Investigating the relationship of increased biomass and nutrient content of soybean (*Glycine* max merr)

by

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Abstract

Declining nutrient concentration in higher yielding cultivars is evident in literature meta-analyses assessing cultivars developed over the last century and studies performed under elevated CO_2 to artificially increase yields and study nutrient concentrations. In meta-analyses covering soybean yield improvement and nutrient decrease, data has been collected over decades during which farm management and varieties have changed concurrently. More efficient agricultural management and breeding for higher yielding cultivars greatly improved overall soybean production but also resulted in unintended mineral nutrient decreases. Whether the nutrient decrease is due to management practices, cultivar improvements or a combination of both is still unknown. For this reason, it is necessary to study nutrient and yield relationships with old and new cultivars grown under similar conditions and practices. By growing old and new cultivars under 4 different nutrient regimes under equivalent farm practices, differences in yield, soil nutrient availability and nutrient uptake can be studied with the aim of understanding if the nutrient decrease observed in high yielding cultivars is due to a dilution of minerals, increase nutrient efficiency, or to a limitation of nutrient absorption by roots. Using these cultivars, we then determined an old and a new cultivar with the greatest difference in nutrient uptake for which we then grew under elevated CO_2 concentrations ($[CO_2]$) in open top chambers. This increased yield in both cultivars, allowing us to study the nutrient response in cultivars that accumulate nutrients differently under ambient $[CO_2]$. Using elevated $[CO_2]$ allowed us to study nutrient and yields under conditions known to alter photosynthesis and transpiration. Decreased nutrient concentrations and transpiration rates were observed in the larger biomass produced under elevated $[CO_2]$. Therefore, transpiration should not be excluded as a factor behind dilution

in larger yields. Understanding climate change factors that influence nutrient content is essential for meeting future food demands.

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List of Abbreviations

Ca	Calcium
$[CO_2]$	Carbon dioxide concentration
Fe	Iron
V_{cmax}	Maximum rate of Rubisco carboxylation
J_{max}	Maximum rate of RuBP regeneration
MG	Maturity group
N	Nitrogen
OTC	Open Top Chamber
P	Phosphorus
K	Potassium
g_s	Stomatal conductance
Zn	Zn

Chapter 1. Literature Review

History of Soybean

Soybean [*Glycine* max (L.) Merr.] most likely originated in China with domestication occurring during the Zhou dynasty (ca. 1125-256 BCE) (Hymowitz, 2008). By the 15th and 16th century, soybean use spread and took root in other Asian countries (i.e., Japan, Indonesia, Philippines, Vietnam, Thailand, Malaysia, Burma, Nepal, and northern India) as landraces were developed (Hymowitz, 1990). In the Western world, soybean was documented by European travelers as a staple food product: miso, soy sauce, and tofu (Hymowitz, 1990). In China, cultivated soybean became a staple for oil extracts used for industrial processes and bean cake used largely as fertilizer, but human consumption remained the primarily use of soybean (Prodohl, 2013). By the 18th century, soybean was introduced to Europe and documented for various uses, such as basic gardening, ornamental purposes, and animal feed (Hymowitz, 1990). Introduction of soybean to the New World had multiple routes, including both Benjamin Franklin and Samuel Bowen (planting in Georgia) during the 18th century (Hymowitz, 1990). By 1851, soybean was introduced to Illinois followed by a large expansion across Canada due to the large potential value as animal feed (Hymowitz, 1990).

During the 20th century, the U.S. government encouraged soybean cultivation to fill wartime needs thereby expanding the United States' role in the global soybean market (Prodohl, 2013). By the mid-20th century, United States soybean production boomed (with much of it being exported) resulting in the United States emerging as the world's leading soy-producing country (Pordohl, 2013). In 2020, soybean was the largest agricultural export from the United States highlighting its economic importance (USDA, 2020). Today, the top three soybean producing countries are Brazil, United States, and Argentina, and the largest soybean importer is China

(FAOSTAT, 2020). With soybean ranking as the fourth most important crop in the world, it is a key component for doubling global food production by 2050 (Ainsworth et al. 2012). Soybean plays a vital role in global food security as a major source for animal feed and for over half the world's oilseed production (Ainsworth et al., 2012). Economic development of countries (e.g., China, Brazil, India, etc.) further increased meat consumption which led to increased demand for animal feed. With growing economic affluence, meat plays a larger role in diets, which elevates the need for animal feed and soybean production.

Meeting global soybean demands and possible solutions

Given an annual growth rate of 77 million people per year (Carvalho, 2006), the world population is expected to reach ~9.73 billion by 2050 (FAO, 2017). Since population increase relates directly to global food demand, food production will also need to increase (Cleland, 2013). To meet global food and fiber demands of the projected 2050 population, current crop production will need to double (Tilman et al., 2011). There are two general strategies to increase food production: 1) Increasing agricultural land, which is very limited due to lack of land suitable for agriculture (Brown, 1997) and environmental impacts associated with land use change; or 2) Producing more food from the same amount of agricultural land, thereby closing the gap between actual yield and yield potential (Godfray et al., 2010). To double production by 2050, average soybean yield needs to increase at a rate of ~2.4% per year (Ray et al., 2013). To increase our yields rapidly, we need to understand how yields were increased during the last century (Ainsworth et al., 2012; Koester et al., 2014; 2016).

Over the last century, soybean yield greatly increased due to improved agricultural management practices and plant breeding (Sacks & Kucharik, 2011). Agronomic practices that increased soybean yields include earlier planting dates, higher planting density, pesticide and fertilizer use, and post-harvest loss reductions (Rowntree et al., 2013). Yield gains from breeding are due to intended or un-intended selection for stress tolerance, higher nutrient and water use efficiencies, disease resistance, reduced lodging, shattering, and other agronomic characteristics related to yield increases (Sacks & Kucharik 2011). One example is the use of disease resistant cultivars in locations where the targeted disease is prevalent. The presence of a disease is dependent on the suitability of the local climate, which means cultivar success will vary by disease resistance. Currently, soil infertility is the primary crop yield constraint in developing nations (Mohammadi & Sohrabi, 2012). Chemical fertilizers are the major inputs used to increase soil fertility and crop yield. However, excessive use of chemical fertilizers leads to environmental pollution and soil structure degradation (Savci, 2012). In this context, researchers are studying management and breeding strategies to improve nutrient absorption by plants to reduce chemical fertilization (Pilbeam, 2015).

Importance of mineral nutrients for plant growth

Nutrients are essential for plant growth and health; this directly relates to crop productivity. Here, I will focus on the six macro-nutrients [nitrogen (N), potassium (K), phosphorus (P), calcium (Ca), sulfur (S), and magnesium (Mg)] and two micro-nutrients [iron (Fe) and zinc (Zn)] since these are needed in the greatest amounts and/or have been previously related to greater yields response (Parvin et al., 2019). Nitrogen and P are essential building blocks of proteins, sugars, and nucleic acids used in all plant developmental stages (Ohyama, 2010). Since N plays a vital role in plant development, N deficiency can cause chlorosis, reduced growth, and accelerated maturation that can result in lower yields (Ohyama, 2010). Jeuffory and Bouchard

(1999) found that intensity and duration of N deficiency determine the level of yield reduction in wheat, but any N deficiency reduced overall yield relative to the control. Phosphorus is mainly used during pod and seed development; without sufficient available P, growth is stunted and yield is reduced (Imas & Hagen, 2007). Potassium, Ca, and Mg are present in plants as cations which control osmotic pressure, pH, and enzymatic activity (Ohyama, 2010). Low K+ ion transport in guard cells leads to a drop in CO_2 diffusion, which ultimately leads to photosynthesis down-regulation (Singh & Reddy, 2018). Since K is involved in functional and structural roles of photosynthesis, this nutrient was investigated in Chapter 2. Calcium is taken up via the xylem and is not redistributed within phloem tissue, which makes the plant dependent on a long-term supply of Ca (White & Broadley, 2003). For this reason, Ca largely affects developing tissue and eventually the harvestable portion of the crop (White & Broadley, 2003). Since Mg is involved with chlorophyll pigments and enzyme cofactors in photosynthesis, Mg deficiency ultimately leads to diminished carbon fixation and crop yields (Guo et al. 2015). Iron and Zn play a role in maintaining metabolic and physiological processes due to their unpaired electrons (Zargar et al., 2015). Iron is essential in the electron transport chain and a deficiency can trigger oxidative reductive reactions (Zargar et al., 2015). Zinc concentration is vital to its uptake and concentration of RNAses and starch which helps control RNA degradation (Zargar et al., 2015). The vital role played by Fe and Zn in maintaining photosynthetic processes highlights their influence on overall crop yield (Zargar et al., 2015).

Breeding for agronomic and physiological traits to increase yields

Great efforts are aimed at understanding the physiological and agronomic factors driving higher yields in newer cultivars [later year of release (YOR)] when grown side by side with earlier YOR cultivars (Koester et al., 2014). The Monteith equation has been used to explain which physiological and agronomic characteristics are responsible (Monteith, 1972; 1977; Ort, 2011; Koester et al., 2014). In the absence of biotic and abiotic stresses (such as extreme weather events or disease), yield potential is defined by four factors (efficiencies) outlined in the Monteith equation:

$$Yp = 0.487S_t \text{ x } \varepsilon_i \text{ x } \varepsilon_c \text{ x } \varepsilon_p$$

Where Yp is the yield potential. S_t is the total incident solar radiation absorbable by the plant during the growing season (this is reduced to 48.7% since only this percentage is photosynthetically active light). ε_i is light interception efficiency of the plant and depends on leaf area and on how fast the plant closes the canopy. Cultivars that have more leaf area and close the canopy earlier in the season have a higher ε_i (Koester et al. 2014; Slattery et al. 2013). ε_c is the energy conversion efficiency which describes how much of the absorbed light is transformed into aboveground crop biomass. ε_c is comprised by all the processes involved in the conversion of the received light into carbohydrates and plant biomass. Therefore, ε_c depends on the light and dark reactions of plant photosynthesis, respiration, photorespiration, and carbohydrate usage and partitioning (Zhu et al., 2008; 2010; Slattery et al. 2013; Koester et al. 2014). ε_p is the partition efficiency (also called the harvest index) which refers to the amount of total aboveground biomass that is partitioned as seed (Monteith, 1977). Cultivars with higher ε_p tend to have higher yields (Koester et al., 2014).

Breeding efforts have historically improved ε_i and ε_p by targeting longer growing seasons, faster canopy closure, lodging resistance, and harvest index; all of which progressed yields (Koester et al. 2014). From 1924 to 2010, soybean yields increased 22.2 kg ha^{-1} annually (Ainsworth et al. 2012), which resulted from light interception and partitioning efficiency reaching close to their theoretical maxima (Zhu et al., 2010; Koester et al. 2014). Although light interception and partitioning efficiency are close to their theoretical maxima, ε_c remains at nearly half of its theoretical maximum leaving it as a significant target for crop improvement (Slattery et al. 2013; Koester et al., 2014). Abiotic stresses, greenhouse gas concentrations, nutrient inputs and farm management influence photosynthetic efficiency and potential crop yield (Slattery et al. 2013). We need to understand how climate change may influence these parameters and resulting yields. Gray et al. (2016) found drought stress abated expected increases in water use efficiency and further reduced soil moisture in soybean grown under elevated [CO_2]. Elevated [CO_2] decreases plant water use by reducing stomatal conductance due to higher inter-cellular [CO_2], under drought conditions combined with e[CO_2], greater canopy temperature and leaf area offset e[CO_2] growth benefits (Gray et al., 2016). Multiple changes in growth conditions associated with climate change will affect factors comprising photosynthetic efficiency and potential crop yield.

Decreased mineral concentrations as an unintended outcome of breeding for higher yields

Soybean production has increased ten-fold from 1961 to 2014 reaching more than 306 million Mg globally (Balboa et al., 2018) and has reached ~2.9 tons/ha in 2020 (USDA, 2020). Increases in crop yield have been driven by more efficient production management, environmental conditions, and genetic improvements (Balboa et al., 2018; Koester et al., 2014; Garvin et al., 2006). Specifically, genetic improvements focused on longer pod-filling periods, decreased lodging, disease resistance, and overall biomass that all targeted yields (Balboa et al. 2018; Koester et al., 2014; Garvin et al., 2006). These breeding targets overlooked nutrient concentration, nutrient efficiency, and nutrient content throughout the whole plant (i.e., seeds, stover, stems, etc.) (Balboa et al. 2018).

Balboa et al. (2018) evaluated literature published from 1931 to 2017 to characterize historical shifts in soybean nutrient content, nutrient use efficiency, and nutrient stoichiometry. From 1931, seed and stover N concentration remained stable, seed P concentrations fell, while K concentration decreased in seed and increased in stover (Balboa et al., 2018). For internal efficiency (also called nutrient use efficiency or amount of nutrient used per unit of biomass), N and P increased while K decreased (Balboa et al. 2018). Concentrations and efficiencies were used to compare nutrient amounts in whole plants or within individual plant organs (Balboa et al., 2018). This revealed that soybeans were able to increase yields with similar amounts of N and P but needed greater amounts of K (Balboa et al. 2018). Garvin et al. (2006) grew 14 cultivars of hard red winter wheat (*Triticum*) (YOR spanning 100 years) and compared yield, YOR, and micronutrient content. While yields increased with more recent YOR, a negative regression with YOR was revealed for newer cultivars, Fe and Zn (Garvin et al., 2006). Although the magnitude of decreasing micronutrient concentration varied with location, annual percent decreases ranged from 0.16% y^{-1} to 0.38% y^{-1} highlighting an on-going pattern of falling nutrient concentrations in more productive newer varieties (Garvin et al., 2006). This pattern in grain crops was further assessed by Fan et al. (2008) who observed declining Zn, Mg, Fe, and copper (Cu) in wheat cultivars developed between 1840 and 2000. This decline was especially apparent after 1960, which marks the introduction of semi-dwarf high yielding cultivars during the Green Revolution (Fan et al., 2008). Davis et al. (2004) found a similar decline pattern associated with cultivar changes across 43 garden crops for protein, Ca, Fe, riboflavin, and ascorbic acid, which further reveals a possible trade-off between yield and nutrient content. This trade off occurs because crop breeding alters characteristics to increase yield, but nutrient concentrations decrease with biomass accumulation (Balboa et al., 2018; Garvin et al., 2006; Fan et al., 2008; Davis et al., 2004). Since crops deliver the necessary calories and essential mineral nutrients for human and animal nutrition, this highlights the importance of understanding the mechanisms driving this trade-off.

To test this trade-off, Reis et al. (2020) evaluated the effects of supplemental N fertilizer application on protein and amino acid concentrations in old and new soybean cultivars. Additional N fertilizer did not improve protein, and amino acid declined with higher yields; this suggest that the nutrient/yield trade-off was due to a physiological limitation rather than soil nutrient availability (Reis et al., 2020). Given a sufficient nutrient supply, the trade-off stems from a physiological limitation such as impediments in root-nutrient absorption and allocation/partitioning of nutrients between plant organs. Multiple theories have been proposed to explain physiological plant functions driving nutrient decreases. In this literature review, the three hypotheses to be covered are: 1) lower transpiration limits nutrient uptake due to reduced mass flow (McGrath & Lobell, 2013); 2) nutrient dilution caused by increased accumulated carbohydrates with nutrient content remaining steady (Chaturvedi et al., 2017); and 3) reduced density of root nutrient transporters in cultivars displaying greater biomass accumulation (Jauregui et al., 2016). Whether the decline in nutrient concentration with greater carbon accumulation is a matter of increased efficiency or plant physiological limitations, these processes may act together rather than being mutually exclusive.

Understanding mechanisms underlying this pattern of increasing yields and decreasing nutrient concentrations is vital since plants are the primary means of nutrient delivery to animals and humans. Two billion people suffer from Fe and Zn deficiencies annually, but this number excludes other nutrient deficiencies such as N via protein (Myers et al., 2014). Further, most human diets are not diversified enough to rely on multiple food groups to fulfill essential nutrient requirements, which further highlights the importance of understanding and preventing nutrient concentration decreases in future crops (Loladze, 2014).

Effect of elevated CO_2 on soybean yield and mineral concentration

Carbon dioxide (CO_2) concentrations are currently at the highest levels observed in the past 800,000 years (NOAA, 2020). Beginning with the Industrial Revolution, [CO_2] increased at an unprecedented rate due to accelerated fossil fuel use (NOAA, 2020). The rate of increasing global [CO_2] continues today. With the most conservative emission projections and hopeful mitigation strategies, [CO_2] is predicted to reach 500 ppm by the end of the century (IPCC, 2013). As [CO_2] increases at faster rates, understanding physiological limitations of crop nutrient uptake and yield is urgent to meet future global food demands.

With increasing [CO_2], C_3 plants are generally expected to increase in biomass (Ainsworth et al., 2002; Ainsworth & Rogers, 2007). Biomass stimulation is driven by greater photosynthetic activity and reduced stomatal aperture (Ainsworth & Rogers, 2007). A greater concentration of CO_2 around Rubisco increases carboxylation rate and reduces oxygenation resulting in increased sugar production, which ultimately produces greater biomass accumulation and thus yield (Ainsworth & Rogers, 2007). Soybean is one of the most studied plants grown under elevated [CO_2] conditions (Ainsworth et al., 2002). Since elevated CO_2 increases yield, growing plants in artificially increased [CO_2] environments could be another way to study reduced mineral concentrations as a result of higher yields. Within elevated CO_2 studies, a decline in mineral concentration is further observed (Loladze, 2014; Myers et al., 2014; McGrath & Lobell, 2013).

In a meta-analysis performed by Loladaze (2014), a pattern of declining nutrient content was robust across artificially (chambers, greenhouse) and field (FACE) studies, temperate and subtropical/tropical locations, and a vast number of crops important to human diet and health. With wheat, rice, barley, potatoes, and other C_3 plants decreasing in mineral nutrient content, animal and human diets will be further at risk for mineral deficiencies (Myers et al., 2014). For example, Fe and Zn concentrations of C_3 crops and legumes decrease under elevated [CO_2], which further contributes to the Fe and Zn deficiencies affecting nearly 2 billion people (Myers et al., 2014).

While mineral concentrations continue to decline with higher yields, the magnitude of decrease under elevated CO_2 depends on other environmental (i.e., water and nutrient availability) and genetic factors. In relation to yield, cultivar variations in total nutrient uptake allows us to study genetic differences that may underlie these variations. With these factors playing a part, identifying the mechanisms affected can help determine physiological pathways controlling nutrient uptake, partitioning, and differences between each nutrient mineral. Robustness of findings on declining mineral levels with greater yields (due to elevated [CO_2] and cultivar changes) underscores the importance of understanding underlying mechanisms to combat nutrient deficiencies impacting human and animal health.

Elevated [CO_2] induces decreases in grain mineral concentrations, specifically Zn, Fe, P, and S (Parvin et al., 2019). When elevated [CO_2] is combined with drought stress, decreases in Fe and Zn were exacerbated (Parvin et al., 2019). However, exposure to elevated [CO_2] under wetter environments still resulted in a dilution effect in lentils (*Lens* culinaris) and faba beans (*Vicia* faba) as shown by falling mineral (i.e., Fe, Zn, P, S, K, Mg) to carbon ratios (Parvin et al., 2019). When reduction of minerals under elevated [CO_2] occurs concurrently with drought, minerals that rely on diffusion and mass flow are highly affected by decreases in stomatal conductance and transpiration rate (Parvin et al., 2019). Nutrients that have higher concentration under elevated [CO_2] and drought appear to be less influenced by reductions in mass flow, which suggests multiple mechanisms involved in this phenomenon (McGrath & Lobell, 2013). More broadly, the dilution effect was evident since nutrient concentration generally decreased when elevated [CO_2] stimulated carbohydrate production. Much like findings of Parvin et al. (2019), the magnitude of decline varied by nutrient and by crop suggesting that multiple mechanisms affect nutrients and crops in different ways (Parvin et al., 2019; Myers et al., 2014). If all nutrient uptake across crops were driven by passive dilution, the percent change in decline should be equal for all minerals, which was not seen in several crops (Parvin et al., 2019; Myers et al., 2014).

There are numerous, hypothesized mechanisms aimed to explain why nutrient content decreases in high yielding cultivars and under elevated [CO_2]. However, no studies have compared the effect of elevated [CO_2] on mineral content of newer and older cultivars. Studies have largely focused on three theories concering how elevated [CO_2] and/or high yields affect mineral nutrient concentration: 1) decreased transpiration; 2) mineral dilution; and 3) reduction in mineral absorption.

1) *Decreased Transpiration:* The decrease in mineral content in seeds under elevated [CO_2] is a consequence of decreased transpiration that reduces the transfer of nutrients from roots to shoots (McGrath & Lobell, 2013). Minerals travel as dissolved molecules in the xylem and therefore depend on the transpiration stream to pull them from roots to aboveground biomass. Under elevated [CO_2], decreases in stomatal conductance reduces canopy transpiration and mass flow of nutrients to leaves (Leakey et al., 2009; Bernacchi et al., 2007; McGrath & Lobell,

2013). Jauregui et al. (2016) observed reduction of Zn and Fe concentrations in *Arabidopsis* leaves with reduced transpiration under elevated [CO_2].

2) *Mineral Dilution*: Mineral nutrient content decreases in plant organs as a consequence of dilution due to an increase in carbohydrate content (and yield) under elevated [CO_2] (Chaturvedi et al., 2017).

3. *Reduction in Mineral Absorption and Expression of Transporters*: Previous work has suggested that a reduction in mineral absorption in root tissue occurs under elevated [CO_2], while another hypothesis is that Zn and Fe transporters decrease in root, stem, and leaf tissue of plants grown under elevated [CO_2], which may influence the flux of these nutrients in a mineral- and organ- specific manner (Leakey et al., 2009; Jaugerui et al., 2016).

As seen with K, elevated [CO_2] can heighten the effects of nutrient deficiencies depending on severity (Singh & Reddy, 2018). Even with a dilution effect under elevated [CO_2], soybean photosynthetic processes were largely affected under severe K deficiencies (Singh & Reddy 2018). Under elevated [CO_2] and severe K deficiency, soybean photosynthetic processes were restricted due to diffusional limitations such as stomatal closure, whereas biochemical limitations occurred under sufficient and moderately K deficient conditions (Singh & Reddy, 2018). Under severe K deficiency, photosystem is inhibited by reduced photosynthetic pigments and light absorption since photorespiration is upregulated (Singh & Reddy, 2018). Since K plays such an important role in photosynthesis and transpiration by regulating stomata opening and also many photosynthetic enzymes (Singh & Reddy, 2018), the effect of elevated [CO_2] on K concentration requires more in-depth study.

Research Objectives

Objective 1: Test if new and old cultivars differ in yield and nutrient concentrations while ruling out involvement of insufficient soil nutrient availability

Objective 2: Test if decreased transpiration led to nutrient content changes with altered mass flow

Objective 3: Investigate yield and mineral nutrient responses of old and new cultivars grown under ambient and elevated atmospheric CO_2 with deficient and supplemental soil K

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Chapter 2. Soybean seed mineral nutrient concentration is dependent on yield potential and elevated CO_2 response

Abstract

Global CO_2 concentrations ($[CO_2]$) are predicted to increase within this century, which can affect plant photosynthesis and biomass production. Historical breeding efforts targeted aboveground biomass accumulation and harvest index to increase yields. However, yield increases have coincided with declining mineral nutrient concentration in seeds of newer/higher yielding cultivars. This decline in seed nutrient concentration could affect human and animal nutrition. The current study tested if newer cultivars with higher yields resulted in lower nutrient concentration and if this was limited by nutrient availability. For this testing, 8 soybean (*Glycine max* (L.) Merr.) cultivars (3 older cultivars, 3 newer conventional cultivars, and 2 transgenic commercial cultivars as checks) were grown under 4 fertilizer treatments (including a control) in the field. Mineral nutrient concentrations of newer cultivars declined with higher yields and were not limited by nutrient availability. To test if the reduced nutrient concentration in high yielding cultivars was caused by a dilution effect and/or a reduction in transpiration, we selected one old (Wabash) and one new (LD00-3309) cultivar for growth under elevated $[CO_2]$ in open top field chambers (OTC) since CO_2 was expected to increase yield in both cultivars. The OTC experiment confirmed a dilution effect under elevated $[CO_2]$ for both cultivars. However, soybeans grown under elevated $[CO_2]$ showed a significant reduction in transpiration and nutrient concentrations. Therefore, reduced transpiration under elevated $[CO_2]$ cannot be ruled out as having influenced lower mineral concentrations and overall mineral nutrient dilution.

Understanding nutrient content changes with progressing yields is critical for fitting future crop production to a changing global climate.

Keywords: carbon dioxide, mineral nutrient dilution, transpiration, soybean, cultivars

Abbreviations: Carbon dioxide concentrations ([CO_2]), maturity group (MG) nitrogen (N), open top chamber (OTC), phosphorus (P), potassium (K)

Introduction

To meet food demands of a projected 9.7 billion global population, crop yields need to increase at a rate of ~2.4% per year (Ray et al., 2013; FAO, 2017). As soybean contributes largely to current food production, its plays a vital role in meeting future demands. In the last century, breeding efforts developed soybean varieties with disease resistance, stress tolerance, and length of growing season. These breeding efforts combined with increased efficiency of farm management drove biomass accumulation and therefore yield increases (Sacks & Kucharik, 2011; Koester et al., 2014). Understanding the physiology behind mechanisms increasing yield in the last century is essential to further enhance production and meet the United Nations goal of feeding the world population (Koester et al., 2014).

Less understood is soybean nutrient demand, use, and efficiency as these factors increase with higher yields (Balboa et al., 2018). Comparing soybean varieties released from 1931 to 2017, Balboa et al. (2018) found that newer cultivars had higher yields, but seed nutrient concentrations decreased and allocation changed relative to older cultivars. For example, seed phosphorus (P) and potassium (K) concentrations fell in newer cultivars while yield and seed nutrient uptake increased (Balboa et al., 2018). This trend of mineral nutrient concentration decrease in new high yielding cultivars has been observed in a wheat cultivar collection spanning 100 years (Garvin et al., 2006). The trade-off between yield and nutrient concentration has been further highlighted in a study comparing wheat cultivars bred before and after the Green Revolution (Fan et al., 2008). In this study, semi-dwarf high yielding cultivars (after the Green Revolution) showed higher yields and lower nutrient concentration than their Green Revolution counterparts (Fan et al., 2008).

Scientist have theorized that the nutrient concentration decrease observed in seeds of high yielding cultivars may be due to higher nutrient demand of these cultivars and inadequate plant-available nutrient concentrations in the soil (Balboa et al., 2018; Reis et al., 2021). To test if soil nutrient availability affected seed nutrient (protein) and yield in a soybean population developed from 1980 to 2014, Reis et al., (2021) performed a two-year experiment where all cultivars were grown with no nitrogen (N) or with additional N fertilizer. This effort showed that newer cultivars produced 50% higher yields and 1.2% lower protein concentrations compared to older cultivars, but protein concentration decrease was not alleviated by additional N fertilizer application. These results suggest that N application did not alleviate decreased concentration in higher yielding cultivars and therefore this reduction in seed mineral concentration may be due to limitations in root absorption or partitioning between different organs and the seed (Balboa et al., 2018). Although nutrient availability has not been demonstrated to be a limiting factor in the case of N (Reis et al., 2021), very little is known about the effects of K and P fertilizer applications which can be more important for soybean yield response as this crop already fixes atmospheric N (Balboa et al., 2018).

Although experiments using old and new varieties can be useful for understanding nutrient concentration declines in high yielding cultivars, these studies compare cultivars with very different genetic backgrounds. With different genetics, it is difficult to conclude which mechanism is behind nutrient decline in high yielding cultivars since differences could be caused by genetic determinants not related to greater biomass accumulation and/or yield (Mohamed et al., 1991). Increasing atmospheric CO_2 concentration ([CO_2]) can be a means of increasing yield and testing if nutrient composition decreases in the same cultivar (Sanz-Saez et al., 2017; Soba et al., 2020). With elevated [CO_2], increased photosynthetic activity results in greater sugar production, biomass accumulation, and yield (Ainsworth et al., 2002; Ainsworth & Rogers, 2007). Therefore, comparisons of low and high yields in the same cultivar (attributed to changing [CO_2]) can help determine if differences in yield are due to physiological responses rather than cultivar. Multiple studies have shown a negative relationship between yield increase and mineral depletion under elevated [CO_2] for crops such as soybean, wheat, rice, barley, potatoes and other C_3 plants (McGrath & Lobell, 2013; Loladze, 2014; Myers et al., 2014; Parvin et al., 2019). However, cultivar response to elevated [CO_2] has been found to be significant for aboveground biomass, yield, and some mineral nutrients in the seed (Myers et al., 2014; Bishop et al., 2015). As not all cultivars respond similarly to elevated [CO_2], in terms of yield increase and nutrient decrease, multiple mechanisms may be underlying the trade-off leading to changes in mineral nutrient concentration.

Multiple theories aim to explain physiological plant functions driving nutrient decreases under elevated [CO_2]: 1) lower transpiration under elevated [CO_2] may limit nutrient uptake via reduced mass flow (McGrath & Lobell, 2013); 2) nutrient dilution results from stimulated biomass accumulation with nutrient content remaining constant (Chaturvedi et al., 2017); and 3) cultivars with larger biomass have a reduced density of root nutrient transporters (Jauregui et al., 2016). Whether these mechanisms act alone or in concert is unknown and requires further investigation.

To investigate theories 1 and 2 in soybeans we developed experiments with three overall objectives. The first objective was to test if new and old cultivars differ in yield and nutrient concentrations while ruling out involvement of insufficient soil nutrient availability. To this end, a field experiment with three old, three new, and two commercial cultivars were grown under five treatments (a sufficient fertilizer rate as the control; additional P; additional K; additional P and K; and a control fertilizer rate with an anti-transpirant spray applied during pod-filling). As a second objective, this last treatment tested if decreased transpiration led to nutrient content changes by altering mass flow. Decreased transpiration's role was further investigated by growing two cultivars under ambient and elevated atmospheric CO_2 in which the last objective was also tested. A third objective, testing dilution's role, investigated yield and mineral nutrient responses of an old and new cultivar grown under ambient and elevated atmospheric CO_2 with deficient and supplemental soil K.

Materials and Methods

Field Study

Experiment Location and field management

The first field study was conducted in 2019 and 2020 at E.V. Smith Research Station in Shorter, AL. Weather data was collected by the Agricultural Weather Information Service, Inc. at a weather station located 1 mile from the field site. Weather data and irrigation quantity are summarized in Table 1. In the 2019 field season, plants were grown under rain fed conditions and experienced a considerable drought (Table 1). In 2020, the experiment was performed using lateral irrigation, but due to a wet season, the field was only irrigated twice during the growing season.

The soil was classified as a Compass loamy sand (coarse-loamy, siliceous, subactive, thermic Plinthic Paleudults) consisting of 76.4% sand, 20.4% silt and 3.2% clay. Field management followed standard practices with Crimson Clover (*Trifolium incarnatum*) planted as cover crop on 2019 winter season and Black Oats (*Avena strigose*) planted as cover crop during the 2020 winter season. Crimson Clover and Black Oats were terminated 21 days and 12 days before planting, followed by strip tillage. Planting of soybean occurred on 30 April 2019 and 28 April 2020. Fertilizer applications in 2019 and 2020 were based on Alabama Extension recommendations. In 2019, 112 kg ha^{-1} of 0-0-60 (N-P-K) was applied 2 days after planting (May 2nd) and 145 kg ha^{-1} of 0-46-0 and 112 kg ha^{-1} of 0-0-60 were applied to the specific treatment plots. In 2020, 34 kg ha^{-1} of 28-0-0-5 (N-P-K-Fe) was applied 46 days (March 13th) before planting, 1 ton ha^{-1} of lime applied 12 days (April 16th) before planting, and 33 kg ha^{-1} of 33-0-0 applied 41 days (June 8th) after planting. To the specific treatment plots, 145 kg ha^{-1} of 0-46-0 and 112 kg ha^{-1} of 0-0-60 were applied 45 days (June 12th) after 2020 planting.

Experimental setup and design

Each experimental unit consisted of a plot with four rows which were 6.1 m long, 3.7 m wide, with a row separation of 0.9 m. Since we hypothesized that nutrient concentration declines in newer cultivars, six maturity group IV cultivars were selected based on year of release and known shoot nutrient content (Dhanapal et al., 2018). Three old cultivars (year of release = 1952 or earlier) with known relatively higher nutrient concentrations (Wabash, 1948; Perry, 1952; Chief, 1940) and three new cultivars with known relatively lower nutrient concentrations (LD00-3309, 2005; Flyer, 1998; Stressland, 1994) were selected based on the work of Dhanapal et al. (2018). Additionally, two commercial cultivars (S13-10590C and LG055087-5; both MG IV) were used for current, standard yield and nutrient concentration to compare with the other six cultivars.

To test if newer cultivars have a limited mineral nutrient absorption capacity, 3 fertilizer treatments plus a control were implemented. The control treatment was fertilized following Alabama Extension recommendations (112 kg ha^{-1} of 0-0-60) at sowing, 2 May 2019. The rest of fertilizer treatments were added 4 and 6 weeks after planting in 2019 and 2020, respectively, and consisted of additional P (control + 146 kg ha^{-1} of 0-46-0), additional K (control + 112 kg ha^{-1} of 0-0-60), and additional P and K (control + 146 kg ha^{-1} of 0-46-0 and 112 kg ha^{-1} of 0-0-60). To test if transpiration may limit nutrient uptake and concentration, a fifth treatment consisted of the control fertilizer rate with an anti-transpirant spray (Vapor Guard ®, active ingredient- 96% di-1-p-Menthene) applied one time in the early morning under absence of wind at the beginning of pod filling-R5 (Ferh et al., 1973) to reduce plant transpiration. The anti-transpirant was applied to the whole plant until run off using a back-pack sprayer at 2.5% (v/v) and the anti-transpirant effect was confirmed one week after application by measuring stomatal conductance with a LI-6400 (LI-COR Biosciences, Lincoln NE, USA) at midday. The five fertility treatments were applied to each of the eight cultivars so each individual plot contained a cultivar by fertilizer treatment. This experiment was conducted using a randomized complete block design with 4 replicates.

Sampling

Plants from the two center rows were harvested on 13 September 2019 and 19 October 2020 using a small plot combine Almaco R1 (Almaco, Nevada, Iowa). Reported yield was adjusted to 13% seed moisture. Seed nutrient testing was performed at Waters Agricultural Laboratory, Inc. (Camilla, GA). Mineral concentrations (mg g^{-1}) of N, P, K, Mg, Ca, Fe, Zn, and S were determined using Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).

Statistical Analysis

Data analysis was conducted using a mixed model procedures of SAS (PROC GLIMMIX, SAS 9.4, Cary, NC, USA; Littell et al., 1996). Nutrient treatments and cultivars were considered fixed effects, while blocks were considered a random effect. When the fix effect of nutrient treatment, cultivars, or their interaction was significant ($p<0.05$), least square means post-hoc tests were performed to compare means (LSMEANS, SAS 9.4, SAS Institute, Cary, NC, USA).

Elevated CO_2 Study

Plant Material and Experimental Conditions

This study was conducted in an open top chamber (OTC) facility located at the USDA-ARS National Soil Dynamics Laboratory, Auburn, AL. The soil bin used in this study (Prior et al., 2003), a detail description of the OTC (Rogers et al., 1983a), and the CO_2 delivery/monitoring system (Mitchell et al., 1995) have all been previously described. Briefly, the OTC consisted of a cylindrical aluminum frame (3 m wide, 2.4 m tall) with the bottom half covered with clear plastic that allowed sunlight penetration to plants. This double-walled plastic chamber cover had 2.5-cm perforations (inner wall) that allowed for even gas distribution throughout the chamber. Plants were exposed to either ambient (~410 $\mu mol\ mol^{-1}$) or elevated (ambient + 200 $\mu mol\ mol^{-1}$) atmospheric CO_2 concentrations during daylight hours. The study utilized four blocks of ambient and elevated paired OTC in a randomized complete block design with blocks occurring along the length of the soil bin.

Two cultivars (Wabash and LD00-3309) were selected from the 2019 field season (described above) based on year of release (1948 and 2005, respectively) and nutrient concentration (high and low in both P and K concentrations simultaneously). Seeds from the 2019 field season were sown into 20-liter black containers filled with the same soil that had been collected from the E.V. Smith Research Station. Seeds were inoculated with commercial *Bradyrhizobium japonicum* (N-dure, Verdesian Inc., Cary, NC, USA) to ensure good nodulation. Containers were placed in OTCs immediately after sowing on May 8. Plants were watered daily with a drip tape irrigation system that applied 1.9L of water every other day for the first 4 weeks and every day afterwards to avoid drought stress. Three different K fertilizer treatments were used: 1) Alabama Extension recommendation (112 kg ha^{-1} 0-0-60); 2) deficient K - consisting of soil with no fertilizer application (112 kg ha^{-1} below the recommended rate); and 3) additional K - consisting of 224 kg ha^{-1} 0-0-60. Each OTC held four containers of each cultivar by K-treatment in order to have two containers for each treatment per OTC to sample at both pod filling (R5) and maturity (R8, Ferh et al., 1971). The experiment was conducted as a three-way factorial in a randomized complete block design with [CO_2], cultivars, and K-level as fixed effects and blocks as a random effect.

Leaf gas exchange measurements

Diurnal measurements were conducted during reproductive growth to measure if cultivars or treatments had any effect on transpiration or photosynthesis. Diurnal measurements of instantaneous leaf photosynthetic CO_2 assimilation (A) and stomatal conductance (g_s) were measured using two LI-6800 Portable Photosynthesis Systems (LI-COR Biosciences, Lincoln NE, USA). Measurements were taken on the most recently fully expanded leaf at the top of the canopy three times over the season: Full flowering (R2, June 29), beginning of pod (R3, July 17), and beginning of pod filling (R5, August 8). Gas exchange measurements were taken approximately every 3h from sunrise to sunset on two plants per cultivar by K-Level by [CO_2] as performed by Soba et al., (2020). Before each time point, light intensity was recorded by the LI-6800 and temperature was measured by an onsite weather station. In the leaf cuvette, conditions were set to match ambient conditions with [CO_2] matching the OTC (~400 ppm or ~600 ppm) and relative humidity was maintained between 60 - 70%. Total daily CO_2 uptake (A`) and stomatal conductance ($g_s$`) were estimated by integrating areas under diurnal curves as in Soba et al. (2020).

In addition, laboratory-based A-Ci curves measurements were conducted over four days during July 18-21 (Full pod, R4, Fehr et al., 1971) to parameterize the Ball et al. (1987) model of g_s to assess if CO_2, cultivar, or K level imposes any limitation in the stomatal response of the plant. Two sub-samples of each cultivar by [CO_2] and K-level were brought into a laboratory on site to maintain steady ambient conditions of relative humidity and temperature (50-65% and 25 ± 1 °C, respectively). Leaf gas exchange measurements were taken using two LI-6800 (LI-COR Biosciences, Lincoln NE, USA) systems and were conducted on a fully expanded leaf at the top of the canopy under light saturated conditions (1750 μmol mol^{-1} photosynthetic active radiation, PAR, Sanz-Saez et al., 2017). After leaf photosynthesis attained a steady state, the effects of varying [CO_2], photosynthetic photon flux density (PPFD), and vapor pressure deficit (VPD) over photosynthesis and stomatal conductance was assessed across three consecutive phases following the protocols of Leakey et al., (2006). First, the [CO_2] of the air entering the cuvette was varied stepwise (i.e., 410, 310, 250, 160, 110, 50, 410, 610, 810, 1010, 1210, 1510 μmol mol^{-1}; Sanz-Saez et al., 2017) as PPFD was held constant at 1750 μmol $m^{-2}s^{-1}$. Due to variation in VPD caused by changes in leaf transpiration, VPD was manually adjusted to keep VPD < 1 kPA with the control of the air flow through the desiccant column. Second, PPFD incident on the leaf was varied stepwise (1750, 1500, 1000, 700, 400, 200, 100, 70, 75, 50 μmol $m^{-2}s^{-1}$) as [CO_2] was held constant as growth conditions in the OTC (~410 ppm or ~610ppm). Variation in VPD was maintained constant manually as mentioned above. Third, VPD was varied stepwise in six increments of 0.5kP from 1.0 kPa to 3.5 kPa while PPFD was held constant at 1750 μmol $m^{-2}s^{-1}$ and [CO_2] held at growth conditions. Between all measurements, gas exchange was allowed to reach steady state before the measurement and next stepwise change. Additionally, a match procedure was performed after any change in CO_2, light, or VPD to correct for deviations between measuring cells.

By altering [CO_2], PPFD and VPD, g_s response is measured to determine if acclimation occurs in elevated [CO_2] growth conditions. These factors are a part of the Equation 1:

(1) $g_s = g_0 + m\,(Ah/[CO_2])$ (Ball et al., 1987),

where A is the net rate of photosynthetic CO_2 assimilation; h is the atmospheric relative humidity, [CO_2] is the concentration of CO_2 at the leaf's surface in the cuvette, g_0 is the y-axis intercept and m is the slope of the line. Using the LI-COR 6800 to alter [CO_2], PPFD, and VPD along with stomatal conductance m and g_0 can be calculated using equation 1. Changing A, h or [CO_2] during the performance of the curves can let us calculate constants of equation 1 (g_0 and m) and then find if our treatments (CO_2, cultivars, K treatments) show any stomatal limitations.

Maximum rates of Rubisco carboxylation (V_{cmax}) and RuBP regeneration rate (J_{max}) were estimated from the response of A to intercellular [CO_2] (C_i). Using the changes in [CO_2], as described above. We then used equations developed by Sharkey et al. (2007) to calculate Vc_{max} and J_{max}.

Canopy Photosynthesis

Total canopy photosynthesis was measured by a modular transparent custom chamber designed as a closed system. The chamber design followed as described in Soba et al., (2020). Once the pot was placed in the canopy chamber, measurements were performed within 90 seconds to avoid temperature changes > 1 °C, avoiding over heating in the canopy chamber. The CO_2 evolution data were analyzed using Soil-Flux-Pro software (LI-COR Biosciences, Lincoln, NE, USA) by fitting a linear regression line to the CO_2 evolution in the chamber and calculating the slope of regression line that is equivalent to the photosynthetic rate. The program also provides

R^2 values to assess accuracy of the measurement. To avoid increased errors due to recent chamber closure, the first 10 s of each measurement were omitted. Canopy photosynthesis measurements were taken at the end of pod filling (R5) and calculated as plant based or leaf base. Average leaf-based photosynthesis was calculated by dividing the canopy photosynthesis over the leaf area collected before measurements.

Crop Growth and Harvest

At beginning of pod filling (R5), 12 plants within each OTC (2 sub-samples of each cultivar by K-level) were destructively harvested and separated into roots, shoots, leaves, and pods. Height, seed and pod count, and ground line diameter (GLD) were measured at harvest. Leaf area (LA) was measured with a LI-3100 leaf area meter (LICOR Biosciences, Lincoln, NE, USA). All plant organs were oven dried for at least 72 h at 60°C before weighing. Organs were then ground and sent to the Waters Lab for nutrient content analysis as described above.

At maturity (R8), the remaining 12 plants per OTC were harvested and separated in stems, leaves and pods and oven dried at 60 degrees for at least 72h. After drying, seeds were separated from pods, counted, and weighed for final yield determination. In the manuscript, above ground biomass refers to the weight of leaves, stems, and pods. Only seeds were sent to Waters Agricultural Laboratory for nutrient analysis.

Statistical Analysis

Data analysis was conducted using a mixed model procedure of SAS (PROC GLIMMIX, SAS 9.4, Cary, NC, USA; Littell et al., 1996). The [CO_2], cultivar, and K-level treatments were considered as fixed effects, while blocks were considered as a random effect. When the main effect of [CO_2], cultivar, K-level, or their interaction was significant, least square means post-hoc tests were performed to compare means (LSMEANS, SAS 9.4, SAS Institute, Cary, NC, USA).

Results

Field Experiment

Seed yield

Yields in 2019 were extremely low due to a severe drought which contrasts with yields in 2020 when the field was irrigated (Table 1,2). In 2019 and 2020, yields between cultivars were significantly different (Table 2) and old cultivars showed a 40% and 33% lower yield than new cultivars, respectively. The old cultivar Perry was an exception and showed similar yields to new cultivars (Stressland and Flyer) in both years (Table 2). In both years, there was no significant effect of the nutrient treatment or anti-transpirant on yield or interaction between cultivars and nutrient treatment (Table 2).

Seed nutrient concentration

In 2019, all measured nutrient concentrations significantly differed between cultivars, while in 2020 there were no differences between them (Table 3). Interestingly in 2019, seed K concentration ([K]) had two significantly different groups with Chief, Flyer, LG055087-5, Perry, S13-10590C, and Stressland showing higher concentration than LD00-3309 and Wabash. In this case, leaf K concentration did not show any difference between new and old cultivars (Table 3). Flyer and LD00-3309 had significantly higher Ca concentrations than other cultivars, while LG055087-5 had the lowest Ca concentration (Fig. 1). Calcium was the only nutrient in which the cultivar by treatment interaction was significant (Table 3; Figure 1).

Fertilizer treatments only affected [K] and [Zn] in 2019 (Table 3). The highest [K] were found in the additional K and additional P + K treatments, while the control, additional P, and anti-transparent spray treatments were not considered different from each other but lower than the above treatments (Table 3). Additional K, additional P + K, and anti-transparent spray showed higher [Zn] than the control and additional P treatment (Table 3). The old cultivar Wabash showed lower yields with similar nutrient concentration in both years compared to LD00-3309. Thus, these two cultivars were selected for the study investigating the effects of elevated [CO_2] on nutrient concentration and whether nutrient concentration would be diluted by increased biomass due to positive growth effects of elevated [CO_2].

Elevated CO_2 Study

Biomass Traits

Compared to ambient [CO_2], elevated [CO_2] significantly increased aboveground biomass by 20 and 25% at pod filling (R5, Ferh et al., 1971) and maturity (R8, Ferh et al., 1971), respectively. The old Wabash cultivar showed a 34% higher aboveground biomass than LD00-3309 (new) at R5, but no differences at R8 possibly caused by small differences in development (Table 4). The additional and recommended K-level treatments showed higher aboveground biomass than the K deficient treatment at R5. However, no differences were noted among K-levels at R8 (Table 4). Pod weight showed a significant 19 and 26% increase under elevated [CO_2] at R5 and R8, respectively. Wabash showed a 28.6% higher pod weight than LD00-3309 only at R5 stage. No differences between cultivars were found at R8 (Table 4). The recommended K-level treatment had the highest pod weight, which was significantly higher than the K deficient treatment at R5, with no differences among treatments at R8.

Elevated [CO_2] and K-rate did not affect leaf area at R5 (Table 5). At that same developmental stage, Wabash showed 24% more leaf area than LD00-3309. Root dry weight was increased by 39% due to elevated [CO_2]. In addition, LD00-3309 showed a 30% increase in root weight in comparison to Wabash. Root dry weight was not influenced by K-level (Table 5). Root shoot ratio was not affected by elevated [CO_2] or K-level. The cultivar LD00-3309 showed a 75% higher root:shoot ratio demonstrating that this cultivar allocated more resources for root system development (Table 5).

Elevated [CO_2] increased seed yield at R5 (16%) and R8 (27%) (Table 4). There were no cultivar differences at either developmental stage for seed yield. At R5, plants fertilized at the recommended K-level showed a higher seed yield than ones grown at the K deficient level (Table 4). Weight per seed was not significantly affected by elevated [CO_2] or K-level at R5, but Wabash seeds weighed significantly more than LD00-3309 at R5 and R8. Weight per seed at R8 was 20% greater with additional K-treatment as compared to recommended or deficient K-levels (Table 4). Ambient [CO_2] weight per seed at R8 was significantly greater by 8% (Table 4). At R8, there was an interaction between [CO_2], cultivar, and K-level for weight per seed demonstrating Wabash in additional K-treatment was highest in ambient and elevated [CO_2] (Fig. 2; Table 4). Harvest index was not affected by any treatment at either developmental stage (Table 4).

Photosynthetic Parameters

Elevated [CO_2] significantly increased diurnal photosynthesis (A'; mol CO_2 m^{-2} d^{-1}) by 22, 16, and 22% at R2, R3, and R5 respectively (Table 6). However, there was no cultivar, K-level or any interaction that affected A' at the three measured developmental stages. In contrast, elevated [CO_2] decreased diurnal stomatal conductance (g_s'; mol H_2O m^{-2} d^{-1}) by 31, 12.3, and 34% at the three respective developmental stages. Diurnal stomatal conductance was higher in Wabash at R2, lower at R5, and not different at R3 compared to LD00-3309 (Table 6). The K-level did not affect g_s' at any developmental stage. However, g_s' showed a significant [CO_2] by K-level interaction at R5 (Fig. 3; Table 6). At this stage, the additional K-level showed higher g_s'

than the recommended or K deficient levels under ambient [CO_2] but the lower g_s' then recommended or K deficient levels under elevated [CO_2] (Fig. 3).

The RuBP regeneration (J_{max}; µmol electrons $m^{-2}s^{-1}$), slope of the ball berry model (unitless; m), and canopy photosynthetic rates (µmol CO_2 $m^{-2}s^{-1}$) were not significantly affected by elevated [CO_2], cultivar, K-Level or any of the treatment interactions (Table 7). In contrast, maximum rates of rubisco carboxylation (V_{cmax}, µmol CO_2 $m^{-2}s^{-1}$) measured at R4 were decreased by 17% under elevated [CO_2], but not affected by cultivar, K-level, or their interaction. The intercept of the ball berry model decreased by 33% (Table 7) under elevated [CO_2] but was not affected by cultivar, K-level, or any interactions.

Seed nutrient concentration and uptake

At maturity, elevated [CO_2] significantly decreased seed [N] by 5%, while a cultivar by K-level interaction was also detected (Table 8). Seed N uptake was significantly increased by 22% under elevated [CO_2] also showing a [CO_2] by K-level interaction trend (Table 8). None of the treatments affected seed [P] or P uptake except for elevated [CO_2], which increased P uptake by 21%. Despite a 5% significant decrease in seed [K], elevated [CO_2] significantly increased K uptake by 14% (Table 8) due to the stimulation of elevated [CO_2] on yield (Table 4). At the same time, Wabash showed higher [K] and uptake than LD00-3309 (Table 8). Overall, the additional and the recommended K treatments showed higher seed [K] than the deficient K treatment, but no effect of K-level was observed on K uptake.

Seed [Fe] was not significantly affected by elevated [CO_2], despite a significant 28% increase in Fe uptake (Table 8). Wabash showed higher seed [Fe] and uptake in comparison to LD00-3309 (Table 8). The K treatments did not affect [Fe] or Fe uptake. Seed [Zn] was not affected by any treatment, while Zn uptake was increased by 20% under elevated [CO_2]. Seed [Mg] and uptake was not affected by elevated CO_2 or K treatments. However, Wabash had a lower [Mg] concentration than LD00-3309, which did not translate into higher seed Mg uptake (Table 8). Similar to Mg, the seed [Ca] was not affected by [CO_2] or K treatments, but this was significantly lower in Wabash. The lower seed [Ca] in Wabash did not translate to a significantly lower Ca seed uptake (Table 8). Seed [S] was not affected by elevated CO_2 or K treatments, while S uptake was increased by elevated [CO_2]. Additionally, Wabash had higher seed [S] and uptake.

Biomass nutrient content

Elevated [CO_2] decreased leaf [N] by 12% but did not affect N uptake (Table 9). Wabash had slightly lower leaf [N] than LD00-3309 with difference in N uptake. Leaf P and Zn concentrations (and associated uptakes) were not affected by any treatment (Table 9). In contrast, leaf [K] decreased by 29 % under elevated [CO_2] with no impact on leaf K uptake. Wabash had lower leaf [K] than LD00-3309, which did not translate to lower K uptake (Table 9). Leaf [K] was higher in plants supplemented with additional K in comparison to the recommended and K deficient treatments. This difference was more accentuated in leaf K uptake, which was higher in the additional and recommended treatment in comparison to the K deficient treatment. Leaf Fe concentration and uptake were increased by 33 and 66 %, respectively, under elevated [CO_2], but were not affected by K treatment. Leaf [Fe] was significantly lower in Wabash compared to LD00-3309 (Table 9), but this did not translate into differences in Fe uptake. Leaf Mg concentration and uptake were not affected by elevated [CO_2], but they were 46 and 79% higher, respectively, in Wabash than LD00-3309. Leaf [Mg] was also higher in the K deficient treatment compared to the other K treatments (Table 9). Leaf [Ca] was only affected by K treatments and was higher in the K deficient treatment; K uptake was not affected by any treatment (Table 9).

Elevated [CO_2] decreased leaf [S] by 13% but did not affect leaf S uptake. Wabash showed a lower [S] than LD00-3309 (Table 9), but leaf S uptake was unaffected. Leaf [S] was higher in the K addition than the recommended treatment (Table 9).

Although elevated [CO_2] did not modify mineral concentrations in roots, nutrient uptake of minerals (except Fe) was significantly enhanced (Table 10) due to more root biomass under elevated [CO_2] (Table 5). Root nutrient concentration was only higher in Wabash for Zn, Mg, and Ca. Root nutrient uptake was similar between cultivars (Table 10) probably due to greater root biomass accumulation in LD00-3309 (Table 5). Root nutrient concentration and uptake were unaffected by K treatments except for Ca uptake (Table 10) where it was highest in the recommended K level and lowest in the K deficient treatment.

Discussion

Field Experiment

The 2019 and 2020 field experiment revealed yield cultivar differences (Table 2) as previously reported by Balboa et al. (2018), where newer cultivars exhibited higher yields than older cultivars. In these two years, the older Wabash cultivar always had lower yield than the newer LD00-3309 cultivar (Table 2); for this reason, these two cultivars were selected for the OTC experiment (discussed below).

Differences in seed nutrient concentration among cultivars were only noted in 2019 (Table 3) during which E.V. Smith experienced severe drought without irrigation (Table 1). Generally, seed zinc [Zn] tended to be higher in old cultivars, while seed calcium [Ca] tended to be higher in new cultivars (Table 3). Similarly, Garvin et al. (2006) noted a trend for lower seed [Zn] in newer cultivars of hard red winter wheat in a two-year experiment without drought stress.

Drought stress tolerance is partially controlled by Zn and Ca's role in osmolyte, stomatal, and hormone regulation (Hassan et al., 2020; Wang & Komatsu, 2018) and Zn and Ca differences may indicate cultivar variation in drought stress tolerance. As drought further alters nutrient acquisition, understanding nutrient accumulation's role in drought tolerance in higher yielding cultivars may be an important target for breeding programs.

In general, neither yield nor nutrient concentrations in 2019 and 2020 increased when more K and P fertilizers were added (Table 3). Bender at al. (2015) found a 2% increase in biomass and yield with supplemental fertilizer treatments and not increased nutrient concentrations. Collectively, these findings indicate that higher yielding cultivars were not limited by soil nutrient availability since supplemental fertilization did not increase yield or nutrient uptake. Therefore, lower nutrient concentrations in higher yielding new cultivars may be due to differences in plant physiology rather than limited nutrient availability.

Elevated CO_2 Study

Elevated [CO_2] increased biomass and yield in both the Wabash and LD00-3309 cultivars (Table 4). Elevated [CO_2] usually stimulates photosynthetic carbon gain leading to overall biomass and yield increases (Roger et al., 1983b; Amthor, 1995; Kimball et al., 2002; Leakey et al., 2009). In our OTC study, Wabash and LD00-3309 did not differ in yield or R8 biomass, which is in contrast to E.V. Smith field study where LD00-3309 showed higher yields than Wabash. Without cultivar differences, Wabash and LD00-3309 responded similarly to elevated [CO_2] resulting in no [CO_2] by cultivar interactions for yield and biomass. The similar response of Wabash and LD00-3309 to elevated [CO_2] may be due to a container effect. Researchers have suggested that containers may limit plant response to elevated [CO_2] due to physical restriction which may explain the lack of cultivar difference in yield and biomass (Arp, 1991; Ainsworth et al., 2002). However, since elevated [CO_2] increased yield in both cultivars, the lack of yield differences between new and old cultivars may come from differential yield plasticity in low planting densities. In the E.V. Smith field study, plants were grown in rows where seeds were separated by ~5 cm within a row. In contrast, the OTC experiment provided a lower planting density where each 20 L pot (30 cm diameter) contained a single plant. Lower density conditions provide more resources (e.g., water, nutrients, and light) for plant growth. Cultivar variation in yield plasticity has been demonstrated, wherein some cultivars display a greater response to low planting densities resulting in higher plant growth than less plastic cultivars (Shimono et al., 2014). In the OTC experiment, Wabash may be more plastic than LD00-3309 resulting in a greater yield response to the lower planting density of the containers, thus explaining the lack of yield differences between older and newer cultivars.

Differences in A, V_{cmax}, or J_{max} (Tables 6, 7) can influence yield response to ambient and elevated [CO_2] (Bernacchi et al., 2013; Koester et al., 2016; Sanz-Saez et al., 2013). Sanz-Sáez et al. (2017) demonstrated that a cultivar with greater diurnal photosynthesis and J_{max} under elevated [CO_2] showed a more significant yield increase than another cultivar not showing as large an increase in photosynthetic parameters under elevated [CO_2]. In the present study, Wabash and LD00-3309 did not differ in V_{cmax}, J_{max} or A' (Tables 6, 7), which may help explain the lack of differences in yield and biomass between these cultivars (Table 4). However, both cultivars showed a diurnal photosynthesis increase under elevated [CO_2], which could explain the higher biomass and yield under elevated [CO_2].

Increased sugar accumulation and biomass production under elevated [CO_2] has been shown to dilute nutrient concentrations in plant organs such as leaves, stems, and seeds (Taub and Wang, 2008; McGrath and Lobell, 2013; Myers et al., 2014; Soba et al., 2020). In the present study, elevated [CO_2] resulted in dilution of N and K as evidenced by lower concentrations in leaves and seeds of larger plants (Tables 8, 9). This supports previously published data pointing to a dilution effect due to increase carbohydrate and biomass production (Taub and Wang, 2008; McGrath and Lobell, 2013). However, in our study root N and K concentrations were not decreased as previously reported in a meta-analysis (McGrath and Lobell, 2013). The lack of dilution in roots could be due to changes in partitioning among organs caused by alterations in expression of nutrient transporters in roots that could limit nutrient absorption (Jauregui et al., 2016; Soares et al., 2021a,b) or transport from the roots to shoot. Effects of elevated [CO_2] on nutrient transporters in different organs requires investigation since this could ultimately influence food quality and human and animal nutrition (Myers et al., 2014).

Elevated [CO_2] has also been shown to decrease transpiration at both leaf (Ainsworth and Rogers 2007; Soba et al., 2020) and canopy levels (Leakey et al., 2009). A meta-analysis by McGrath and Lobell (2013) indicated that decreased transpiration could provoke decreased mass flow absorption of nutrients, which could help explain decreased nutrient concentrations under elevated [CO_2]. Further, our study did not observe changes in stomatal sensitivity between ambient and elevated [CO_2] (Table 7) which aligns with a study by Leakey et al. (2006) parameterizing the Ball Berry model. Since N and K are water soluble, they have been demonstrated to move in the soil and plant predominantly by mass flow (McGrath and Lobell, 2013). In our study, we showed decreased stomatal conductance under elevated [CO_2] at all measurement periods (Table 6) and decreased seed and leaf N and K concentrations. Therefore, mass flow could be contributing to a reduction in N and K concentrations in these organs.

Greater biomass due to elevated [CO_2] did not result in a dilution of Fe or Zn in any organ (Tables 8, 9, 10). This suggests that plants were able to sustain comparable Fe and Zn absorption at rates similar to carbohydrate accumulation stimulated by elevated [CO_2]. In contrast, McGrath & Lobell (2013) and Myers et al. (2014) observed Fe and Zn dilution under elevated [CO_2]. The different results between experiments may be due to the influence of growth conditions and how different nutrients are absorbed by the plants. Experiments where Fe and Zn concentration have been observed to decrease were performed under FACE field conditions (Myers et al., 2014; Soares et. al, 2021b) in which nutrients can be more mobile in the soil and roots may not be closer to nutrients. However, in container studies roots have limited growth volume where nutrients are confined which may increase nutrient accessibility. This could be the reason why Fe and Zn were not diluted in higher biomass in elevated [CO_2]. Some elevated [CO_2] studies using container-grown soybean documented increased levels of Fe and/or Zn in seeds or leaves (Soba et al., 2020; Soares et al., 2021b). The different mechanisms of plants to absorb different nutrients may be another reason why N and K diluted but Fe and Zn concentrations remained similar in elevated [CO_2]. Contrary to the influence of mass flow on N and K absorption, Fe and Zn are less soluble in water and rely on diffusion for soil and plant translocation (McGrath and Lobell, 2013). Since diffusion is not exclusively dependent on plant transpiration, reduced g_s' in elevated [CO_2] may not affect Fe and Zn as it does in the case of N and K. In fact, Soares et al. (2021b) found that higher Fe levels in leaves under elevated [CO_2] were related to an increased expression of ferritin proteins that regulate Fe transport in plants.

The K fertilizer treatments in the OTC experiment did not affect aboveground biomass or yield, demonstrating that K was naturally abundant in the soil we used and soybean did not need additional K fertilizer for adequate yield performance. Higher levels of K in leaves and seeds were observed in the additional K treatment than in the deficient K treatment (Tables 8, 9). However, we did not observe a [CO_2] by K treatment interaction that would have alleviated the decrease of K under elevated [CO_2]. This could mean that root absorption or translocation (root to shoot) was altered under elevated [CO_2] and insensitive to additional K. This strengthens the theory that under elevated [CO_2] there may be some limitation in root transporters that could impact absorption of some minerals such as N and K (Jauregui et al., 2016).

Conclusion

The E.V. Smith field study confirmed that older soybean cultivars have lower yields than newer ones. We also showed that older cultivars tended to exhibit higher nutrient concentrations in a very dry season. This field experiment was useful in demonstrating that the lower nutrient concentrations historically shown in new cultivars was not due to soil nutrient limitations since we found the addition of P and K did not result in higher yields or seed nutrient concentrations. In the OTC experiment, Wabash and LD00-3309 showed the same yield even though LD00-3309 was expected to have higher yields. This phenomenon was possibly caused by differences in planting density in the field vs. the container study. However, in both cultivars, elevated [CO_2] increased yield and decreased leaf and seed K and N concentrations. This decrease in nutrient concentration was associated with a dilution effect caused by increased growth from higher photosynthesis. It is also possible that decreased transpiration could have decreased bulk flow of K and N under elevated [CO_2]. The fact that root K and N concentrations were not decreased under elevated [CO_2], in combination with the lack of yield and nutrient effect of K-fertilization, points to some limitations regarding specific nutrient transporters that could limit nutrient absorption in high yielding cultivars and/or under elevated [CO_2] conditions.

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Table 9. Leaf nutrient concentrations (mg g^{-1} or mg kg^{-1}) and uptake (g $plant^{-1}$ or mg $plant^{-1}$) at R5 (pod-filling stage) from soybean grown at USDA-ARS National Soil Dynamics Laboratory (Auburn, AL). Leaf nutrient concentrations and nutrient uptake includes nitrogen (N), phosphorus (P), potassium (K), iron (Fe), zinc (Zn), magnesium (Mg), calcium (Ca) and sulfur (S). Means are grouped into [CO_2], cultivar, K-Level and represent individual treatment within the treatment group. Letters indicate significant differences of means within [CO_2], cultivar, and K-Level treatment groups. Below the means are p-values generated from a three way ANOVA and represent differences within [CO_2], cultivar, K-Level and treatment interactions. Asterisks (*) represent significant p-values ($p<0.05$).

Table 10. Root nutrient concentrations (mg g^{-1} or mg kg^{-1}) and uptake (g $plant^{-1}$ or mg $plant^{-1}$) measured at R5 (pod-filling stage) from soybean grown at USDA-ARS National Soil Dynamics Laboratory (Auburn, AL). Nutrients measured for root nutrient concentrations and root nutrient uptake are nitrogen (N), phosphorus (P), potassium (K), iron (Fe), zinc (Zn), magnesium (Mg), calcium (Ca) and sulfur (S). Means are grouped into [CO_2], cultivar, K-Level and represent

individual treatment within these treatment groups. Letters indicate significant differences within the respective treatment group. P-values below the means were generated from a three way ANOVA and represent differences within [CO_2], cultivar, K-Level and the treatment interactions. Asterisks (*) represent significant p-values ($p<0.05$).

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Figure 1. Field seed calcium concentration for eight soybean cultivars grown at E.V. Smith (Shorter, AL) under five fertilizer treatments in 2019. Bars represent cultivar by fertilizer treatment means. The legend key shows fertilizer treatment by color. Capital letters indicated significant differences among cultivars ($p<0.001$) and lower-case letters indicate significant differences among cultivar by fertilizer treatment means ($p=0.05$); bars followed by same lower case letters are not significantly different. P-values were calculated using a two way ANOVA.

Figure 2. R8 weight per seed of soybeans grown in open top chambers at USDA-ARS National Soil Dynamics Laboratory, Auburn, AL. Bars represent [CO_2] by cultivar by K-level means. The legend key shows K-levels by shade of color, [CO_2] treatment by horizontal or vertical lines and cultivar by orange or blue. Capital letters indicate significant differences between [CO_2] treatments and lower-case letters indicate significant differences between [CO_2] by cultivar by K-level means ($p<0.05$). P-values were calculated using a two-way ANOVA.

Figure 3. The daily stomatal conductance (g_s') measured in soybeans grown at USDA-ARS National Soil Dynamics Laboratory, Auburn, AL. Bars represent [CO_2] by K-level means. The legend key shows K-level by color and [CO_2] treatment by no lines or diagonal lines. Capital letters indicated significant differences between [CO_2] treatments and lower-case letters indicate significant differences between [CO_2] by K-level means ($p<0.05$). P-values were calculated using a two-way ANOVA.

Tables

Table 1.

Temperature and water input (irrigation and rain)				
Year	**Avg. daily max. temperature (°C)**	**Avg. daily min. temperature (°C)**	**Accumulated Rain (mm)**	**Accumulated Irrigation (mm)**
2019 EVS	33.2 ± 2.8	20.1 ± 2.8	319	0
2020 EVS	30.6 ± 3.4	19.5 ± 4.2	393	22.3
2020 Auburn	30.2 ± 3.1	20.4 ± 3.9	384	41.1

Table 2.

Field Yield (kg/ha)			**2019**	**2020**
Cultivar	**Old**	**Chief**	139.88 d	1585.11 d
		Perry	359.79 bc	1794.93 c
		Wabash	189.65 d	1398.15 d
	New	**LD00-3309**	304.65 c	2359.17 ab
		Flyer	313.39 c	2159.43 abc
		Stressland	350.38 c	2094.20 bc
	Commercial	**LG055087-5**	615.35 a	2307.38 ab
		S13-10590C	448.56 b	2409.61 a
Treatment		**Control**	329.53	2088.82
		Additional P	312.04	2162.79
		Additional K	332.89	2009.46
		Additional K + P	361.14	1850.08
		Anti-transpirant Spray	365.17	2082.09
Cultivar			<0.001*	<0.001*
Treatment			0.561	0.115
Cultivar x Treatment			0.676	0.844

Table 3.

Field Seed Nutrient Concentrations									
2019			**P** (mg g^{-1})	**K** (mg g^{-1})	**Fe** (mg kg^{-1})	**Zn** (mg kg^{-1}))	**Mg** (mg g^{-1})	**Ca** (mg g^{-1})	**S** (mg g^{-1})
Cultivar	**Old**	**Chief**	0.711 b	2.085 a	76.92 cd	48.96 bc	0.309 b	0.361 cd	0.315 c
		Perry	0.728 ab	2.088 a	83.04 ab	49.87 abc	0.302 b	0.408 b	0.345 a
		Wabash	0.653 d	1.987 b	76.92 cd	52.73 a	0.308 b	0.361 cd	0.336 ab
	New	**LD00-3309**	0.665 cd	1.972 b	80.55 bc	45.67 d	0.3463 a	0.466 a	0.317 c
		Flyer	0.682 c	2.059 a	80.18 bc	47.53 cd	0.304 b	0.460 a	0.341 a
		Stressland	0.672 cd	2.067 a	78.90 c	48.50 bcd	0.299 b	0.382 c	0.329 b
	Commercial	**LG055087-5**	0.653 d	2.081 a	75.10 d	48.35 bcd	0.267 c	0.342 d	0.339 a
		S13-10590C	0.669 cd	2.058 a	86.51 a	50.71 ab	0.303 b	0.377 c	0.318 c
Treatment		**Control**	0.693	2.032 c	79.78	48.59 bc	0.307	0.391	0.2933
		Additional P	0.689	2.035 bc	81.07	47.08 c	0.305	0.388	0.2871
		Additional K	0.693	2.081 a	80.47	49.51 ab	0.305	0.398	0.2886
		Additional K + P	0.681	2.067 ab	79.94	48.84 abc	0.303	0.396	0.2998
		Anti-transparent Spray	0.695	2.032 c	77.64	51.18 a	0.304	0.4	0.2897
Cultivar			<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Treatment			0.478	0.011*	0.204	0.019*	0.96	0.589	0.546
Cultivar x Treatment			0.359	0.229	0.939	0.778	0.349	0.050*	0.200
2020			**P** (mg g^{-1})	**K** (mg g^{-1})	**Fe** (mg kg^{-1})	**Zn** (mg kg^{-1})	**Mg** (mg g^{-1})	**Ca** (mg g^{-1})	**S** (mg g^{-1})
Cultivar	**Old**	**Chief**	0.641	1.92	104.00	63.20	0.358	0.424	0.351
		Perry	0.649	1.931	104.40	65.15	0.359	0.428	0.357
		Wabash	0.649	1.895	105.75	61.15	0.361	0.431	0.353
	New	**LD00-3309**	0.632	1.912	102.90	66.10	0.356	0.421	0.346
		Flyer	0.662	1.941	105.10	65.85	0.365	0.450	0.363
		Stressland	0.642	1.900	105.70	67.05	0.363	0.441	0.348
	Commercial	**LG055087-5**	0.633	1.887	107.15	63.25	0.346	0.411	0.349
		S13-10590C	0.637	1.886	108.85	63.75	0.354	0.436	0.349
Treatment		**Control**	0.633	1.902	105.53	64.344	0.35	0.419	0.348
		Additional P	0.639	1.907	105.91	63.688	0.356	0.426	0.353
		Additional K	0.638	1.912	106.72	62.594	0.355	0.429	0.35
		Additional K + P	0.658	1.923	102.03	65.281	0.3616	0.432	0.356
		Anti-transparent Spray	0.645	1.902	107.22	66.281	0.364	0.442	0.353
Cultivar			0.203	0.099	0.877	0.509	0.132	0.133	0.099
Treatment			0.077	0.739	0.506	0.544	0.063	0.256	0.469
Cultivar x Treatment			0.622	0.754	0.583	0.872	0.287	0.285	0.379

Table 4.

		Aboveground Biomass (g plant^{-1})		Pod Weight (g plant^{-1})		Seed Yield (g plant $^{-1}$)		Weight per seed (g seed^{-1})		Harvest Index	
		R5	**R8**	**R5**	**R8**	**R5**	**R8**	**R5**	**R8**	**R5**	**R8**
CO_2	**Ambient**	47.06 b	48.07 b	29.96 b	35.63 b	17.23 b	22.31 b	0.091	0.117 a	0.382	0.471
	Elevated	56.62 a	60.62 a	35.60 a	44.92 a	20.12 a	28.44 a	0.084	0.108 b	0.446	0.473
Cultivar	**Wabash (Old)**	59.60 a	56.09	36.89 a	41.19	19.91	26.35	0.092 a	0.127 a	0.340	0.470
	LD00-3309 (New)	44.08 b	52.59	28.67 b	39.35	17.44	24.39	0.081 b	0.097 b	0.488	0.475
K-Level	**Additional**	52.61 ab	54.33	33.22 ab	41.23	18.71 ab	26.45	0.084	0.125 a	0.359	0.495
	Recommended	58.12 a	52.17	36.59 a	39.08	20.88 a	24.19	0.088	0.105 b	0.374	0.469
	Deficient	44.79 b	56.53	28.53 b	40.51	16.43 b	25.49	0.089	0.106 b	0.509	0.454
CO_2		0.015*	0.002*	0.018*	<0.001*	0.048*	<0.001*	0.116	0.039*	0.469	0.929
Cultivar		<0.001*	0.339	<0.001*	0.448	0.089	0.246	0.010*	<0.001*	0.096	0.848
K-Level		0.021*	0.624	0.022*	0.767	0.049*	0.555	0.480	<0.001*	0.309	0.332
CO_2 x Cultivar		0.164	0.108	0.154	0.354	0.166	0.244	0.459	0.096	0.311	0.461
CO_2 x K-Level		0.480	0.209	0.392	0.142	0.261	0.093	0.363	0.255	0.536	0.811
Cultivar x K-Level		0.089	0.749	0.209	0.486	0.491	0.558	0.992	0.002*	0.489	0.298
CO_2 x Cultivar x K-Level		0.502	0.614	0.585	0.664	0.546	0.378	0.435	0.016*	0.339	0.070

Table 5.

		Leaf Area (cm^2)	Root Weight (g $plant^{-1}$)	Root: Shoot ratio
CO_2	**Ambient**	1515.1	7.49 b	0.1702
	Elevated	1583.5	10.44 a	0.1912
Cultivar	**Wabash (Old)**	1717.6 a	7.78 b	0.1313 b
	LD00-3309 (New)	1380.9 b	10.16 a	0.2301 a
K-Level	**Additional**	1498.5	8.99	0.1786
	Recommended	1804.2	10.31	0.1922
	Deficient	1345.1	7.61	0.1713
CO_2		0.668	0.008*	0.331
Cultivar		0.041*	0.030*	<0.001*
K-Level		0.068	0.125	0.721
CO_2 x cultivar		0.211	0.619	0.084
CO_2 x K-Level		0.889	0.820	0.311
Cultivar x K-Level		0.188	0.381	0.830
CO_2 x Cultivar x K-Level		0.427	0.591	0.836

Table 6.

		Full bloom (R2)		Beginning Pod (R3)		Beginning Seed (R5)	
		A'	g_s'	A'	g_s'	A'	g_s'
CO_2	**Ambient**	0.773 b	34.6 a	0.8451 b	26.9 a	0.7763 b	26.4 a
	Elevated	0.945 a	23.4 b	0.9831 a	18.1 b	0.9495 a	17.9 b
Cultivar	**Wabash (Old)**	0.842	30.8 a	0.9026	23.9	0.8453	19.2 b
	LD00-3309 (New)	0.880	27.2 b	0.9257	21.2	0.8804	25.1 a
K-Level	**Additional**	0.857	27.7	0.9202	23.9	0.8568	23.7
	Recommended	0.844	20.1	0.9377	20.6	0.8834	21.8
	Deficient	0.833	29.2	0.8846	23.1	0.8484	20.9
CO_2		0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
Cultivar		0.443	0.019*	0.545	0.233	0.465	0.014*
K-Level		0.801	0.431	0.513	0.438	0.823	0.577
CO_2 x cultivar		0.508	0.657	0.143	0.369	0.534	0.394
CO_2 x K-Level		0.120	0.829	0.910	0.601	0.116	0.012*
Cultivar x K-Level		0.809	0.697	0.083	0.269	0.842	0.705
CO_2 x Cultivar x K-Level		0.939	0.431	0.694	0.629	0.906	0.860

Table 7.

		V_{cmax} at 25°C	J_{max} at 25°C	Ball Berry (m)	Ball Berry (g_o)	Canopy photo per plant	Canopy Photo per leaf area
CO_2	**Ambient**	124.45 a	223.04	4.13	0.490 a	15051	10.86
	Elevated	103.47 b	208.19	6.63	0.332 b	15973	10.78
Cultivar	**LD**	117.08	210.53	5.58	0.390	13826	10.59
	Wabash	110.84	220.7	5.19	0.432	17197	11.05
K-Level	**Adequate**	112.67	220.85	6.89	0.428	15477	10.55 ab
	Recommended	104.89	201.62	5.30	0.374	15141	8.76 b
	Deficient	124.32	224.37	3.96	0.432	15918	13.15 a
CO_2		0.023*	0.209	0.086	0.005*	0.619	0.956
Cultivar		0.482	0.387	0.784	0.437	0.076	0.745
K-Level		0.195	0.224	0.249	0.603	0.942	0.048*
CO_2 x cultivar		0.790	0.381	0.341	0.196	0.671	0.339
CO_2 x K-Level		0.995	0.737	0.752	0.0867	0.652	0.463
Cultivar x K-Level		0.060	0.536	0.772	0.6414	0.347	0.207
CO_2 x Cultivar x K-Level		0.670	0.336	0.787	0.7836	0.673	0.385

Table 8.

R8 Seed Nutrient Concentrations		**N (mg g^{-1})**	**P (mg g^{-1})**	**K (mg g^{-1})**	**Fe (mg kg^{-1})**	**Zn (mg kg^{-1})**	**Mg (mg g^{-1})**	**Ca (mg g^{-1})**	**S (mg g^{-1})**
CO$_2$	**Ambient**	6.25 a	0.65	2.19 a	109.85	63.74	0.34	0.58	0.37
	Elevated	5.93 b	0.61	2.08 b	112.61	60.69	0.32	0.55	0.35
Cultivar	**Wabash (Old)**	6.17	0.63	2.19 a	117.29 a	62.47	0.31 b	0.45 b	0.37 a
	LD00-3309 (New)	6.01	0.62	2.08 b	105.17 b	61.96	0.35 a	0.68 a	0.35 b
K-Level	**Additional**	6.19	0.63	2.22 a	110.17	61.13	0.34	0.58	0.36
	Recommended	6.01	0.62	2.18 a	118.50	61.16	0.33	0.53	0.36
	Deficient	6.06	0.63	1.20 b	105.02	64.36	0.33	0.58	0.36
CO$_2$		0.016*	0.065	0.028*	0.540	0.152	0.094	0.223	0.057
Cultivar		0.217	0.668	0.032*	0.011*	0.805	0.002*	<0.001*	0.010*
K-Level		0.463	0.899	0.002*	0.064	0.346	0.650	0.332	0.808
CO$_2$ x Cultivar		0.589	0.786	0.819	0.170	0.123	0.587	0.261	0.739
CO$_2$ x K-Level		0.584	0.766	0.310	0.133	0.628	0.409	0.268	0.635
Cultivar x K-Level		0.046*	0.406	0.685	0.204	0.673	0.764	0.845	0.089
CO$_2$ x Cultivar x K-Level		0.591	0.445	0.922	0.572	0.493	0.818	0.614	0.991
R8 Seed Nutrient Uptake		**N (g plant^{-1})**	**P (g plant^{-1})**	**K (g plant^{-1})**	**Fe (mg plant^{-1})**	**Zn (mg plant^{-1})**	**Mg (g plant^{-1})**	**Ca (g plant^{-1})**	**S (g plant^{-1})**
CO$_2$	**Ambient**	1.39 b	0.14 b	0.49 b	2.46 b	1.42 b	0.075	0.126	0.082 b
	Elevated	1.70 a	0.17 a	0.59 a	3.17 a	1.71 a	0.091	0.155	0.100 a
Cultivar	**Wabash (Old)**	1.63	0.16	0.57 a	3.08 a	1.62	0.082	0.119	0.097 a
	LD00-3309 (New)	1.46	0.15	0.50 b	2.54 b	1.51	0.085	0.162	0.085 b
K-Level	**Additional**	1.64	0.17	0.58	2.92	1.62	0.088	0.149	0.096
	Recommended	1.45	0.15	0.53	2.64	1.47	0.078	0.127	0.087
	Deficient	1.54	0.16	0.50	2.88	1.61	0.083	0.146	0.089
CO$_2$		0.024*	0.026*	0.006*	<0.001*	0.031*	0.059	0.052	0.003*
Cultivar		0.100	0.111	0.049*	0.009*	0.273	0.589	<0.001*	0.037*
K-Level		0.359	0.375	0.126	0.436	0.425	0.418	0.235	0.450
CO$_2$ x Cultivar		0.383	0.171	0.210	0.885	0.039*	0.408	0.519	0.181
CO$_2$ x K-Level		0.056	0.089	0.109	0.248	0.061	0.328	0.466	0.038
Cultivar x K-Level		0.337	0.463	0.662	0.235	0.689	0.862	0.942	0.394
CO$_2$ x Cultivar x K-Level		0.189	0.245	0.284	0.633	0.498	0.529	0.469	0.276

Table 9.

R5 Leaf Nutrient Concentration		N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mg (mg g^{-1})	Ca (mg g^{-1})	S (mg g^{-1})
CO₂	**Ambient**	3.25 a	0.23	1.02 a	287.04 b	68.04	0.491	2.251	0.204 a
	Elevated	2.85 b	0.20	0.72 b	384.80 a	65.33	0.485	2.209	0.176 b
Cultivar	**Wabash (Old)**	2.93	0.20	0.78 b	278.30 b	68.54	0.578 a	2.272	0.181 b
	LD00-3309 (New)	3.18	0.23	0.96 a	393.50 a	64.83	0.398 b	2.188	0.199 a
K-Level	**Additional**	3.23	0.22	1.06 a	295.19	69.19	0.364 b	2.010 b	0.202 a
	Recommended	3.00	0.20	0.70 b	351.75	59.44	0.458 b	2.148 b	0.183 b
	Deficient	2.92	0.23	0.86 b	360.81	71.44	0.642 a	2.532 a	0.186 ab
CO₂		0.004*	0.061	<0.001*	0.039*	0.722	0.896	0.711	<0.001*
Cultivar		0.052	0.061	0.029*	0.016*	0.627	0.001*	0.455	0.012*
K-Level		0.123	0.172	0.003*	0.451	0.397	<0.001*	0.002*	0.047*
CO₂ x Cultivar		0.372	0.537	0.559	0.203	0.987	0.896	0.651	0.316
CO₂ x K-Level		0.293	0.977	0.856	0.199	0.198	0.493	0.842	0.184
Cultivar x K-Level		0.408	0.159	0.284	0.233	0.582	0.865	0.919	0.343
CO₂ x Cultivar x K-Level		0.622	0.876	0.784	0.32	0.844	0.485	0.187	0.729
R5 Leaf Nutrient Uptake		**N (g plant^{-1})**	**P (g plant^{-1})**	**K (g plant^{-1})**	**Fe (mg plant^{-1})**	**Zn (mg plant^{-1})**	**Mg (g plant^{-1})**	**Ca (g plant^{-1})**	**S (g plant^{-1})**
CO₂	**Ambient**	0.25	0.0166	0.072	1.928 b	0.537	0.0386	0.169	0.0155
	Elevated	0.25	0.0172	0.063	3.299 a	0.585	0.0429	0.193	0.0154
Cultivar	**Wabash (Old)**	0.27	0.0179	0.068	2.503	0.634	0.0523 a	0.202	0.0165
	LD00-3309 (New)	0.23	0.0159	0.067	2.724	0.488	0.0291 b	0.160	0.0144
K-Level	**Additional**	0.27	0.018	0.087 a	2.346	0.593	0.0304	0.168	0.0169
	Recommended	0.27	0.017	0.072 a	2.496	0.562	0.0446	0.177	0.0163
	Deficient	0.21	0.015	0.045 b	2.999	0.527	0.0472	0.198	0.0132
CO₂		0.946	0.700	0.244	<0.001*	0.611	0.516	0.307	0.940
Cultivar		0.189	0.272	0.903	0.561	0.130	0.001*	0.073	0.236
K-Level		0.161	0.386	<0.001*	0.341	0.848	0.095	0.527	0.189
CO₂ x Cultivar		0.067	0.082	0.356	0.404	0.324	0.432	0.144	0.056
CO₂ x K-Level		0.395	0.544	0.557	0.255	0.221	0.659	0.635	0.396
Cultivar x K-Level		0.412	0.746	0.921	0.124	0.820	0.179	0.209	0.438
CO₂ x Cultivar x K-Level		0.479	0.387	0.317	0.048*	0.97	0.971	0.931	0.465

Table 10.

R5 Root Nutrient Concentrations		N (mg g^{-1})	P (mg g^{-1})	K (mg g^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mg (mg g^{-1})	Ca (mg g^{-1})	S (mg g^{-1})
CO_2	**Ambient**	1.990	0.177	0.217	1441	35.96	0.188	0.530	0.220
	Elevated	2.069	0.197	0.268	1597	36.29	0.225	0.546	0.244
Cultivar	**Wabash (Old)**	2.075	0.189	0.238	1480	39.50 a	0.226 a	0.609 a	0.238
	LD00-3309 (New)	1.984	0.185	0.246	1559	32.75 b	0.188 b	0.467 b	0.227
K-Level	**Additional**	2.159	0.190	0.310	1335	35.69	0.236	0.545	0.257
	Recommended	1.987	0.181	0.214	1801	34.94	0.196	0.553	0.217
	Deficient	1.943	0.190	0.203	1422	37.75	0.189	0.516	0.223
CO_2		0.454	0.143	0.212	0.376	0.914	0.053	0.526	0.114
Cultivar		0.391	0.776	0.845	0.653	0.034*	0.043*	<0.001*	0.456
K-Level		0.220	0.796	0.072	0.081	0.729	0.092	0.459	0.062
CO_2 x Cultivar		0.256	0.924	0.364	0.198	0.626	0.717	0.841	0.818
CO_2 x K-Level		0.274	0.090	0.626	0.738	0.943	0.400	0.202	0.045
Cultivar x K-Level		0.583	0.759	0.747	0.029*	0.390	0.396	0.519	0.591
CO_2 x Cultivar x K-Level		0.066	0.368	0.631	0.480	0.181	0.553	0.171	0.546
R5 Root Nutrient Uptake		**N (g $plant^{-1}$)**	**P (g $plant^{-1}$)**	**K (g $plant^{-1}$)**	**Fe (mg $plant^{-1}$)**	**Zn (mg $plant^{-1}$)**	**Mg (mg $plant^{-1}$)**	**Ca (mg $plant^{-1}$)**	**S (mg $plant^{-1}$)**
CO_2	**Ambient**	0.145 b	0.013 b	0.016 b	12.96	0.26 b	14.38 b	38.71 b	15.62 b
	Elevated	0.210 a	0.019 a	0.027 a	18.07	0.37 a	23.37 a	55.48 a	24.56 a
Cultivar	**Wabash (Old)**	0.161	0.015	0.020	12.92	0.30	18.61	47.93	18.50
	LD00-3309 (New)	0.195	0.017	0.023	18.11	0.32	19.14	46.26	21.68
K-Level	**Additional**	0.192	0.017	0.029	13.34	0.33	21.74	48.22 ab	22.67
	Recommended	0.199	0.018	0.021	20.08	0.35	20.44	55.54 a	21.48
	Deficient	0.141	0.013	0.016	13.12	0.37	14.45	37.53 b	16.12
CO_2		0.003*	<0.001*	0.037*	0.124	0.005*	0.005*	0.005*	<0.001*
Cultivar		0.105	0.125	0.445	0.119	0.626	0.859	0.768	0.176
K-Level		0.053	0.115	0.106	0.153	0.223	0.122	0.043*	0.059
CO_2 x Cultivar		0.909	0.647	0.510	0.227	0.449	0.777	0.433	0.948
CO_2 x K-Level		0.657	0.300	0.906	0.965	0.635	0.765	0.604	0.268
Cultivar x K-Level		0.451	0.680	0.817	0.110	0.097	0.578	0.210	0.710
CO_2 x Cultivar x K-Level		0.912	0.732	0.710	0.813	0.995	0.850	0.803	0.872

Figures

Figure 1.

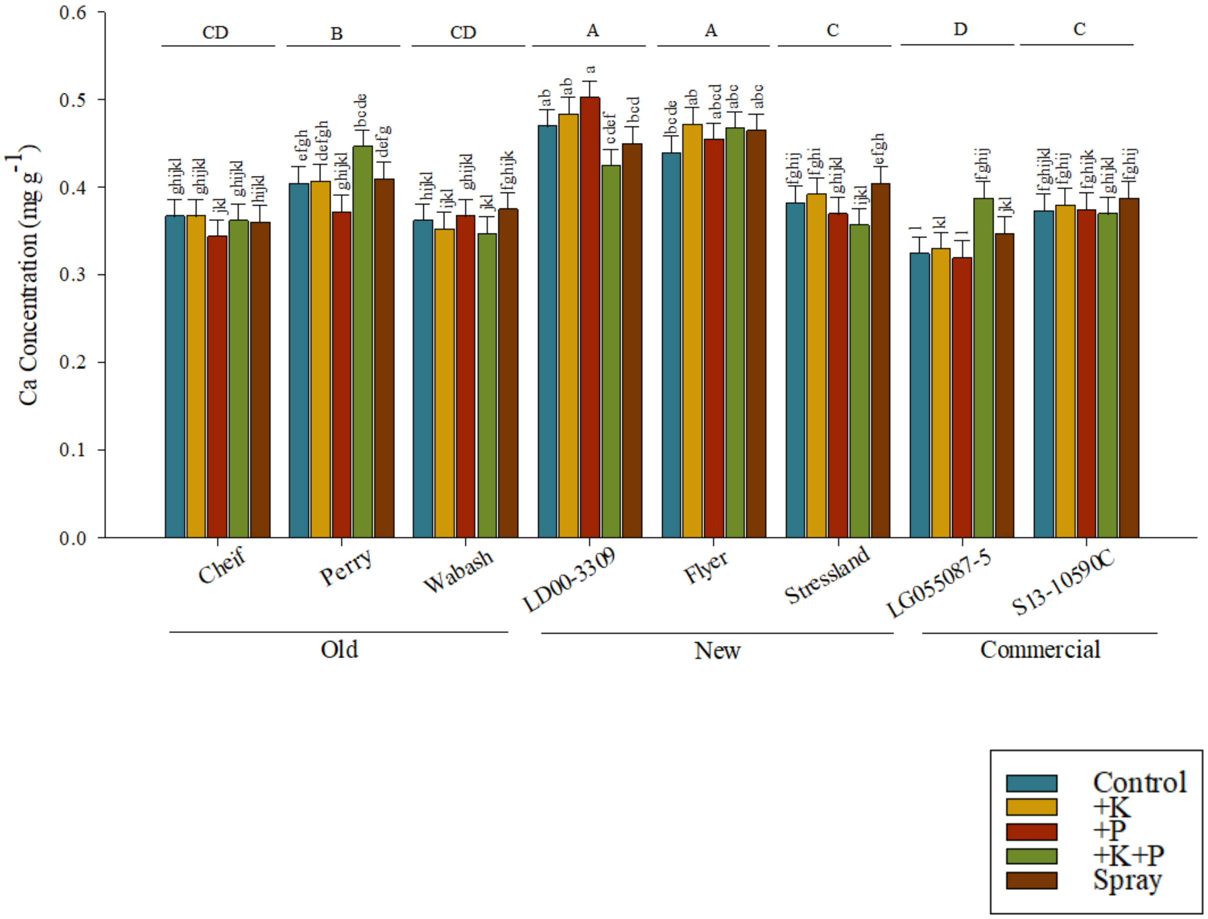


Figure 2.

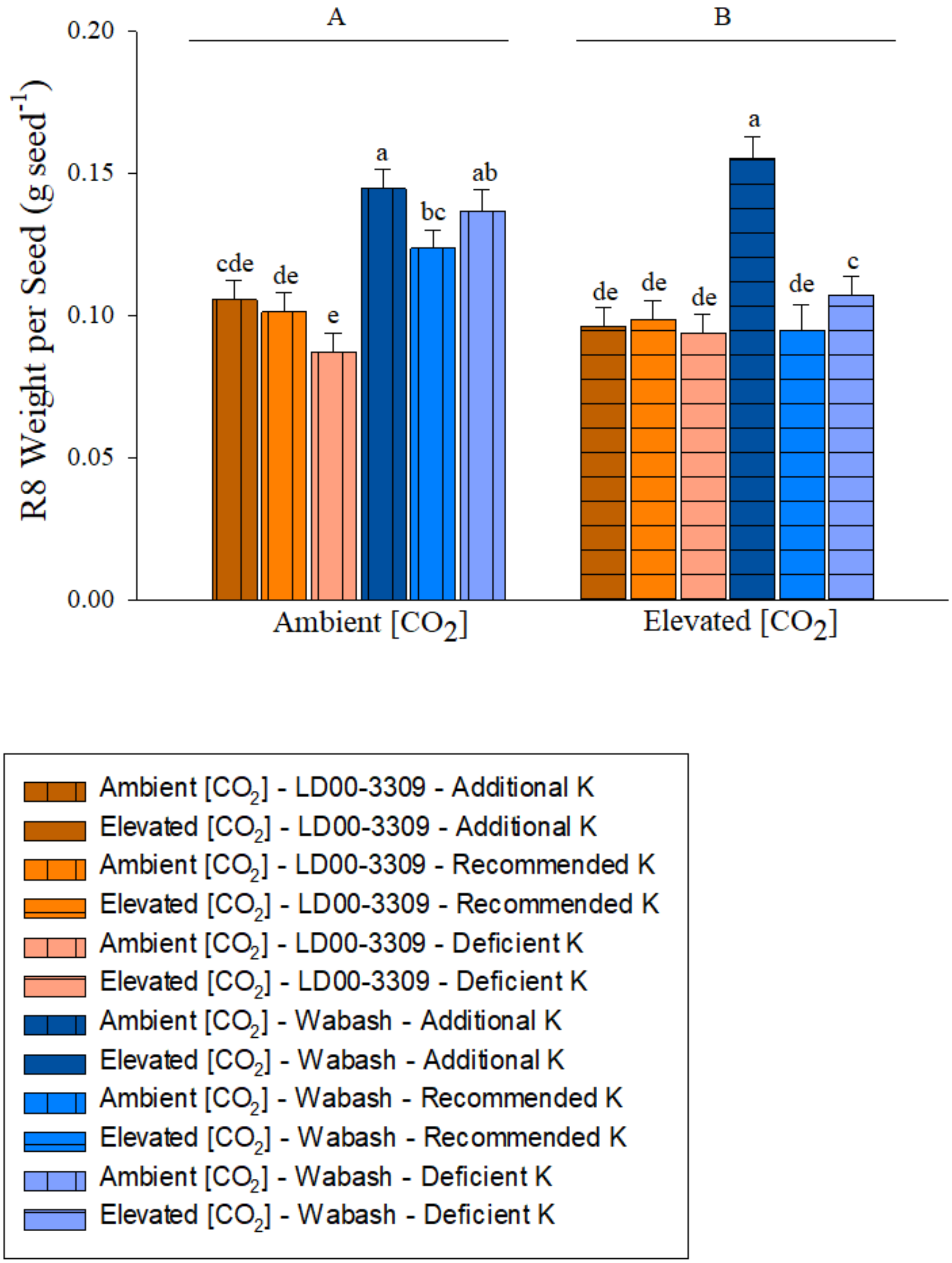
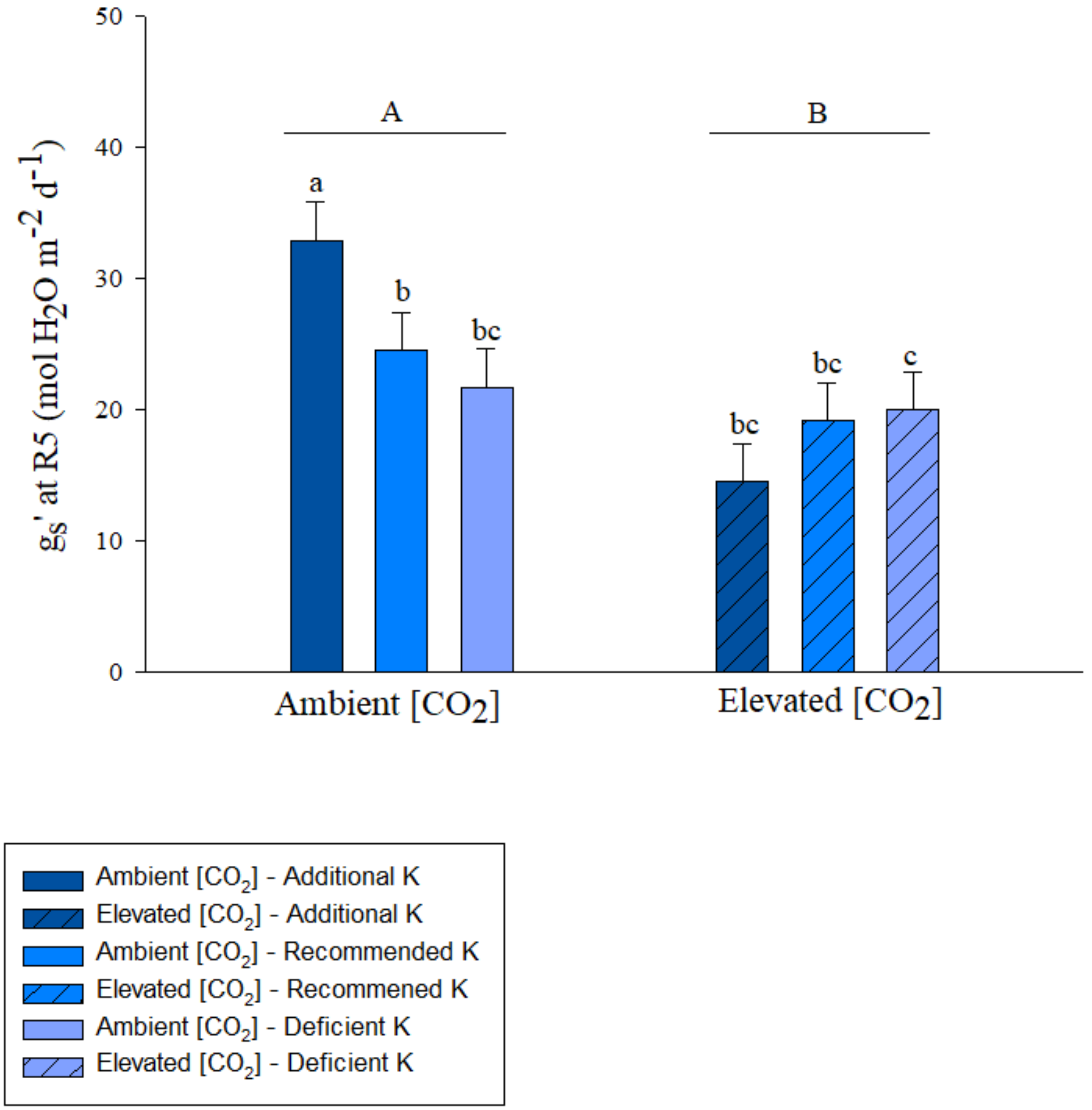

A
B
0.20
0.15
0.10
0.05
0.00
R8 Weight per Seed (g seed^{-1})
cde
de
e
a
bc
ab
de
de
de
a
de
c
Ambient [CO_2]
Elevated [CO_2]
Ambient [CO_2] - LD00-3309 - Additional K
Elevated [CO_2] - LD00-3309 - Additional K
Ambient [CO_2] - LD00-3309 - Recommended K
Elevated [CO_2] - LD00-3309 - Recommended K
Ambient [CO_2] - LD00-3309 - Deficient K
Elevated [CO_2] - LD00-3309 - Deficient K
Ambient [CO_2] - Wabash - Additional K
Elevated [CO_2] - Wabash - Additional K
Ambient [CO_2] - Wabash - Recommended K
Elevated [CO_2] - Wabash - Recommended K
Ambient [CO_2] - Wabash - Deficient K
Elevated [CO_2] - Wabash - Deficient K

Figure 3.


50
40
30
20
10
0
g_s' at R5 (mol H_2O m^{-2} d^{-1})
A
B
a
b
bc
bc
bc
c
Ambient [CO_2]
Elevated [CO_2]
Ambient [CO_2] - Additional K
Elevated [CO_2] - Additional K
Ambient [CO_2] - Recommended K
Elevated [CO_2] - Recommened K
Ambient [CO_2] - Deficient K
Elevated [CO_2] - Deficient K

Supplemental Table 1. P-values comparing individual diurnal photosynthetic and stomatal conductance measurements taken from soybeans grown at USDA-ARS National Soil Dynamics Laboratory (Auburn, AL). P-values compare means within [CO_2], cultivar, and K-level treatments and compare means of treatment interactions. P-values calculated from a three-way ANOVA. Asterisks represent significant differences ($p<0.05$).

						June 29th						
			A (umol CO_2 m^{-2} s^{-1})						**g_s (mmol H_2O m^{-2} s^{-1})**			
	7:30am	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**	**7:30am**	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**
CO_2	0.006*	<0.001*	<0.001*	<0.001*	0.015*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.001*	0.434
Cultivar	0.964	0.601	0.058	0.461	0.091	0.026	0.834	0.119	0.004*	0.008*	0.050	0.129
K-Level	0.256	0.598	0.809	0.026	0.582	0.896	0.880	0.706	0.701	0.119	0.261	0.820
CO_2 x cultivar	0.970	0.039	0.197	0.199	0.537	0.713	0.601	0.613	0.604	0.628	0.765	0.396
CO_2 x K-Level	0.955	0.779	0.297	0.905	0.997	0.729	0.635	0.908	0.748	0.987	0.955	0.913
Cultivar x K-Level	0.471	0.387	0.676	0.043	0.729	0.719	0.943	0.875	0.983	0.353	0.936	0.060
CO_2 x Cultivar x K-Level	0.610	0.100	0.993	0.537	0.272	0.642	0.801	0.870	0.288	0.017*	0.752	0.741
						July 17th						
			A (umol CO_2 m^{-2} s^{-1})						**g_s (mmol H_2O m^{-2} s^{-1})**			
	7:30am	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**	**7:30am**	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**
CO_2	0.212	0.004*	<0.001*	<0.001*	<0.001*	0.014	<0.001*	0.005*	0.041*	0.032	0.006*	<0.001*
Cultivar	0.197	0.412	0.194	0.709	0.696	0.130	0.023*	0.093	0.142	0.841	0.236	0.052
K-Level	0.735	0.071	0.727	0.495	0.753	0.186	0.840	0.196	0.931	0.231	0.412	0.477
CO_2 x cultivar	0.583	0.365	0.704	0.954	0.887	0.375	0.529	0.203	0.958	0.724	0.875	0.601
CO_2 x K-Level	0.989	0.301	0.749	0.229	0.490	0.277	0.800	0.546	0.540	0.453	0.040*	0.071
Cultivar x K-Level	0.888	0.155	0.224	0.048	0.728	0.361	0.579	0.615	0.506	0.164	0.839	0.656
CO_2 x Cultivar x K-Level	0.435	0.287	0.391	0.412	0.657	0.414	0.487	0.485	0.359	0.711	0.288	0.292
						August 8th						
			A (umol CO_2 m^{-2} s^{-1})						**g_s (mmol H_2O m^{-2} s^{-1})**			
	7:30am	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**	**7:30am**	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**
CO_2	0.020*	<0.001*	0.019*	0.002*	0.093	0.002*	0.026*	0.005*	0.058	0.021*	0.018*	0.077
Cultivar	0.772	0.815	0.435	0.838	0.973	0.938	0.189	0.032	0.295	0.061	0.227	0.129
K-Level	0.481	0.288	0.782	0.521	0.861	0.694	0.762	0.731	0.582	0.544	0.907	0.199
CO_2 x cultivar	0.966	0.663	0.539	0.095	0.274	0.136	0.703	0.647	0.947	0.059	0.177	0.652
CO_2 x K-Level	0.228	0.998	0.146	0.607	0.351	0.169	0.407	0.024*	0.011*	0.134	0.250	0.021*
Cultivar x K-Level	0.938	0.106	0.650	0.739	0.511	0.840	0.943	0.360	0.716	0.113	0.690	0.594
CO_2 x Cultivar x K-Level	0.361	0.805	0.931	0.859	0.646	0.217	0.818	0.738	0.958	0.898	0.489	0.350

Supplemental Table 2. Instantaneous photosynthetic (A, umol CO_2 m^{-2} s^{-1}**)** and stomatal conductance (g_s, mmol H_2O $m^{-2}s^{-1}$) measured on soybeans grown at USDA-ARS National Soil Dynamics Laboratory (Auburn, AL). Values represent means grouped into [CO_2], cultivar and K-level treatments and represent individual treatment values.

June 29th													
		A (umol CO_2 m^{-2} s^{-1})						**g_s (mmol H_2O m^{-2} s^{-1})**					
		7:30am	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**	**7:30am**	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**
CO_2	**Ambient**	14.77	20.62	26.02	24.79	22.31	8.05	0.920	1.094	1.790	1.019	0.651	0.115
	Elevated	16.48	24.79	32.78	30.97	25.65	12.16	0.655	0.796	0.807	0.727	0.402	0.123
Cultivar	**LD00-3309**	15.61	22.51	28.54	27.62	22.85	9.40	0.792	0.903	0.872	0.791	0.460	0.110
	Wabash	15.64	22.89	30.27	28.14	25.1	10.81	0.783	0.987	1.114	0.955	0.593	0.128
K-Level	**Additional**	15.58	23.18	29.43	26.49	23.08	10.26	0.794	0.958	1.010	0.785	0.474	0.115
	Recommended	15.03	22.66	29.04	28.48	24.08	9.91	0.798	0.914	0.948	0.914	0.504	0.119
	Deficient	16.26	22.27	29.74	28.68	24.77	10.13	0.771	0.963	1.022	0.919	0.602	0.123
July 17th													
		A (umol CO_2 m^{-2} s^{-1})						**g_s (mmol H_2O m^{-2} s^{-1})**					
		7:30am	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**	**7:30am**	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**
CO_2	**Ambient**	16.12	24.96	24.08	24.42	22.79	20.35	0.544	0.880	0.683	0.834	0.759	0.436
	Elevated	17.48	29.07	29.93	30.69	28.22	23.28	0.274	0.526	0.511	0.665	0.550	0.258
Cultivar	**LD00-3309**	16.09	26.46	26.12	27.36	25.31	20.99	0.353	0.601	0.536	0.757	0.611	0.304
	Wabash	17.5	25.57	27.89	27.75	25.69	22.64	0.464	0.805	0.658	0.742	0.697	0.391
K-Level	**Additional**	16.52	28.1	27.02	27.41	26.00	22.56	0.422	0.818	0.611	0.779	0.720	0.379
	Recommended	16.49	24.81	26.34	26.87	25.14	20.41	0.389	0.564	0.575	0.658	0.641	0.315
	Deficient	17.38	28.14	27.65	28.39	25.37	22.47	0.415	0.727	0.605	0.812	0.603	0.348
August 8th													
		A (umol CO_2 m^{-2} s^{-1})						**g_s (mmol H_2O m^{-2} s^{-1})**					
		7:30am	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**	**7:30am**	**9:30am**	**11:30am**	**1:30pm**	**3:30pm**	**5:30pm**
CO_2	**Ambient**	8.79	24.08	22.41	22.70	23.33	17.86	0.530	0.971	0.701	0.650	0.690	0.440
	Elevated	10.61	30.67	27.92	29.97	28.08	22.44	0.371	0.670	0.447	0.469	0.448	0.328
Cultivar	**LD00-3309**	9.59	27.56	26.05	26.56	25.66	20.10	0.495	0.932	0.643	0.632	0.629	0.432
	Wabash	9.81	27.20	24.29	26.11	25.76	20.20	0.405	0.710	0.505	0.487	0.510	0.336
K-Level	**Additional**	9.20	29.15	24.22	26.45	26.66	20.63	0.459	0.873	0.665	0.565	0.601	0.460
	Recommended	9.63	26.48	26.11	27.82	24.81	19.33	0.416	0.776	0.560	0.607	0.553	0.324
	Deficient	10.27	26.50	25.17	24.74	25.66	20.49	0.476	0.813	0.497	0.506	0.554	0.369

Supplemental Table 3. Correlation p-values between organ dry weight and nutrient content. Measured biomass includes the plant organs leaves, roots and R8 seeds. Nutrient content includes uptake and concentration for potassium, iron and zinc. Biomass and nutrient content were measured from soybeans grown at USDA-ARS National Soil Dynamics Laboratory (Auburn, AL). P-values calculated using a Pearson correlation.

Potassium- Leaves	Concentration	Dry Weight	Stomatal Conductance	**Potassium- Roots**	Concentration	Dry Weight	Stomatal Conductance	**Potassium - Final Harvest Seeds**	Concentration	Dry Weight	Stomatal Conductance
Uptake	r=0.54 p<0.0001	r=0.59 p<0.0001	r=0.17 p=0.25	Uptake	r=0.82 p<0.0001	r=0.52 p=0.0002	r=-0.21 p=0.15	Uptake	r=0.12 p=0.43	r=0.83 p<0.0001	r=-0.3016 p=0.049
Concentration		r=-0.29 p=0.049	r=0.24 p=0.094	Concentration		r=-0.0017 p=0.99	r=-0.066 p=0.66	Concentration		r=-0.34 p=0.025	r=0.15 p=0.34
Dry Weight			r=-0.070 p=0.64	Dry Weight			r=-0.31 p=0.035	Dry Weight			r=-0.40 p=0.0074
Stomatal Conductance				Stomatal Conductance				Stomatal Conductance			
Iron- Leaves	Concentration	Dry Weight	Stomatal Conductance	**Iron- Roots**	Concentration	Dry Weight	Stomatal Conductance	**Iron- Final Harvest Seeds**	Concentration	Dry Weight	Stomatal Conductance
Uptake	r=0.76 p<0.0001	r=0.37 p=0.0087	r=-0.37 p=0.0088	Uptake	r=0.88 p<0.0001	r=0.89 p<0.0001	r=-0.25 p=0.092	Uptake	r=0.47 p=0.0016	r=0.78 p<0.0001	r=-0.32 p=0.037
Concentration		r=-0.23 p<0.12	r=-0.29 p=0.047	Concentration		r=0.65 p<0.0001	r=-0.20 p=0.18	Concentration		r=-0.12 p=0.45	r=-0.075 p=0.63
Dry Weight			r=-0.070 p=0.64	Dry Weight			r=-0.31 p=0.035	Dry Weight			r=-0.40 p=0.0074
Stomatal Conductance				Stomatal Conductance				Stomatal Conductance			
Zinc- Leaves	Concentration	Dry Weight	Stomatal Conductance	**Zinc- Roots**	Concentration	Dry Weight	Stomatal Conductance	**Zinc- Final Harvest Seeds**	Concentration	Dry Weight	Stomatal Conductance
Uptake	r=0.7080 p<0.0001	r=0.801 p<0.0001	r=0.031 p=0.83	Uptake	r=0.24 p=0.1077	r=0.84 p<0.0001	r=-0.27 p=0.067	Uptake	r=0.19 p=0.22	r=0.85 p<0.0001	r=-0.43 p=0.0039
Concentration		r=0.18 p=0.21	r=0.073 p=0.62	Concentration		r=-0.23 p=0.11	r=0.16 p=0.29	Concentration		r=-0.28 p=0.068	r=-0.18 p=0.24
Dry Weight			r=-0.070 p=0.64	Dry Weight			r=-0.31 p=0.035	Dry Weight			r=-0.40 p=0.0074
Stomatal Conductance				Stomatal Conductance				Stomatal Conductance			