpiration due to reduced stomatal conductance. Lower rate of transpiration might have resulted lower Ca concentration in inner leaves resulting tipburn (Both et al., 1997). Although there was no significant difference in Ca concentration of plant grown in ambient and elevated CO₂ during whole plant nutrient analysis, there might be difference in inner and outer leaves Ca

concentration. Mattson (2015) reported that tipburn in lettuce is not generally associated with lower Ca availability. Lower rate of transpiration and high humidity are the environmental factors affecting Ca uptake and causing localized tipburn. Calcium supply to the inner leaves of quickly growing plant (due to elevated CO₂, high light intensity, and greater fertilizer rate) fail to meet the demand causing lower distribution of Ca to inner leaves, which develops necrotic brown spots in margin of developing leaves (Mattson, 2015). Since environmental factors (lower transpiration and higher humidity) could be the cause of tipburn under elevated CO₂, vertical air flow within greenhouse could be a feasible solution for the tipburn problem (Lee et al., 2013).

Although elevated CO₂ results in increased growth, a meta-analysis showed lower nutrient content and protein level in major food crops when grown under elevated CO₂ (Taub et al., 2008). Thus, global increment in atmospheric CO₂ concentration could be threatening to human nutrition in future (Myers et al., 2014). Future studies should be focused on identifying suitable CO₂ concentration in combination with different nutrient levels for greater production while maximizing the nutritional quality.

Conclusion

Elevated CO₂ has significant potential to increase growth of leafy greens in hydroponics. Increased growth rate could result in early harvest and more crop cycles each year. Growth response of different species varies, but, this study showed increased growth of all three species. Lower leaf N concentration might affect the available protein and greater Fe concentration in our food is a desired quality. The study suggests both positive and negative effect of elevated CO₂ in nutritional quality of leafy greens. Thus, future studies should examine the nutritional aspect of crops grown in elevated CO₂.

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Table 3.1. Effect of CO₂ treatments (ambient at 400 ppm and elevated at an average of 800 ppm) on height, width, SPAD, and atLEAF values of lettuce ‘Auvona’, Swiss chard ‘Magenta Sunset’ and basil ‘Cardinal’.

Species	Height (cm)		Width (cm)		Fresh weight (g)		Dry weight (g)	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Basil	34.0b ^z	36.9a	26.2b	29.8a	123.1b ^y	158.9a	11.9b ^y	15.0a
Lettuce	26.6a	26.5a	25.2a	25.7a	203.8b	254.2a	19.1b	23.8a
Swiss chard	48.4b	52.8a	33.3b	35.4a	296.8b	414.1a	28.4b	28.4b

^zMeans (n=30) within a parameter of an individual species followed by same letter are not significantly different at $P \leq 0.05$.

Table 3.2. Effect of CO₂ treatments (ambient at 400 ppm and elevated at an average of 800 ppm) on fresh weight, dry weight, total leaf area, and specific leaf area values of lettuce ‘Auvona’, Swiss chard ‘Magenta Sunset’, and basil ‘Cardinal’.

Species	SPAD		atLEAF		Total leaf area (cm ²)		Specific leaf area ^z (cm ² ·g ⁻¹)	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Basil	44.44a ^y	42.28b	52.64a ^y	49.60b	1644.4b ^x	2333.8a	260.7a ^x	160.0b
Lettuce	48.05a	45.13b	45.83a	48.60a	4884.5b	5988.5a	271.1b	321.6a
Swiss chard	50.67a	47.43b	54.81a	51.05b	2836.4b	3801.1a	105.9a	105.8a

^zSpecific leaf area is the ratio of leaf area of one side of an individual leaf to the dry weight of the same leaf.

^yMeans (n=30) within a parameter of an individual species followed by same letter are not significantly different at $P \leq 0.05$.

^xMeans (n=10) within a parameter of an individual species followed by same letter are not significantly different at $P \leq 0.05$.

Table 3.3. Effect of CO₂ treatments (ambient at 400 ppm and elevated at an average of 800 ppm) on nutritional composition of lettuce ‘Auvona’, Swiss chard ‘Magenta Sunset’, and basil ‘Cardinal’ grown under nutrient film technique (NFT).

CO ₂ Concentration	N (%)	P (%)	Ca (%)	K (%)	Mg (%)	S (%)	B (ppm)	Mn (ppm)	Fe (ppm)	Zn (ppm)
Basil										
Ambient	5.1a ^z	0.6a	2.6a	2.97a	0.86a	0.30a	36.70a	57.96a	135.38b	49.90a
Elevated	4.6b	0.5a	2.5a	3.21a	0.84a	0.29a	40.46a	55.93a	239.30a	48.75a
Lettuce										
Ambient	4.3a	0.6b	1.8a	5.68a	0.63a	0.28a	49.93a	99.90a	154.40a	38.90a
Elevated	3.7b	0.4a	1.6a	5.12a	0.81a	0.26a	47.51a	95.20a	195.01a	32.45a
Swiss chard										
Ambient	4.6a	0.3b	1.7a	4.38a	1.14a	0.37a	61.93a	108.03a	97.01a	35.75a
Elevated	4.4a	0.5a	1.4a	4.55a	0.70b	0.33a	59.68a	71.08a	137.55a	39.96a

^zFor each species, means (n=6) within a column with the same letters are not significantly different at $P \leq 0.05$.

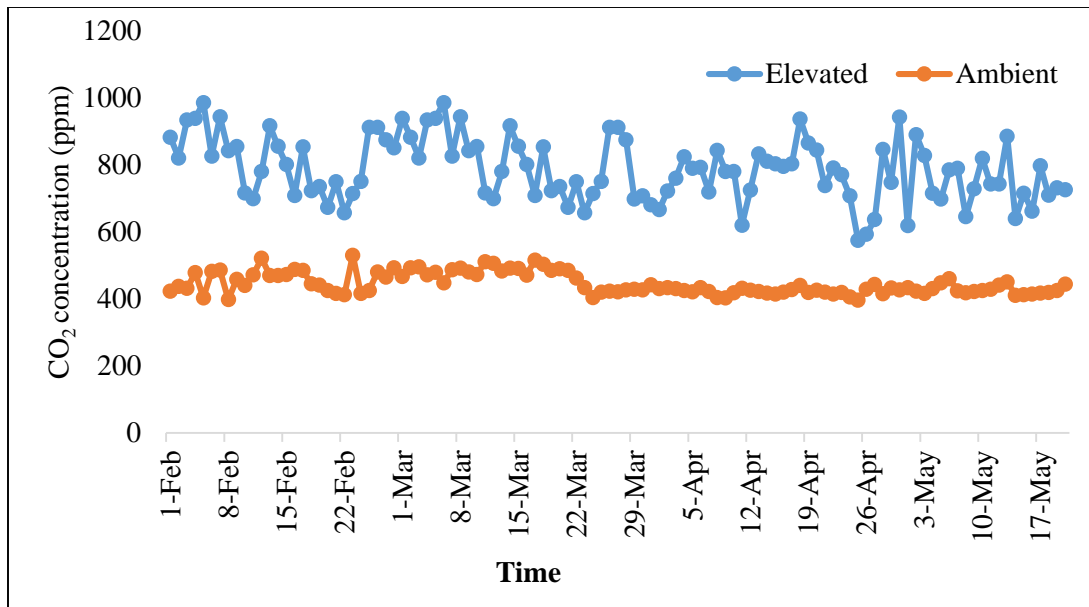


Fig. 3.1. Average daily value of ambient and supplemental CO₂ levels in greenhouses at Stillwater, OK, in which leafy greens were grown using hydroponics.



Fig. 3.2. Basil, lettuce, and Swiss chard (left to right in both figures) grown in ambient (A) and elevated CO₂ (B) condition.

CHAPTER IV

EFFECTS OF ELEVATED CO₂ ON ROOTING OF *EUONYMUS* 'MOONSHADOW' UNDER DIFFERENT CONCENTRATION OF ROOTING HORMONE

Abstract

Although effect of rooting hormone in the propagation of ornamental species has been studied by many scientists, very few have contributed to understanding the effect of supplemental CO₂ in rooting of plants. With the aim of understanding the effect of CO₂ in rooting and its interaction with rooting hormone treatment, a greenhouse study was conducted. Two identical greenhouses were used in which, one was supplied with an average of 800 ppm of carbon dioxide and the other one was at about 400 ppm (ambient) throughout the rooting period. Rooting hormone treatments were control, 1000, 3000, and 5000 ppm concentrations of Dip'N Grow. *Euonymus* 'Moonshadow' cuttings were dipped in rooting hormone and grown for 45 days under mist. Each greenhouse had four replications of each treatment and each treatment had 12 samples. The whole experiment was repeated and rooting percentage, root number, root length, root diameter, and root dry weight were measured. In the study, elevated CO₂ did not promote root development when compared to ambient condition. However, increasing rooting hormone concentration significantly affected the rooting parameters. Rooting percentage was not improved under elevated CO₂.

Ambient CO₂ with 5000 ppm concentration of Dip'N Grow showed greater root number. Thus, application of elevated CO₂ might not be beneficial in propagation of *Euonymus* 'Moonshadow'.

Introduction

Propagation of ornamental shrubs is an important aspect of the nursery and ornamental industry. Among different methods of asexual propagation, taking cutting is a widely used method for cloning shrubs and trees (Witcher et al., 2014). Cutting propagation is important in tree and shrub improvement programs to reduce production time, helps in mass vegetative production, and ensures the establishment of clonal seed orchard (Kesari et al., 2009). Furthermore, use of cuttings eliminates the long dormancy period, which is exhibited by some plants grown through seeds. Plants grown through cuttings resemble mother plants and retain special qualities and characteristics (Henrique et al., 2006; Somashekhar and Manju, 2002). Depending upon the availability of cutting material, a single cutting may cost a few cents to thousands of dollars for rare species. Propagation through cutting could be an expensive operation, so a high percentage of rooting and good quality root system is highly desired (Meredith et al., 1970). There are numerous factors which affect root development. Quality of stock plants, the season of propagation, environmental factors, size of the container, rooting substrate, use of rooting hormones, and watering frequency may affect root development (Witcher et al., 2014). Hartmann et al. (2002) reported the entire process of root formation is based on three stages: initiation, the growth of root initials, and development and establishment of the root system. Various studies have shown the role of auxin in stem growth and

adventitious root formation (Henrique et al., 2006; Kesari et al., 2009). Indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) are the most widely used auxins and can be naturally occurring or synthetic, but are equally effective (Zimmerman and Wilcoxon, 1935). However, rooting success may depend on the timing of application, methods adopted, types of auxin used, and concentration of auxin applied on the cuttings. Basal quick dip and basal powder application are the most common methods of auxin application (Blythe et al., 2007). For different types of cuttings, different concentrations of rooting hormones are required. Tip cutting or softwood cutting may require a lower concentration of auxin, whereas species like magnolia (*Magnolia acuminata* L.) may require up to 30000 ppm of IBA for successful rooting (Sharma et al., 2006). Even within a single plant species, tip cutting or softwood cuttings may require a lower concentration of hormone than hardwood cuttings (Blythe et al., 2007).

Temperature, light, moisture, humidity, CO₂, substrate, container size, and other environmental factors influence the success of cuttings. Among these, CO₂ plays a crucial role in root development of cuttings. Grant et al. (1992) stated that increased CO₂ resulted in increased photosynthesis and carbohydrate storage in the plant stem. The accumulated carbohydrate is assumed the source of energy for adventitious root development (Davis and Potter, 1983; French, 1990). However, increased concentrations of CO₂ have shown both positive and negative effect in terms of root development. French (1990) reported a decrease in rooting percentage of rhododendron (*Rhododendron* L. 'Anna Rose Whitney') when a mixture of CO₂ and water was misted during propagation. However, propagation of sweet potato (*Ipomoea batatas* L.) (Bhattacharya et al., 1985) and raspberry (*Rubus idaeus* L.) had increased rooting percentages when the CO₂

concentration was greater (Laforge et al., 1991). Variation among the studies has been found for the concentration of CO₂ maintained during propagation. Studies on the effect of CO₂ have been conducted from double ambient (i.e. 700 ppm) to 3000 ppm (Kirkham, 2011). Similarly, methods of CO₂ supplementation have also changed over time. Before the 1970's, a mixture of CO₂ and water was supplemented through mist (Davis and Potter, 1983; Kirkham, 2011), but these days CO₂ generators are used to produce CO₂ in gaseous form. Increase in rooting percentage, root dry weight, and reduction in the timing of root initiation for early production of cuttings are some reported advantage of CO₂ supplementation during propagation (Rogers et al., 1999).

Euonymus fortunei (Turcz.) Hand.-Maz. 'Moonshadow' commonly known as the spindle, Fortunei's spindle, winter creeper, or wintercreeper is a bushy shrub belonging to the family Celastraceae and is native to East Asia. The genus consists of more than 176 species and varieties that are mostly evergreen shrubs and have landscape importance (Hou, 1975). The genus is a fast-growing plant able to grow in different habitats. The evergreen nature and variegated pattern of the leaves makes the plant an integral part of the nursery industry and has a high consumer preference (Boyer et al., 2008). Many species of this genus are seedless, thus taking cutting is a good option for mass production (Poston, 2007). Generally, cuttings are taken in spring and summer root early (Lee and Tukey, 1971) and the genus *Euonymus* L. is considered an easy to root species (Hartmann et al., 2002). The timing of cuttings during the year determines the rate of rooting hormone required for rooting. Generally, 1000-3000 ppm of IBA in the spring and up to 8000 ppm of IBA in the fall is required for rooting of *Euonymus alatus* (Thunb.) Siebold in 5-7 weeks (Whitcomb, 1978). However, studies on the influence of CO₂ elevation on *Euonymus* propagation has

not been reported. Thus, this study was conducted with the objective of studying how elevated CO₂ concentrations affects the rooting of *Euonymus* and how CO₂ interacts with exogenous application of rooting hormone.

Materials and Methods

Plant materials and growth conditions

On 15 December 2016 and 5 January 2017, two different shipments of 6 cm cuttings of *Euonymus* 'Moonshadow' were shipped from Greenleaf Nursery Co. (Parkhill, OK). The cuttings were kept in a cooler overnight and the next day cuttings were inserted into 5.08 cm × 5.71 cm × 8.25 cm flats (Johny's selected seed, Winslow, ME). The flats were filled with a 1:1 perlite and vermiculite mixture. About 2-3 cm of each cutting was dipped in respective treatments for 15 seconds and placed in media. The flats were placed on mist benches and a timer was set to turn on the system every 16 minutes for 8 seconds. In the greenhouse, day/night temperature was set at 21 °C/18 °C, respectively. Both groups were left for rooting for 45 days then roots were harvested. On 30 Jan 2017, the first experiment was harvested and on 20 February 2017, the second was harvested.

Experimental setup and treatments:

Rooting of 'Moonshdow' stem cutting was studied in a replicated experiment conducted in the Department of Horticulture and Landscape Architecture research greenhouse at Oklahoma State University, Stillwater, OK. Cuttings were grown in a split-plot design in which, two identical greenhouses were used. One of the greenhouse was

supplemented with an average of 800 ppm of CO₂ (Fig. 4.1) and other was at the ambient CO₂ condition (400 ppm). A CO₂ generator (Johnson Gas Appliance Company, Cedar Rapids, IA) was used for CO₂ generation and was monitored by CO₂ Monitor (FLIR Commercial System Inc., Nashua, NH). The CO₂ generator was set to produce an daily average of 800 ppm of CO₂ by burning natural gas (Fig 4.1). The generator was automatic and turned on from 6:00 AM to 2:00 PM.

In addition to the CO₂ treatment, the effect of different concentrations of a commercially available liquid product (Dip'N Grow (Dip'N Grow Inc., Clackamas, OR) was used in rooting. The product consists of 1% Indole-3-Butyric Acid (IBA) and 0.5% Naphthaleneacetic acid (NAA). Control, 1000, 3000, and 5000 ppm concentration of rooting hormone was applied to basal part of cuttings to promote adventitious root development. In each greenhouse, four replications of all treatments were made and each treatment had 12 samples of cuttings.

Measurements and statistical analyses:

In each treatment, cuttings having adventitious roots were cleaned with water and measured. All other measurements were made from cuttings that had developed roots. Rooting percentage, root number, length of roots (average of two longest roots), root diameter, and dry weight of roots were measured. For dry weight, roots were harvested, cleaned, and dried at 60 °C for 72 hours. All data were subjected to two-way Analysis of Variance using SAS (Statistical Analysis System) version 9.4 (SAS Institute, Cary, NC). The two ways interaction between CO₂ and different concentrations of rooting hormone was studied in two different sets of cuttings. Means were computed using PROC MIXED

and pdmix800 macro program was used for mean separation between the treatments. In the case where interactions were found insignificant, means of the main effect were computed at 5% level of significance.

Results

Interaction of CO₂ x Rooting Hormone was significant for most of the parameters except rooting percent and root diameter (Table 4.1). There was no significant difference in rooting percentage between ambient and elevated CO₂ as well as rooting hormone treatments had similar rooting percentage (data not shown). In the study, root number decreased with increasing level of CO₂ (Table 4.2). The cuttings placed in 5000 ppm concentration of rooting hormone in ambient CO₂ condition had a significantly greater number of roots when compared to the elevated CO₂ condition. Root numbers in ambient condition were greater (27.8%) when compared to the elevated condition in 5000 ppm rooting hormone treatment. However, root number for 3000 ppm treatment in both ambient and elevated CO₂ and 5000 ppm treatment in elevated CO₂ were statistically similar, but lower than 5000 ppm rooting hormone treatment at the ambient CO₂ condition. However, for the control and 1000 ppm rooting hormone treatment, the difference in root number was similar and was the lowest. Root length was greater in the 5000 ppm rooting hormone treatment at ambient CO₂ (Table 4.2). However, the values for root length was statistically similar to all other treatments except the control treatment at ambient CO₂ condition. The greatest root length of 2.3 cm and smallest root length of 1.6 cm was measured in ambient CO₂ in 5000 ppm and control treatments, respectively. Similarly, root dry weight also increased with increasing concentration of rooting hormone. Cuttings treated with 5000 ppm rooting hormone in both CO₂ treatments and

cuttings with 3000 ppm rooting hormone under elevated CO₂ had the highest root dry weight. Root dry weight was nearly 200% greater in the 5000 ppm treatment when compared with the control in ambient CO₂ condition. The control and 1000 ppm treatment had the smallest root dry weight and were statistically similar in both ambient and elevated CO₂ conditions.

Rooting hormone treatments were significantly different as a main effect for root diameter. The root diameter increased with increasing concentration of rooting hormone and was greatest with 3000 and 5000 ppm rooting hormone (Table 4.3). Cuttings under 3000 and 5000 ppm rooting hormone had a diameter of 0.11 and 0.12 cm, respectively, and were similar. Similarly, the control and 1000 ppm rooting hormone treatments were statistically similar and had the smallest value for root diameter. Besides the measured parameters, significant difference in leaf senescence was observed between CO₂ treatments (Fig. 4.2 and 4.3). The cuttings treated with supplemental CO₂ had complete leaf senescence but the cuttings under ambient CO₂ had intact leaves and more shoot growth (data not shown).

Discussion

Both ambient and elevated CO₂ concentrations showed gradual increments in root numbers with increasing rooting hormone concentration. Root number was the greatest at the highest concentration of root hormone under ambient CO₂ condition. However, there was no significant effect of either CO₂ or rooting hormone in rooting percentage. Poston (2007) reported that *Euonymus* sp. is an easy to root species and even rooting hormone at lower concentration is sufficient to promote rooting (Poston, 2007). Swamy et al. (2002)

reported that the response of rooting to different rooting hormone concentration is species specific and affected by season of cutting. Since carbohydrate level in cuttings plays a significant role in rooting response; the change in carbohydrate content is seasonal and may affect the rooting response (Davis and Porter, 1983). However, cuttings placed in elevated CO₂ had early leaf senescence, which might have affected the rate of photosynthetic accumulation in cuttings under elevated CO₂. In contrast, the leaves were intact in ambient CO₂ and may have had a greater carbohydrate level in the stem due to more leaves, which might have resulted in more root numbers. However, a study in rooting of *Rhododendron* 'Anna Rose Whitney' showed no relation between carbohydrates level in the stem and rooting (French, 1990). The author reported inhibition of rooting with increasing carbohydrate level, but the mechanism is still unknown. Similarly, Lee and Tukey (1971) also reported no significant difference in root number of *Euonymus alatus* 'Compactus' with increasing concentration of IBA. In support of Lee and Tukey (1971), a study in rose (*Rosa hybrid* L. 'Madelon') reported no role of auxin in the promotion of rooting but reported a significant role of auxin in cell elongation. Yet, Lee and Tukey (1971) reported increased root length with increasing IBA concentration during rooting. Similar to our study, Bhattacharya et al. (1985) reported an interaction effect of CO₂ and IBA for root number and root length in sweet potato 'Georgia Jet'. The authors suggested that certain balance needs to be maintained between stem carbohydrate and auxin level for promotion of rooting.

An increase in root dry weight in higher rooting hormone concentration in both ambient and elevated CO₂ could be the result of greater number and length of roots in these treatments. For each rooting hormone treatment, the dry weight was not different

when compared to ambient and elevated CO₂ condition. Similar to our study, Patterson et al. (1988) in cotton (*Gossypium hirsutum* L.) and Kaushal et al. (1989) also reported no effect of supplemental CO₂ in root dry weight in black pine (*Pinus nigra* L. 'Corsicana'). In contrast, Laforage et al. (1991) reported an increase in root dry weight of raspberry (*Rubus ideaus* L.) by 173-245% when compared between ambient and 1600 ppm CO₂ concentration. Although leaf abscission was not considered as one of the measured parameters in the study, the effect was clearly visible between ambient and elevated CO₂ condition in our study (Fig. 1 and 2). French (1989) reported similar leaf senescence in *Rhododendron* 'Mortha Isaacson' when propagated in fall and only 5% of leaves were intact in cuttings misted with 1200 ppm of CO₂ but more than 50% were intact in case of ambient condition. Application of supplemental CO₂ in sunflower (*Helianthus annus* L.) promoted ethylene production from plant tissue (Dhawan et al., 1981). Since, ethylene is responsible in senescence, the increased ethylene in tissue level might have resulted leaf abscission (French, 1989). However, the author also mentioned that ethylene production in elevated CO₂ might affect leaf abscission but promoted rooting. A similar negative effect could have happened in our supplemental CO₂ study too, which resulted in leaf senescence but no difference in rooting percentage. In the future, more studies are needed with multiple CO₂ levels, different types of rooting hormone, and at different concentrations in multiple species to fully understand the effect in root development.

Conclusion

Elevated CO₂ had a negative effect in rooting of *Euonymus* 'Moonshadow'. Although many studies have reported positive effect of elevated CO₂, the response is species specific. Application of rooting hormone improved root numbers and cuttings had a well-established root system with 3000 or 5000 ppm hormone. From the study, supplemental CO₂ could not be recommended for better rooting of *Euonymus* 'Moonshadow'. However, future research in supplemental CO₂ should consider the interaction with various environmental factors, which might result in better rooting.

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Table 4.1. Analysis of variance (ANOVA) showing the effect of CO₂ (ambient at 400 ppm and elevated at an average of 800 ppm) and Dip'N Grow rooting hormone (control, 1000, 3000, and 5000 ppm) on different root parameters of *Euonymus* 'Moonshadow'.

Effect	Rooting %	Root number	Root length	Root diameter	Root dry weight
CO ₂	ns ^z	ns	ns	ns	ns
Rooting hormone	ns	***	***	***	***
CO ₂ × Rooting hormone	ns	*	*	ns	*

^z*, *** indicate the level of significance at $P < 0.05$ and $P < 0.0001$, respectively and ns indicates that the treatments are not significant.

Table 4.2. Effect of CO₂ (ambient at 400 ppm and elevated at an average of 800 ppm) and Dip'N Grow rooting hormone (control, 1000, 3000, and 5000 ppm) on different root parameters of *Euonymus* 'Moonshadow'.

Rooting hormone (ppm)	Root number		Root length (cm)		Root dry weight (g)	
	Ambient	Elevated	Ambient	Elevated	Ambient	Elevated
Control	12.9d ^z	11.8d	1.6b	1.8ab	0.008d	0.008d
1000	14.8d	19.1cd	1.9ab	1.8ab	0.010cd	0.014bcd
3000	23.6bc	24.3bc	2.1ab	1.9ab	0.016bc	0.020ab
5000	35.4a	27.7b	2.3a	1.9ab	0.024a	0.020ab

^zMeans (n=24) within a parameter followed by same letters are not significantly different at $P \leq 0.05$.

Table 4.3. Effect of Dip'N Grow rooting hormone (control, 1000, 3000, and 5000 ppm) on root diameter of *Euonymus* 'Moonshadow'.

Rooting hormone (ppm)	Root diameter (cm)
Control	0.10b ^z
1000	0.10b
3000	0.11a
5000	0.12a

^zMeans (n=48) within a column followed by same letters are not significantly different at $P \leq 0.05$.

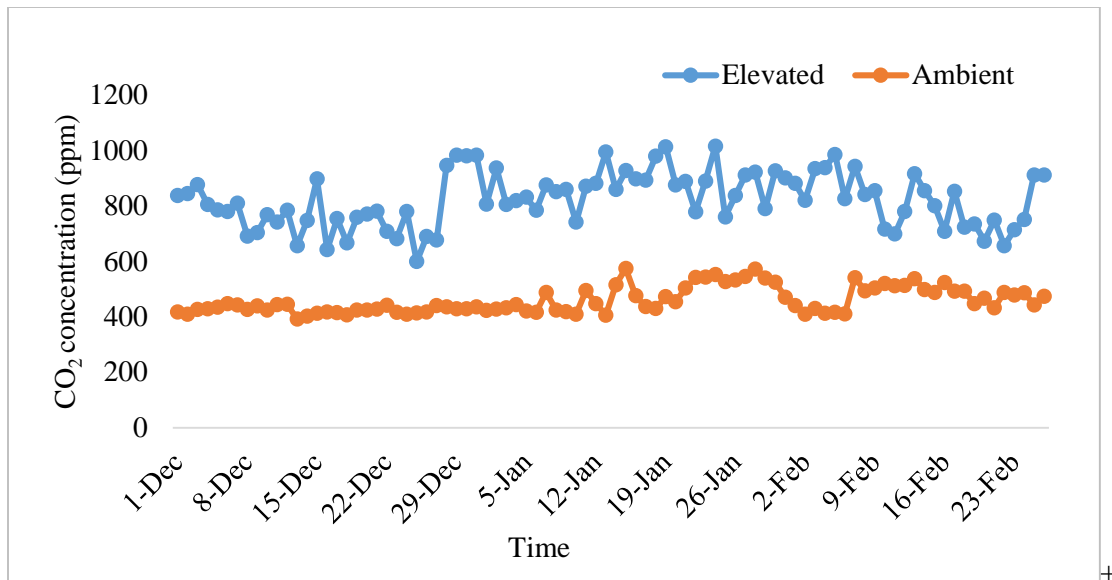


Fig. 4.1. Ambient and Elevated CO₂ levels maintained during propagation of *Euonymus* 'Moonshadow'



Fig. 4.2 Cuttings of *Euonymus* 'Moonshadow' at ambient (400 ppm) CO₂. Control, 1000, 3000, and 5000 ppm of Dip'N Grow rooting hormone from left to right, respectively.



Fig. 4.3. Cuttings of *Euonymus* 'Moonshadow' at elevated (an average of 800 ppm) CO₂. Control, 1000, 3000, and 5000 ppm of Dip'N Grow rooting hormone (from left to right, respectively)

CHAPTER V

CONCLUSION

Application of supplemental CO₂ in commercial greenhouses is increasing. Small CO₂ generators are sufficient to increase CO₂ concentration up to 800-1000 ppm, which can have a significant effect in plant growth. The studies herein were designed to understand the application of small CO₂ generator in greenhouse production system. Since the greenhouse industry includes multiple crops requiring different growth environments, the research tried to cover irrigation and fertilizer management in pot grown ornamentals, propagation of an ornamental shrub through asexual propagation, and production of hydroponic leafy greens under supplemental CO₂ condition.

A replicated study of geranium and fountain grass showed increased plant growth with supplemental CO₂. Supplemental CO₂ could be beneficial in increasing plant size and might be used in the nursery industry to manipulate the growing cycle. Similarly, delayed flowering in geranium signifies that supplemental CO₂ could be beneficial in manipulating flowering time but could be species-specific. As expected, plants in well-wetted irrigation did not perform well. When irrigation is managed through an automated tensiometer, the type of soilless media used and crop water requirement must be considered. Potted plants grown in a greenhouse might require moderate irrigation with

greater fertilizer rates to proportionate increased growth under supplemental CO₂ condition.

Application of supplemental CO₂ in hydroponically grown leafy greens showed greater growth of plants and plants were ready to harvest earlier compared to ambient CO₂ condition. However, some species might show physiological disorder like tipburn in lettuce under supplemental CO₂. Thus, growers need to be cautious about potential damage before application of supplemental CO₂. Similarly, low tissue N level in plants suggests future studies should examine the plant nutritional quality of leafy greens when grown in supplemental CO₂.

No difference in rooting percentage of *Euonymus* cuttings in supplemental CO₂ suggests that application of supplemental CO₂ might not be beneficial for propagation of euonymus. However, rooting hormone at 5000 ppm could be beneficial in increasing root numbers and development of well-established root system.

CHAPTER VI

APPENDICES

Appendix I: Figure illustrating the hourly measured ambient and elevated CO₂ concentration in a typical cloudy and sunny day.

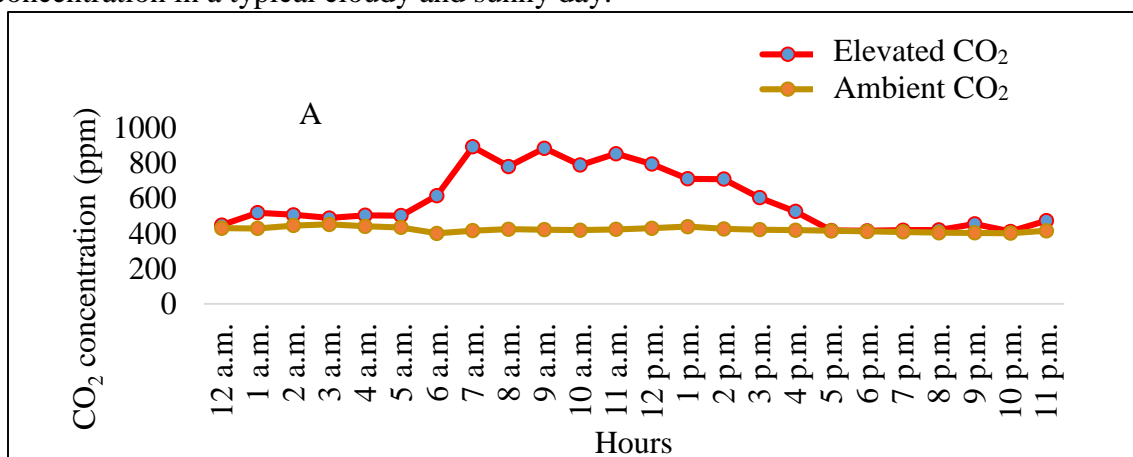


Fig. A. Hourly measured CO₂ concentration in a typical cloudy day in February 2017, when average daily temperature inside the greenhouse was 22 °C.

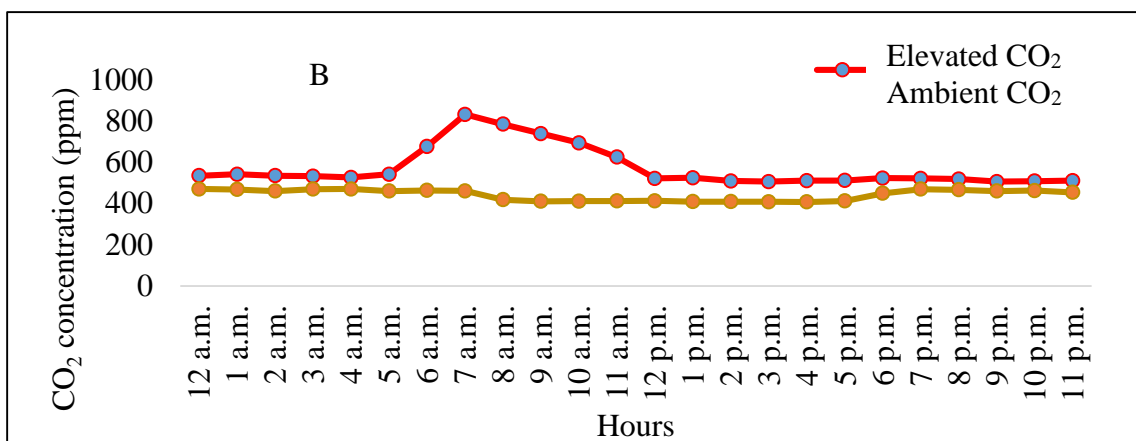


Fig. B. Hourly measured CO₂ concentration in a typical sunny day in March 2017, when average daily temperature inside the greenhouse was 28 °C.

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