

RESPONSES OF C3 AND C4 WEED SPECIES TO TEMBOTRIONE AND
DICAMBA HERBCIDE UNDER ELEVATED ATMOSPHERIC CO₂ AND WATER
DEFICIT

A Thesis

by

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ABSTRACT

The current atmospheric CO₂ concentration of 400 ppm will increase to 700-1000 ppm by the year 2100, according to the IPCC. Since chemical control is the most common method to control weeds in cropping systems, and climate change can influence weed growth and herbicide efficacy, its effect on agricultural production will likely be critical. It is also known that C3 and C4 species react differently to changing climate, with C3 species having an advantage over C4 species. Therefore, to evaluate changes in weed responses and herbicide efficacy between photosynthetic systems, two C3 species, velvetleaf and weedy rice, and two C4 species, Johnsongrass and Palmer amaranth were studied. Greenhouse studies were conducted to evaluate herbicide efficacy under elevated CO₂ concentration and water deficit on these species. Firstly, tembotrione, an HPPD herbicide, was applied at 0, ½, and ¾ the recommended rate for weedy rice, Johnsongrass, and Palmer amaranth. Velvetleaf plants received it at 0, ⅓, and ½ rates. Secondly, dicamba, an auxin herbicide, was applied on velvetleaf and Palmer amaranth at similar rates. In both studies, herbicide efficacy was evaluated at 10 and 21 DAT. At 10 DAT, carbon assimilation rate (*A*) and stomatal conductance (*g_s*) measurements were taken on plants with non-necrosed leaves. At 21 DAT, total biomass and root to shoot ratio were measured. Results showed tembotrione application under elevated CO₂ provided efficient weed control in weedy rice, Johnsongrass, and Palmer amaranth. However, lower herbicide efficacy was seen when velvetleaf plants were sprayed with

tembotrione. Although lower herbicide rates were applied on velvetleaf, it showed increased tolerance to tembotrione, which could be explained by plant anatomical and physiological features. Dicamba showed higher efficacy under ambient CO₂ concentration in both, velvetleaf and Palmer amaranth. In addition, the application of ½ dicamba rate increased the tolerance of velvetleaf under elevated CO₂, which exemplifies the need for full herbicide rate to completely control this species and avoid resistance. Therefore, these findings suggest that under future climate scenarios, plant specific features can have a bigger interference on herbicide efficacy than a generalization on photosynthetic pathway.

DEDICATION

To my mother, Rosinha, for all her support and love. Thank you so much for putting my dreams before yours.

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All other work conducted for the thesis was completed by the student independently.

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NOMENCLATURE

ppm	Part per Million
CO ₂	Carbon Dioxide
U. S	United States
PGA	3-phosphoglycerate
ATP	Adenosine Triphosphate
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
HCO ₃	Bicarbonate
<i>g_s</i>	Stomatal Conductance
C _i	Internal Carbon
[CO ₂]	Carbon Dioxide Concentration
ALS	Acetolactate Synthase
PSII	Photosystem II
EPSPS	5-enolpyruvylshikimate-3-phosphate
HPPD	4-Hydroxyphenylpyruvate dioxygenase
HGA	Homogentisate
ROS	Reactive Oxygen Species
UV	Ultraviolet
HRAC	Herbicide Resistant Action Committee
MCPA	2-methyl-4-chlorophenoxyacetic acid

IAA	Indole-3-acetic acid
ABA	Abscisic acid
2,4- D	2,4-Dichlorophenoxyacetic acid
IPCC	Intergovernmental Panel on Climate Change
eCO ₂	Elevate CO ₂
FC	Field Capacity
WD	Water Deficit
MSO	Modified Seed Oil
CRD	Complete Randomized Design
DAT	Days After Treatment
H ₂ O ₂	Hydrogen peroxide
O ₂ ^{•-}	Superoxide
HO•	Hydroxyl Radicals
¹ O ₂	Singlet Oxygen
PSI	Photosystem I
R/S	Root to shoot ratio
<i>A</i>	Assimilation Rate
OTT	Over-the-top

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1. INTRODUCTION

Climate change is known as “the long-term changes in the climate during a period of time”(IPCC, 2014). Although climate change is a natural process, the number of extreme climatic events resulting from climate change has increased over the past decades.

Periods of drought, extreme temperatures, flooding, windstorms, and hurricanes resulting from rapid climatic changes are directly affecting human lives (Houghton, 2001). For example, during the devastating hurricane Harvey in the southern U.S. during 2017, more than 80,000 homes were flooded (Wang et al., 2018). The economic damage left from Harvey is estimated between \$90–\$160 billion dollars (Blake & Zelinsky, 2018).

Climate change can affect the future of food production and our ability to feed the world’s increasing population. The world’s population is expected to reach to 9 billion by the year 2050 and about 66% of this population will live in urban areas (Li et al., 2019). Additionally, the global demand for food is estimated to rise by 70 to 80% by 2050 (FAO, 2009). At the same time, increased temperature, long periods of drought in some places and hurricanes and flooding in other places are threatening the agriculture industry and the future of food security (Jones et al., 2012). It is predicted that climate change can cause hunger to affect an additional 600 million people by 2080 (Warren et al., 2006). An enhanced greenhouse effect is known to be one of the main drivers of severe climate change. The planet’s surface absorbs part of the radiation emitted by the sun (i.e. short wavelengths) and reflects long and infrared wavelengths into space. The

greenhouse effect occurs when those long wavelengths are absorbed by gases such as water vapor, carbon dioxide, methane, nitrous oxide and clouds and then emit the energy back to the Earth's surface. Thus, the accumulation of long and infrared wavelengths in the lower atmosphere provokes a rise in the temperature at the Earth's surface (Houghton, 2001; IPCC, 2013).

Carbon dioxide (CO₂) is the main atmospheric gas contributing to the enhanced greenhouse effect. The CO₂ concentration is expected to rise linearly from the current concentration of 412 ppm to 700 to 1000 ppm by the year 2100 (IPCC, 2013; Refaat et al., 2018). The increased level of CO₂ is primarily associated with enhanced fossil fuel consumption since the industrial revolution, while changes of the biophysical properties of the land (i.e. deforestation, agriculture, and urbanization) is considered as a secondary cause of increased CO₂ (Ciais et al., 2014; Flato et al., 2013). Accumulation of high levels of CO₂ can lead to a rapid increase in temperature. Without any global regulation on CO₂ emissions, the global average temperature may increase by up to 1.5°C by 2030, which can impact species distribution and affect food security (Ciais et al., 2014; Dusenge et al., 2019).

Crop growth and yield would be greatly affected by climate change. Many studies have shown that increased CO₂ above the current level can increase crop biomass and yield by stimulating photosynthesis (fertilizer effect) and reducing transpiration, mainly for C₃ species (Adams et al., 1998; Ainsworth et al., 2006; Kimball et al., 2002; Tubiello & Ewert, 2002). However, most of these studies have focused on the effect of elevated CO₂ on crop production alone. In fact, biotic (pathogens, pests and weeds) and abiotic

(increased temperature, flooding) stressors can influence crop production under predicted elevated CO₂ scenarios. For example, high temperature during the flowering period of a crop may reduce grain number, size, and quality and decrease the positive effect of the elevated CO₂ (Caldwell et al., 2005). Additionally, pollen grains lose their viability at high temperature, causing the failure of seed set, even if the elevated CO₂ level is in favor of the crop (Hatfield & Prueger, 2015).

Weed species are one of the most important biotic stressors that can undermine the positive effect of elevated CO₂ on crop production. Although the impact of unmanaged weeds on reducing crop yield is not new, whether the future climate change can increase the impact of weeds to the cropping systems is concerning because weeds are known to be highly adaptive to environmental changes because of their great population diversity when compared to crops (Korres et al., 2016). Many weed species can already be found in a wide range of environmental conditions, however, under future climate change they are expected to expand their geographical ranges. Due to their rapid dispersal and colonization ability, they could become successful invaders occupying locations that they have not previously colonized (Waryszak et al., 2018). For example, it was predicted that Johnsongrass (*Sorghum halepense*) and velvetleaf (*Abutilon theophrasti*) which are currently predominant weeds in the southern U.S., can invade the northeastern U.S. crop fields (e.g. corn) under future climatic conditions (McDonald et al., 2009). Chemical control is one of the most common tools used for managing weeds. Chemical control is the preferred method, though not sustainable, because of its rapid control response and cost-effectiveness compared to the other practices of weed management.

As a consequence of high reliance on chemical control, the number of herbicide resistant weed species are rapidly increasing. The number of weed species that developed multiple resistance has increased quicker over the past years

(<http://www.weedscience.org/>) due to an intense selection pressure that makes weed species evolve adaptive traits that overcome herbicide effects (Délye et al., 2013).

However, the efficacy of herbicides for weed control can be dependent on the environmental conditions. Temperature, precipitation, light intensity, relative humidity, dew, wind, and soil moisture are known to affect herbicide efficacy (Varanasi et al., 2016). In addition, a number of studies showed that the level of herbicide resistance can vary with the environmental conditions that weeds are exposed to (Johnson & Young, 2002; Zanatta et al., 2008). This is mostly because of the changes in the anatomical and physiological characteristics of the weed species that can influence herbicide uptake, translocation, retention and efficacy (Johnson & Young, 2002). For example, the glyphosate efficacy for controlling Canada thistle (*Cirsium arvense*) was reduced when plants were grown under increased concentrations of CO₂ (Ziska et al., 2004). Water deficit also impacts herbicide efficacy, and improved weed control was observed when sulfosulfuron was applied at field capacity than at one-third soil capacity (Olson et al., 2000).

Therefore, the future of weed management is highly dependent on our understanding of the weed species' response and adaptation to climate change (McDonald et al., 2009).

Given that elevated CO₂ is one of main drivers of the future climate, an important question is how weed species respond to the elevated CO₂ and whether herbicide

efficacy would be influenced by the increased atmospheric CO₂ level. Another question is how the interaction of elevated CO₂ and drought may change the future of weed control. In this study, the biological, physiological and growth characteristics of four economically important weed species, velvetleaf (*Abutilon theophrasti*), red rice (*Oryza sativa*), Palmer amaranth (*Amaranthus palmeri*) and Johnsongrass (*Sorghum halepense*), will be evaluated under current and simulated future CO₂ levels. Furthermore, these weed species will be exposed to tembotrione and dicamba to examine the herbicide efficacy under high CO₂ and water deficiency.

1.1 Differences between C3 and C4 photosynthetic pathway

Approximately 85% of higher plant species use the C3 photosynthetic pathway while only 5% of plant species (18 families) have the C4 pathway (Yamori et al., 2014). This might be due to the later evolution of the C4 plants. C4 plants evolved from C3 plants more than 25 million years ago as a result of the low CO₂ concentration in the atmosphere. Most C4 species belong to Poaceae, Cyperaceae and Chenopodiaceae (Edwards et al., 2004). There are only 23 species that have characteristics of both pathways, referred to as C3-C4 intermediate plants (Monson & Moore, 1989).

In the C3 pathway, carbon dioxide reaches the chloroplast through the stomata and the intercellular air space. Carbonic anhydrases then reversibly convert CO₂ into bicarbonate (HCO₃⁻). This step helps to keep the supply of CO₂ high for Rubisco. Rubisco carboxylates CO₂ and produces the first compound of the C3 pathway, 3-phosphoglycerate (PGA). Subsequent reduction is powered by ATP and NADPH

produced by the photosynthetic electron transport chain in thylakoid membranes, and the product can then be used in the synthesis of other sugars and starch, and also for regenerating Rubisco (Yamori et al., 2014).

The C4 leaf includes mesophyll and bundle sheath cells that are both involved in different phases of the photosynthetic process. C4 species carboxylate atmospheric carbon by different enzymes into C4 acids such as malate and aspartate in the mesophyll cells. These C4 acids are then transferred to bundle sheath cells, decarboxylated and the released CO₂ enters the C3 pathway (Edwards et al., 2001). The C4 CO₂ “pump” leads to a 10 to 100-fold increase in CO₂ concentration at the site of Rubisco catalysis. CO₂ is then fixed by Rubisco in the chloroplasts of the bundle sheath cells, which have a normal Calvin cycle, as in C3 plants.

Carbon fixation saturates at a lower CO₂ concentration in C4 plants compared to C3 plants. Increasing stomatal conductance (g_s) in C4 plants may favor an additional loss of water vapor without having the benefit of increasing photosynthesis. On the other hand, C3 species have a higher CO₂ saturation point, and thus increased stomatal conductance can elevate C_i (intercellular carbon concentration) and lead to higher assimilation rates. In conclusion, C4 species have lower g_s and transpiration rates when compared to C3 species. In addition, the water use-efficiency (assimilation/transpiration) is higher in C4 than C3 plants (Pearcy & Ehleringer, 1984).

1.2 Weeds and elevated CO₂

Under the earth's current CO₂ concentration, C₃ species are less efficient at carbon fixation than C₄ species due to photorespiration. With the gradual increase in CO₂ levels, net photosynthesis (and subsequent growth) will be enhanced in C₃ plants as photorespiration declines. However, C₄ plants already have an internal biochemical pump for accumulating CO₂ and increase of the atmospheric CO₂ may have less effect on their growth because they already effectively suppress photorespiration (Taiz et al., 2014).

Weed and crop competition can be directly influenced by the level of CO₂. Most current cultivated crops are C₃ species while world's most troublesome weed species are C₄ (Harlan, 1992; Holm et al., 1977). Under the current climatic condition C₄ weeds are more competitive than C₃ crops while an increase of CO₂ concentration may benefit the C₃ crops when competing with C₄ weeds and vice versa.

In competition between C₃ soybean and C₄ redroot pigweed (*Amaranthus retroflexus*), soybean yield loss was lower under elevated CO₂ than normal CO₂ level. Furthermore, the biomass of redroot pigweed did not change between the normal and elevated CO₂ level (Ziska, 2000). Sorghum (C₄), planted at different densities with C₃ common cocklebur (*Xanthium strumarium* L.), showed a reduction in biomass and leaf area at elevated CO₂ concentration (Ziska, 2001).

There is no evidence that under elevated CO₂ a C₃ crop will overcome the competitive ability of a C₃ weed species. On the contrary, high levels of CO₂ may result in even more severe competition between weedy and cultivated rice when CO₂ is increased

(Ziska et al., 2010). When studying the interactions between common lambsquarters (*Chenopodium album* L.), a C3 species, and soybean at elevated [CO₂], the weed species had a biomass increase of 65%, while the crop had a yield reduction of 39% (Ziska, 2000). Given that the majority of cropping systems are comprised of various C3 and C4 species, future increases in CO₂ may lead to a weed community shift and the rise of new weeds with highly competitive ability.

1.3 Interaction of elevated CO₂ and drought on weeds

Interactions between elevated CO₂ and water availability may change the development and growth of C3 and C4 plants. At current CO₂ levels, C4 plants are more efficient in water use than C3 plants due to their CO₂ concentrating mechanisms. These mechanisms allow C4 species to suppress photorespiration and reduce stomatal conductance (g_s), therefore saving water (Bräutigam & Gowik, 2016). When soil water content is low, C4 plants are better able to overcome the drought stress and produce more shoot, root, and seeds when comparing to C3 species (Long, 1999). However, under elevated [CO₂], C3 species may increase water use efficiency due to the increased level of carbon dioxide. Wullschleger et al. (2002) suggested that plants grown under drought stress and high CO₂ allocate more carbon to root development, altering therefore root and shoot growth. Drought can also impact leaf development and morphology (i.e. increasing leaf cuticle thickness). Durum wheat grown under low water content and elevated CO₂ had the same number of leaves as plants grown under field capacity conditions and elevated CO₂. However, the leaf area index was reduced in plants under low water content but was

unaffected by $[\text{CO}_2]$ (Kaddour & Fuller, 2004). In addition, CO_2 can act as an antitranspirant molecule decreasing transpiration and saving water (Kirkham, 2011). *A. retroflexus* (C4) plants grown under elevated CO_2 and drought conditions did not show any decrease in net photosynthesis when compared to plants grown only under high CO_2 indicating that after drought stress the recovery time from the drought period was much faster under elevated $[\text{CO}_2]$, which shows that high CO_2 may help plants to overcome drought stress (Ward et al., 1999). Similar results were also observed for velvetleaf, a C3 species, where high $[\text{CO}_2]$ increased net photosynthesis after a period of drought (Ward et al., 1999).

Grassland species grown with elevated $[\text{CO}_2]$ presented lower stomatal conductance (g_s) in both water deficiency and well-watered conditions (Owensby et al., 1997). On the other hand, well-watered plants that had higher g_s also showed an increased water potential in the leaf as a consequence of more water in the soil, as well as more root biomass to uptake water.

1.4 Effect of elevated CO_2 on herbicide efficacy and weed control

Herbicides affect different metabolic functions such as photosynthesis, lipid metabolism, amino acid synthesis, and other pathways that are essential for growth and development. Thus, any changes in plant anatomy, morphology, and physiology due to changes in the $[\text{CO}_2]$ in the atmosphere could result in a change in the herbicide efficacy (Fernando et al., 2016). Knowledge about how an increase of CO_2 in the atmosphere can influence herbicide activity could enable us to develop management tools for sustainable weed

control. Identification of future problematic weed species and herbicides with adequate efficacy can help to prevent potential risks to food security (Waryszak et al., 2018).

Several studies have evaluated herbicide efficacy under elevated CO₂ (Jabran & Doğan, 2018; Marble et al., 2015; Zhang et al., 2015; Ziska et al., 2004). Most of these studies have focused on the effect of future climate change on the efficacy of glyphosate.

Application of glyphosate to four invasive species grown under elevated CO₂ (ranging from 675 ppm to 715 ppm) did not provide adequate control and the weeds showed some resistance to glyphosate (Manea et al., 2011). While the reasons are not very clear, the total biomass and root/shoot ratio were higher in the tolerant species than susceptible biotypes.

In another experiment a glyphosate resistant biotype of goosegrass (*Eleusine indica*) grown at elevated CO₂ (800 ppm), showed a reduction of 60% in the resistance level (Zhang et al., 2015). In contrast, the susceptible biotype increased the tolerance to glyphosate by 11% (Zhang et al., 2015). According to the author, the reason was that the resistant biotype had less efficient photosynthesis (reduced maximum net photosynthesis rate (A_{max})) and carboxylation efficiency (CE) and stomatal limitations compared to the susceptible biotype. When testing herbicide efficacy of glyphosate, halosulfuron, and a combination of both herbicides, under elevated CO₂ on yellow and purple nutsedge, Marble et al. (2015), showed that [CO₂] did not have any effect on weed control after three weeks of application for all treatments. In contrast, under elevated CO₂, glyphosate was less effective in controlling Canada thistle (Ziska et al., 2004). However, less herbicide activity had no correlation with less herbicide uptake,

since plants did not show decreased herbicide injury in the aboveground tissue. An increase in the root/shoot ratio was the explanation for regrowth and reduced weed control after six weeks of herbicide application, in elevated [CO₂] (Ziska et al., 2004). In another study, Ziska & Teasdale (2000), verified the efficacy of glyphosate on different cohorts of quackgrass (*Elytrigia repens*) under elevated and ambient levels of carbon dioxide. Although elevated CO₂ had no effect in the youngest cohort, an increase in herbicide tolerance were observed for the intermediary and oldest cohorts due to regrowth after herbicide application.

Taking all this into account, it is important to consider that herbicide efficacy is also dependent on plant species. Each species and its morphological and physiological characteristics can influence herbicide activity.

1.5 Effect of water deficit on herbicide activity

In general, under drought conditions, post emergence herbicides are less effective in controlling weeds due to reduced absorption, translocation, and metabolism (Zhou et al., 2007). Drought stress periods can cause conformational changes in plant morphology. Plants can develop thicker cuticles, which can reduce herbicide penetration, consequently reducing herbicide uptake (Fernando et al., 2016). However, under drought conditions, higher herbicide activity can occur due to reduced soil evapotranspiration. When applying glufosinate-ammonium on barley at low soil moisture, Anderson et al. (1993) verified an increase in herbicide efficacy. Although, reduced soil moisture would

typically decrease herbicide efficacy, in this study the author suggested the herbicide activity was higher because the chemical can remain in the soil, near to the rhizosphere. In addition, when fluroxypyr was applied, a higher herbicide activity was observed in Palmer (*Amaranthus palmeri*) and Kochia (*Kochia scoparia*) when soil moisture content was higher (Lubbers et al., 2007). Furthermore, improved weed control was observed when MON 37500 (sulfosulfuron) was applied at field capacity than at one-third soil capacity (Olson et al., 2000). Moreover, bispyribac caused more plant injury when soil moisture content was higher. As soil moisture increased a biomass reduction between 45 to 99% occurred in barnyardgrass (Koger et al., 2007). Finally, in a field experiment testing pre-emergent herbicides, control of several weed species was ineffective in dry soil conditions (Stewart et al., 2010).

When observing the effects of soil moisture on herbicide efficacy in green foxtail, Boydston (1990) noticed that injury is less noticeable in plants that received a drought stress period before and after herbicide application than in plants in well-watered conditions.

Under greenhouse and growth chamber conditions, Dortenzio & Norris (1980) showed that diclofop presented highest herbicidal activity when soil moisture was maintained close to field capacity. The authors showed herbicide activity decreases as soil moisture decreases, and to obtain better weed control, soil moisture should be near field capacity for at least two days after treatment or should achieve field capacity within four days after herbicide application.

1.6 Studied weed species

To evaluate the change in the herbicide efficacy under elevated [CO₂], four troublesome weed species were selected based on their photosynthetic pathway.

1.6.1 Velvetleaf (*Abutilon theophrasti*)

Velvetleaf belongs to the Malvaceae family and its native to Asia (Hui-Lin, 1970). This species is grown in China as a primary source of fiber to manufacture ropes, bags, fishing nets, etc. and it was brought to America for the same purpose (Spencer, 1984). Earlier reports support the idea that velvetleaf was the 'common yellow mallow' described in the 18th century in Virginia (Miller, 1735; Spencer, 1984).

Velvetleaf is a C₃ annual weed species. Leaves are alternate with long petioles, heart-shaped laminae, and are hairy on both sides with toothed margins. Stems are erect, branched, and contain short hairs. The plant can present a single yellow flower or small clusters that contain five sepals, and the fruit is a capsule. Velvetleaf is an auto-pollinated species that can produce more than 17,000 seeds per plant. Velvetleaf seeds have dormancy due to the hard seed coat and can remain viable in the soil for more than 50 years (John & Heather, 1997; Warwick & Black, 1983).

Velvetleaf is a major problem in corn, soybean, and cotton. Lindquist et al. (1996) showed that when not well controlled, velvetleaf can cause more than 80% yield loss in corn. In cotton, a weed density of 0.44-0.48 plant m⁻¹ would decrease cotton seed production by 50% (Ma et al., 2016).

The first case of herbicide resistance in velvetleaf was reported in 1984, in a corn field. In the past few years, more cases were confirmed. In all reported cases, the resistance developed was to the herbicide atrazine (Photosystem II inhibitor) (Heap, 2018). Due to a natural tolerance to many herbicides and for presenting weedy characteristics such as seed dormancy and germination at deep soil depths, velvetleaf has the potential to become a problematic species in many cropping systems (Spencer, 1984).

1.6.2 Weedy rice (*Oryza sativa*)

Weedy rice or red rice is a C3 species that belongs to the Poaceae family and it is one of the most troublesome weeds in rice production. Rice is one of the most important crops in the world. In 2016, 157 million hectares were harvested, which represents 8% of the world crop land (IRRI, 2016). The crop is grown by 25% of the farmers in the world, and due to its importance, a field with proper weed control is a prerequisite to achieve high yields (IRRI, 2016). If weeds are not well controlled, rice can have a yield loss of 40 to 60% on average (Kistner & Hatfield, 2018). However, with no weed control yield losses can reach 94 to 96% (Chauhan et al., 2011; Dass et al., 2017). Furthermore, the presence of 2, 4, 6, and 8 weedy rice plants per m² reduced rice yield by 24.9, 31.4, 33.7, and 60.1%, respectively (Xu et al., 2018).

There are many hypothesis about the origin of weedy rice. Londo & Schaal (2007) indicate the domestication process selected weedy rice from the wild type in regions where the wild type was present. The opposite occurred where the wild type was not present, which means crop de-domestication gave origin to the weedy rice. Furthermore,

cases of weedy rice in Brazil and Italy, where the wild type was not present are explained by seed contamination (Merotto Jr et al., 2016). Gene flow between crop and weedy rice explains cases of mutation and herbicide resistance in weedy rice and other weed species (Gealy et al., 2003).

Although weedy rice has a similar phenotype and physiology as the crop, it presents singular weedy characteristics such as seed dormancy, prolonged emergence, shattering, and red pericarp in most of the cases (Kanapeckas et al., 2016). The first case of resistance was reported in 2002, in Arkansas. In the past few years, Brazil, Costa Rica, Italy, and Greece also reported herbicide resistance to ALS inhibitor herbicides (Heap, 2018).

1.6.3 Palmer amaranth (*Amaranthus palmeri*)

Palmer amaranth belongs to the Amaranthaceae family. It is a summer annual, with a C₄ photosynthetic pathway. Palmer amaranth is native from the Sonoran Desert region, which includes the areas of the southwestern United States and northern Mexico regions (Kistner & Hatfield, 2018; Sauer, 1950)

Palmer amaranth has ovate shaped and alternate leaves. The petiole is longer than the leaf blade and the stem is reddish-green. Female and male flowers occur on different plants (dioecious) and the inflorescence is a spike (Ward et al., 2013).

Palmer amaranth has become one of the most damaging weed species in several crops. Yield loss due to weed competition has been reported to be as high as 79% in soybean

(Bensch et al., 2003), 65% in cotton (Rowland et al., 1999), and up to 91% in corn (Massinga et al., 2001).

Despite of the small size of the seed, Palmer amaranth has spread its growth area across the Midwestern region of the United States and invaded Europe, Africa, and South America. The large expansion has occurred due to contaminated animal feed, and contaminated equipment such as harvesters, seed planters, and other field utensils (Kistner & Hatfield, 2018).

Palmer amaranth is a dioecious species and the female plant has prolific seed production. In a non-competitive environment, a single Palmer plant can produce 200,000 to 600,000 seeds (Keeley et al., 1987; Ward et al., 2013). The dioecious characteristic of the species enables more variability within the population than among populations (Bravo et al., 2018), which also favors the development of herbicide resistance and other traits that allows Palmer amaranth to adapt and survive in the most adverse environments (Kistner & Hatfield, 2018).

Due to climate change, the Earth's temperature will increase in the future and it will affect plant distribution worldwide. Kistner & Hatfield (2018) developed a map showing the suitable areas for Palmer amaranth expansion. According to their model, Palmer expansion will still occur in areas where the plant is well established and it can reach new areas such as soybean, corn, and cotton fields in Australia, the corn area in Africa (Nigeria, Ethiopia, Tanzania, and South Africa), areas in central and eastern Asia, the Middle East, Madagascar, and the Caribbean Islands.

The first case of herbicide resistance in Palmer amaranth was reported in 1989, in cotton and soybean fields in the United States due to trifluralin applications. Since then many cases have been reported, and resistant biotypes are now present in 25 of the 50 United States. Multiple resistance traits are found in many states, and Georgia has reported resistance to 3 different sites of action (ALS, PSII, and EPSP inhibitors). Argentina, Brazil, and Israel have also reported resistance cases (Heap, 2018).

The fast germination and growth rate, prolific seed production, and high adaptive plasticity of *Amaranthus palmeri* make it one of the most aggressive weed species in cropping systems (Ward et al., 2013). Effective control of this weed is important to guarantee crop yield, and thus, studying herbicide efficacy under changing climates is highly relevant.

1.6.4 Johnsongrass (*Sorghum halepense*)

Johnsongrass was introduced in the 1800s in the southeastern USA as a forage. The common name is derived from the farmer who introduced the species in Alabama, in the 1840s. By 1900, Johnsongrass had spread throughout the USA and was classified as a weed, which led to the first federal appropriation for weed control in the country (Warwick & Black, 1983). The exact origin of Johnsongrass is not certain. However, it is believed that the plant is native to the Mediterranean region, in areas belonging to Iran and Turkey (Monaghan, 1979). Johnsongrass is classified as a noxious weed species in several states in the USA, which means that actions are taken to prevent establishment and plant dispersion (Skinner et al., 2000).

Johnsongrass is a C4 perennial grass species that belongs to the Poaceae family. Johnsongrass leaves have a long blade with a prominent midrib, and a membranous ligule. The stem is round and the inflorescences have an open panicle shape. This weed species has several characteristics that gives the species plasticity to adapt and successfully reproduce in different environments. Rhizome production, seed dormancy, shattering, and longevity are some of the traits that makes the species so aggressive in agriculture systems. Johnsongrass competition for 6, 9, 12, and 25 weeks can reduce cotton yield by 20, 60, 80, and 90%, respectively (Keeley & Thullen, 1989). In soybean fields, Johnsongrass infestation can reduce yield to 60% (Toler et al., 1996). Johnsongrass interference can reduce corn yield to 33% in the United States (Holm et al., 1977). However, if Johnsongrass competes for a long period, corn yield can be reduced from 62 to 83%. (Mitskas et al., 2003).

The first two herbicide resistant biotypes were reported in 1991, in cotton and soybean fields, in Mississippi and Kentucky, respectively. Resistant biotypes have now been confirmed in more than ten countries around the world, including the USA, where Johnsongrass herbicide resistance is present in eight states (Heap, 2018).

Therefore, an understanding of how Johnsongrass responds to herbicide application under elevated [CO₂] is critical to help farmers develop adequate weed management tools to better control this noxious species.

1.7 Studied herbicides

1.7.1 Tembotrione: 4-Hydroxyphenylpyruvate dioxygenase (HPPD) herbicide

After observing that grass growth was inhibited by leptospermone, a natural HPPD inhibitor secreted by *Callistemon citrinus*, L., researchers started to synthesize and analyze it as a potential chemical to control weed species (Ahrens et al., 2013).

Susceptible plants exhibit bleaching due to the loss of carotenoids and plastoquinone synthesis (Nakka et al., 2017). The first HPPD herbicides were developed in the 70's and introduced in the market in 1980. There are 4 chemical families in the HPPD herbicides: isoxazoles, pyrazolones, triketones, and benzoylpyrazoles. In 1982, the first triketone family herbicide was discovered. Later research provided the first HPPD herbicide for corn (first sulcotrione and later mesotrione). In 1999, a combination of the 2-methanesulfonyl-4-trifluoromethyl benzoyl moiety of isoxaflutole with the 5-hydroxyl-1,3-dimethylpyrazole heterocycle of pyrazolinate gave origin to pyrasulfotole. Later alterations in the benzoyl moiety created two new active chemicals: tembotrione and tefuryltrione (Ahrens et al., 2013).

HPPD herbicides inhibit the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD), blocking the conversion of 4-hydroxyphenylpyruvate (HPP) into homogentisate (HGA). This conversion is part of the catabolic degradation of tyrosine, one of the aromatic amino acids produced in the shikimate pathway. HGA is a precursor in the biosynthesis of α -tocopherol and plastoquinone, and the blockage of the HPPD enzyme results therefore, in a deficit of these two compounds (Ahrens et al., 2013). Tocopherols (α , β , γ , and δ -tocopherol) are lipid-soluble antioxidants, called tocochromanols or vitamin E,

which protect plants against the damage caused by reactive oxygen species (ROS) (DellaPenna & Pogson, 2006; Fritsche et al., 2017). Plastoquinone is an important cofactor in the photosynthesis process, which transfers energy in the electron transport chain. Plastoquinone deficiency may cause cellular damage due to the resulting inability of the chloroplast to dissipate light energy through photochemistry. Furthermore, plastoquinone is also a cofactor in carotenoid biosynthesis, which is necessary to protect chlorophyll from UV and excess of light damage (van Almsick, 2009).

The triketone family, which includes as active ingredients tefuryltrione, bicyclopyrone, sulcotrion, benzobicyclon, mesotrione, and tembotrione has a market size estimated as 1,283 million USD, which corresponds to 4.4% of the herbicide market (Ndikuryayo et al., 2017).

Tembotrione started to be commercialized in 2007 as a new option of post emergence herbicide to control broadleaves and grasses in corn fields. An HPPD resistant biotype of Palmer amaranth was first identified in 2009 from corn and sorghum fields, in Kansas. Later on, another biotype of Palmer amaranth presented resistance to mesotrione, topramezone, and tembotrione (Küpper et al., 2017; Nakka et al., 2017).

According to the International Survey of Herbicide Resistant weeds (HRAC), two of the most problematic weed species, Palmer amaranth and tall waterhemp (*Amaranthus tuberculatus*), have developed resistance to HPPD herbicides in the past few years. Although the number of resistant species is still small compared to other herbicides, most reported cases presented resistance to more than one site of action, which makes weed management very difficult. The USA is the only country with weeds resistant to

this group of herbicides, and the resistant biotypes are found in Kansas, Nebraska, and Wisconsin (for Palmer Amaranth) and Illinois, Iowa, and Nebraska for tall waterhemp (Heap, 2018).

1.7.2 Auxin – Growth Regulator Herbicide

The first auxin herbicides were discovered practically at the same time in the UK and in the US. Templeman and collaborators developed the herbicide MCPA in the UK in 1941, whereas Zimmerman and Hitchcock presented 2, 4-D as a growth regulator in the USA, in 1942. Both chemicals started to gain attention in the agricultural sector due to their selectivity, as they kill broadleaf species in grass crops. In 1945, 2, 4-D was the first auxin herbicide introduced into the market by the American Chemical Paint Co. as ‘Weedone’. In the next year, ICI introduced MCPA as ‘Agroxone’. The introduction of these chemicals completely changed the traditional way of controlling weed species and provided a new option of control. In addition, the success of the initial chemical control stimulated the development of many other herbicides (Grossmann, 2010; Troyer, 2001). Auxin is an important phytohormone that regulates plant growth and development. Although the auxin herbicides mimic the natural auxin, indole-3-acetic acid (IAA), excess amounts of auxin are toxic. Auxin herbicides are divided into five chemical families: phoxycarboxylic acids (as 2, 4-D), benzoic acids (as dicamba), pyridine carboxylic acids (as picloram), quinoline carboxylic acids (as quinclorac), and semicarbazones (as diflufenzopyr) (Christoffoleti et al., 2015).

The most common symptoms that plants will present after auxin herbicide application include epinasty, swelling, stem curling, etc. However, the physiological processes behind auxin herbicide action are complex. Grossmann (2010) classifies the response to high concentrations of auxin herbicides in 3 phases. The first phase starts within few hours after herbicide application, and is called as stimulation phase. Ethylene biosynthesis is stimulated and the first symptoms, tissue swelling, stem curling, and leaf epinasty start to appear. Changes in membrane selectivity, H⁺-ATPase function, and accumulation of abscisic acid (ABA) also occur. The second phase, inhibition phase, is initiated within 24 hours after herbicide application and includes reduced leaf area and internode elongation, loss of chlorophyll pigmentation, and inhibition of root growth, followed by inhibition of shoot growth. Furthermore, carbon fixation, evapotranspiration, and starch biosynthesis are reduced due to stomatal closure. In this phase the production of oxygen reactive species (ROS) is also observed. The third phase is called the senescence phase, where the tissues are already damaged enough to cause necrosis and plant death (Grossmann, 2010). The exact mode of auxin herbicide action it is still not clear. However, different auxin receptors, which can regulate gene expression are assumed to be the target of the auxin herbicides (Grossmann, 2010) and may be responsible for the chemical selectivity in the different auxin herbicides (Quareshy et al., 2018).

Dicamba herbicide was first developed by Velsicol Chemical Corporation in 1958, when the company obtained the patent and its use was approved in the US in 1962 (Hartzler, 2017). The first reported case of dicamba resistance occurred in Canada, in 1991.

Farmers were not able to control a biotype of wild mustard (*Sinapis arvensis*) using a mixture of dicamba, 2, 4- D and mecopop (Heap & Morrison, 1992; Jasieniuk et al., 1995). Seven weed species have now developed resistance to dicamba in 6 countries worldwide. The USA has the most problematic scenario, where Kochia (*Kochia scoparia*) developed resistance in more than 5 states and some of the biotypes present multiple site of action resistance (Heap, 2018).

1.8 Research project

1.8.1 Hypothesis

The main hypothesis of this research is that increasing the [CO₂] could allow weeds to become more competitive, depending on their photosynthetic pathway. It is expected that C3 weeds (here *A. theophrasti* and *O. sativa*) will grow better and be competitive than C4 ones (*A. palmeri* and *S. halepense*). Further, these weeds may show less sensitivity to the herbicides tembotrione and dicamba, under elevated [CO₂] (i.e. future scenario) compared with the low level of CO₂ (current condition) due to their enhanced plant growth (higher biomass production). In addition, the interaction of elevated CO₂ and drought may be more advantageous for the C4 weeds than C3 weeds.

1.8.2 Objectives

The overall objectives of these studies are to:

- Evaluate the possible changes of the herbicide efficacy of tembotrione and dicamba, for controlling the above-mentioned weeds under the elevated concentrations of CO₂;
- Examine the interaction of water deficit and elevated CO₂ on herbicide efficacy and control of the selected weeds.

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2. EFFECT OF ELEVATED CARBON DIOXIDE AND WATER DEFICIT ON RESPONSES OF FOUR WEED SPECIES TO TEMBOTRIONE

2.1 Introduction

Recent changes in climatic conditions are associated with anthropogenic activities (Sih et al., 2011). Burning of fossil fuels, and changes in the land-use surface (i.e. deforestation, agriculture) are examples of human activities that can impact the climate. One of the proposed reasons for climate change is the increase in CO₂ ([CO₂]), methane, and nitrous oxide concentrations. According to the IPCC, during the first decade of the 21st century, an average increase of 2.0 ± 0.1 ppm v/v per year in [CO₂] was observed and projections are that if CO₂ emissions remains unregulated, it can reach up to 700 ppm by the year 2100 (IPCC, 2014).

The continued increase in [CO₂] is expected to lead to an increase in the global temperature. The average temperature is predicted to increase by up to 5 °C in several regions of the world by the end of the century. As a direct consequence of global warming, a change in the precipitation patterns and an increase in the occurrence of extreme events are expected (Fuhrer, 2003; IPCC, 2007).

Carbon dioxide and water are essential for plant growth and development, and therefore, changes in these two components can have significant effects on agricultural systems. Although Kimball (1983) reported that the average yield in crops grown under elevated CO₂ (eCO₂) is 33% higher, when combining the effect of eCO₂ and other abiotic stressors (e.g., drought/flooding and heat waves), the overall result is predicted to be a

decrease in crop yield (Xu et al., 2010). Conversely, weed species can benefit from climate change due to their adaptability to grow and reproduce in a large range of environmental conditions (Peters et al., 2014). Further, climate change can also facilitate the expansion and colonization of weed species into new areas (Carboni et al., 2018). Due to their adaptability, weeds are a main reason for yield suppression in cropping systems. Weed infestation can account for more than 50% yield loss in wheat (Khan & Haq, 2002), and a loss of 80% in soybean (Bensch et al., 2003). To prevent yield loss, farmers most often control weeds with herbicides (Gianessi, 2013).

Considering the importance of chemical control to secure food production, scientists have questioned the efficacy of herbicides under future climate conditions. Although most of research has focused on the glyphosate response to eCO₂ alone or in combination with elevated temperature, results indicate plant response is species dependent. Under eCO₂, glyphosate showed no change in herbicide efficacy when controlling three species (Jabran & Doğan, 2018), it increased tolerance in grasses (Manea et al., 2011), and in broadleaves species (Matzrafi et al., 2019; Ziska et al., 2004). Furthermore, reduced herbicide efficacy was observed when applied to resistant biotypes under eCO₂ (Refatti et al., 2019; Zhang et al., 2015).

Although studies of the effect of eCO₂ and elevated temperature have been conducted in recent years, no studies have evaluated the effect of eCO₂ and water deficit on herbicide efficacy. However, it is known that lower soil moisture decreases weed control due to reduced herbicide uptake and translocation (Zhou et al., 2007). Therefore, it is necessary to study how plants respond to herbicides under both eCO₂ and water deficit conditions.

With the increase in the atmospheric [CO₂], it is expected that C3 species will have advantages over C4. Reduced photorespiration, stomatal conductance (g_s) and transpiration rates, increased carbon assimilation rates and improved nitrogen and water use efficiencies are some of the potential impacts of rising [CO₂] on C3 species (Leakey et al., 2009). On the other hand, large changes are not expected to occur for C4 species, since they already have mechanisms to concentrate CO₂. Therefore, scientists have wondered if herbicide efficacy would change according to the photosynthetic pathway (C3 or C4), under a changing climate.

While chemical control has undeniably helped to increase yield, the number of weed species that have developed resistance to herbicides has increased recurrently since herbicide implementation. According to the International Survey of Herbicide Resistant Weeds (<http://www.weedscience.org/>), more than two hundred and fifty species have become resistant to one or more of the fourteen different viable modes of action. The intensive use of glyphosate has resulted in more than 40 species with resistance to its mode of action. As an option to glyphosate resistant weeds, HPPD herbicides (4 hydroxyphenylpyruvate dioxygenase) emerged as alternatives to assist farmers in controlling weed species (broad spectrum herbicide), and have potential value for controlling weeds in the future.

Therefore, the aim of this study was to evaluate how several problematic weed species respond to tembotrione (Laudis®), an HPPD herbicide, under eCO₂ and water deficit, and if the interaction of both could change herbicide efficacy. Four species differing in their photosynthetic pathway as well their taxonomic group, including the C3 weedy rice

(*Oryza sativa*) and velvetleaf (*Abutilon theophrasti*), and C4 Johnsongrass (*Sorghum halepense*) and Palmer amaranth (*Amaranthus palmeri*) were chosen. Plants grown under ambient (aCO₂) and elevated (eCO₂) were subjected to two water level (WL) conditions (field capacity (FC) and water deficit (WD)), and three tembotrione rates. In this study, growth and photosynthetic measurements were taken to estimate herbicide efficacy.

2.2 Material and Methods

The study was conducted in controlled environment chambers (CONVIRON, Model BDW40, Canada) at College Station, Texas, U.S.A., during 2017-2018. Seeds of velvetleaf, Palmer amaranth, and Johnsongrass were obtained from Azlim® Seed Company and seeds of weedy rice were obtained from a survey conducted in Texas in 2015 (Liu, 2018). To obtain uniform germination, velvetleaf seeds were soaked in warm water (40°C) for one hour to break dormancy, and Johnsongrass seeds were soaked in water (ambient temperature) for 12 hours before planting to allow moisture to penetrate the hard seed coat. For this multifactorial experiment, two [CO₂] were chosen, 400 ppm as control (aCO₂) and 700 ppm as an elevated concentration (eCO₂); two water levels were employed, field capacity and water deficit; and three herbicide doses were studied. Seed of each weed species was sowed in a six-cell tray (5.4 cm x 6 cm x 5.7 cm deep). Each six-cell tray was considered as one experimental unit and contained only one of the weed species. Cells were filled with commercial growth medium (Sun Gro® Sunshine®LC1 Grower Mix with RESILIENCE). To minimize growth of fungus gnats,

the growth mixture was saturated with a solution containing *B. thuringiensis* (0.38 g/L of Gnatrol®) before planting. Two to three seeds were planted in each cell and thinned to one plant per cell after germination. Following thinning, the growth mixture in the cells was saturated with fungicide (0.38 g/L of Banrot® 40 WP). Plants were grown under a temperature of 30/26°C – day/night, and photoperiod of 14h light at 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD from incandescent and metal halide lamps. Humidity was set to 60%. Plants were fertilized with Miracle-Growth® Water Soluble All-purpose Plant Food 24-8-16, according to label instructions.

At the two to three expanded leaf stage, water deficit was simulated on half of the plants by withholding water for 3 days before herbicide application and was maintained for the following 24 hours after herbicide application. Field capacity (FC) treatments were watered daily by filling the trays to allow irrigation throughout capillary movement. When plants were acclimated to the water levels, herbicide application was made by an automated spray chamber calibrated to deliver 140 L ha⁻¹. A XR Teejet 80015 nozzle was used. According to the label instructions, tembotrione requires the addition of Methylated Seed Oil (MSO), 1% v/v, and a nitrogen source, ammonium sulfate (AMS) Dupont, 1.68 g/ha.

The recommended label rate of tembotrione (Laudis®) is 92 grams per hectare.

However, lower herbicide rates were chosen as our initial study suggested that using the labelled rate would kill all of the plants (data not shown). Tembotrione was applied at 46 g a.i./ha ($\frac{1}{2}$ rate) and 69 g a.i./ha ($\frac{3}{4}$ rate) of the recommended label rate for

Johnsongrass, weedy rice, and Palmer amaranth. Velvetleaf was treated with 27.6 g/ha ($1/3$ rate) and 46 g a.i./ha ($1/2$ rate) of tembotrione.

Eight replications of each treatment ($\text{CO}_2 \times \text{WL} \times \text{Herbicide rate}$) were used in the experiment. At 10 DAT (days after treatment), herbicide injury was evaluated together taking the average of the six plants in the tray. No herbicide injury was scored as “0%” and a dead plant was considered as “100%”.

For additional analyses, plants that did not present necrosis on their leaves were randomly selected for photosynthetic measurements. Photosynthetic parameters were analyzed using an infrared gas analyzer (model 6400XT, LI-COR Inc., Lincoln, NE, USA) with a chamber CO_2 concentration of $400 \mu\text{mol mol}^{-1}$, as $a\text{CO}_2$, and $700 \mu\text{mol mol}^{-1}$ for $e\text{CO}_2$. An LED source provided $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD for C3 species and $1400 \mu\text{mol m}^{-2} \text{s}^{-1}$ PPFD for C4 species. Block temperature control was set at 30°C and leaf size was adjusted according to each species. The variables analyzed were the carbon assimilation rate (A) and stomatal conductance (g_s). For these photosynthetic analyses, four different leaves from four different plants per treatment were analyzed.

At 21 DAT, herbicide injury was scored again. All plants were collected and roots were separated from the shoots. Roots of 15 random plants per treatment were washed and dried together with their respective shoots in an oven at 60°C for 72 hours before determining their total dry biomass (shoot + root). Total biomass (mg) and R/S (root shoot ratio) were estimated based on the shoot and root dry biomass.

The experiment was repeated twice. The experimental design was a completely randomized design (CRD) with 3 factors ($[\text{CO}_2]$, water level, and herbicide rate). All

analyses were subjected to a three-way ANOVA (proc mixed SAS version 9.4), and means were separated by Fisher's Least Significant Difference (LSD), $\alpha = 0.05$. Data was transformed when needed to achieve normality.

2.3 Results

2.3.1 Johnsongrass

The interactions of $[\text{CO}_2]$ x herbicide rate and water level x herbicide rate were statistically significant for injury at 10 and 21 DAT (see tables 1 and 6 in appendix A). In general, Johnsongrass plants showed more injury at 700 ppm than 400 ppm at all doses (Figure 1A and 1C) whereas the injury level was greater at FC than WD condition (Figure 1B and Figure 1D). At 10 DAT, plants sprayed with $\frac{1}{2}$ herbicide rate showed higher injury at eCO₂ when compared to same rate at aCO₂ ($p=0.0066$). Further, $\frac{3}{4}$ herbicide rate plants presented 18% higher herbicide injury at FC when compared to same treatment at WD ($p=0.0305$). At 21 DAT the injury increased from 36% at aCO₂ to 76% at eCO₂ ($p<0.0001$) in plants sprayed with $\frac{1}{2}$ herbicide rate. A significant difference was also found when comparing the effect of $[\text{CO}_2]$ at $\frac{3}{4}$ herbicide rate ($p<0.0001$) (Figure 1C). Johnsongrass plants had slightly more herbicide injury under FC condition compared to WD treatments. Significant differences were found for plants that received the $\frac{3}{4}$ herbicide rate at 10 DAT and 21 DAT (Figure 1B and 1D) and for untreated plants.

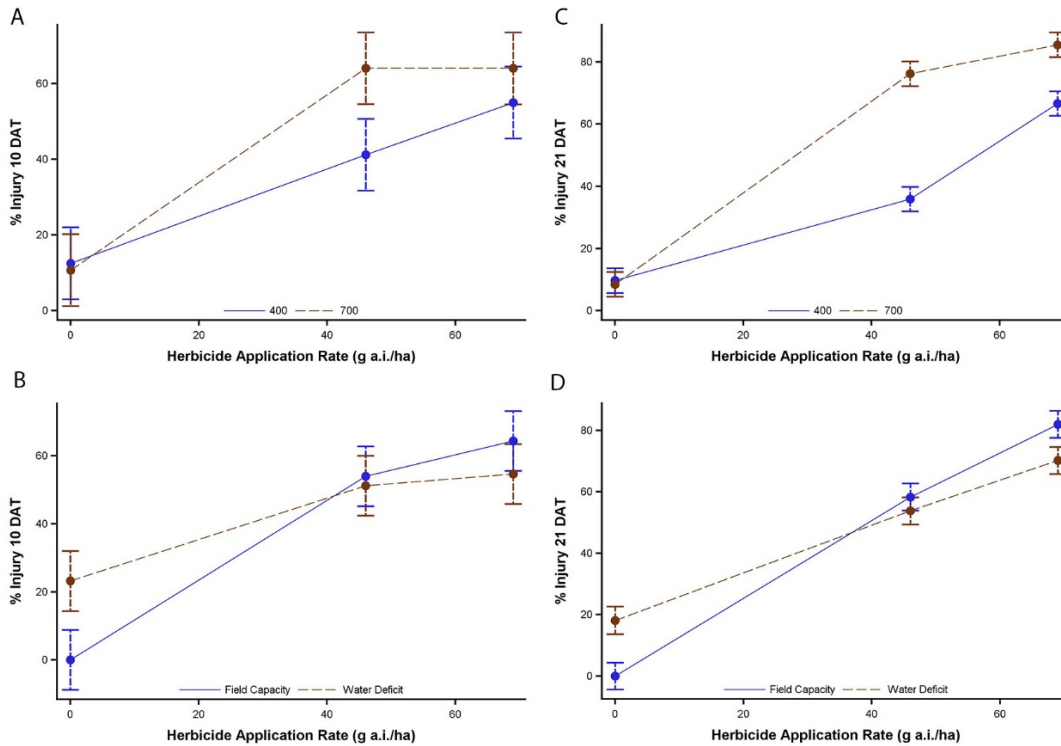


Figure 1. Johnsongrass herbicide injury at 10 DAT (A and B) and 21 DAT (C and D) under different [CO₂] and water levels. Error bars represent ± standard errors of means.

Two-two-way interactions were observed to be significant for total biomass of Johnsongrass (Figure 2A and 2B). Although plants grown at aCO₂ had more biomass than when grown at eCO₂, no differences in biomass were observed for any of the herbicide rates applied when comparing both [CO₂]. Furthermore, no significant differences were observed in total biomass when comparing the two water levels at any applied herbicide rate (Figure 2B).

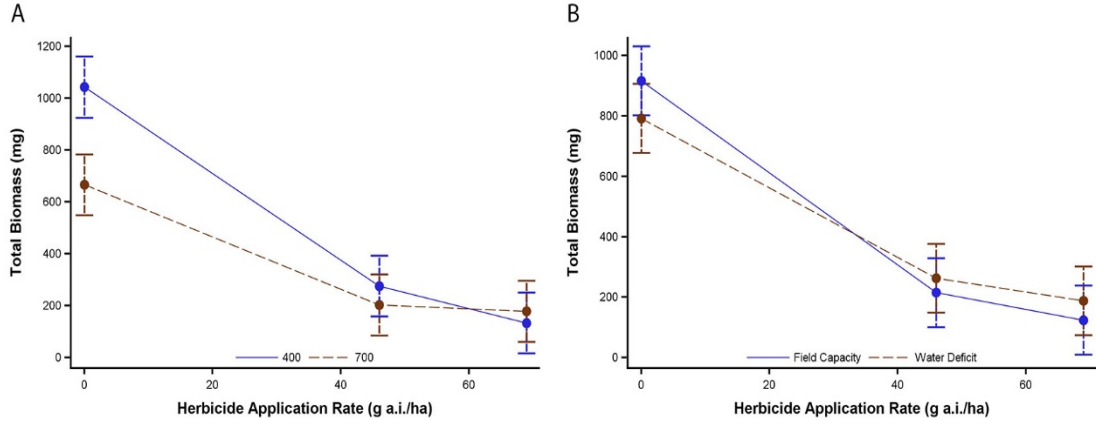


Figure 2. Johnsongrass total biomass and its interaction [CO₂] x Herbicide rate (A) and water level x Herbicide rate (B) obtained 21 DAT. Error bars represent ± standard errors of means.

Johnsongrass plants did not present any differences for the variable root/shoot ratio.

Figure 3A displays the interaction of water level and herbicide rate. Although the interaction of these factors was significant for CO₂ assimilation rate, no difference was observed at ½ and ¾ herbicide rates with either water level (Figure 3A). The only significant difference was found for untreated plants (p=0.0010). A three-way interaction between CO₂, water level, and herbicide rate was significant for stomatal conductance (g_s) (Figure 3B). At FC, g_s did not differ between the herbicide rates and [CO₂] except for untreated plants, in which g_s was greater when plants were grown under aCO₂ than eCO₂ (i.e. double). Plants sprayed with ¾ herbicide rate presented a difference when comparing [CO₂] at WD (p=0.0096). Water level impacted g_s in plants sprayed with ½ herbicide rate at aCO₂ (p=0.0324) and ¾ herbicide rate at eCO₂ (p=0.0470).

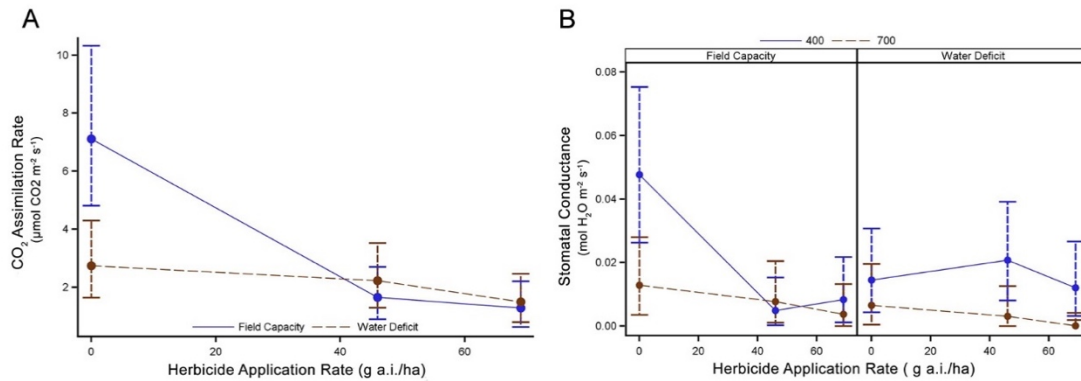


Figure 3. Johnsongrass CO₂ assimilation rate under different water levels and herbicide rates (A); Stomatal conductance response to the interaction of [CO₂], water level and herbicide rate (B) measured 10 DAT. Error bars represent ± standard errors of means.

2.3.2 Weedy Rice

ANOVA test shows that a three-way interaction was found to be significant at 10 ($p=0.0018$) and 21 DAT ($p=0.0385$) (Figure 4). At 10 DAT, herbicide injury was higher under eCO₂ compared to aCO₂. Furthermore, plants exposed to a short period of drought presented significantly less herbicide injury when compared to FC treatments at eCO₂. No statistical differences were found when comparing herbicide rates under both [CO₂], in the WD treatments. Conversely, a significant difference was found when comparing the effect of [CO₂] at ½ herbicide rate under FC ($p=0.0003$). The same pattern was observed at 21 DAT (Figure 4B); herbicide injury was significantly greater at eCO₂ when compared to plants exposed to aCO₂, at FC. Moreover, the increase in herbicide injury from ½ rate application (65%) to ¾ rate application (83%) was found to be significant ($p=0.0044$) in the FC treatment at aCO₂.

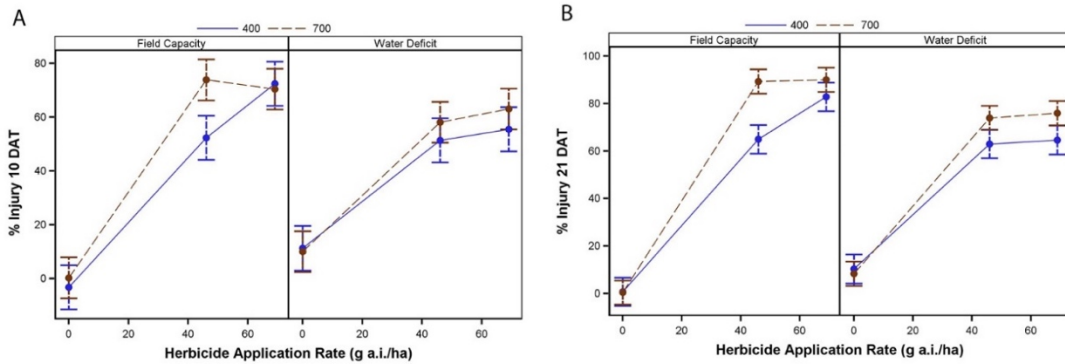


Figure 4. Weedy rice herbicide injury at 10 DAT (A) and 21 DAT (B) under different [CO₂] and water levels. Error bars represent ± standard errors of means.

A three-way interaction was also found to be significant for total biomass, $p=0.0008$ (Figure 5). While WD treatment increased biomass of herbicide treated plants under eCO_2 , the difference was not significant for both herbicide rates. No statistical difference in biomass was found when comparing water levels at aCO_2 , but at eCO_2 a significant difference was found when plants were sprayed with $\frac{1}{2}$ herbicide rate ($p<0.0001$), where plants presented higher total biomass at WD.

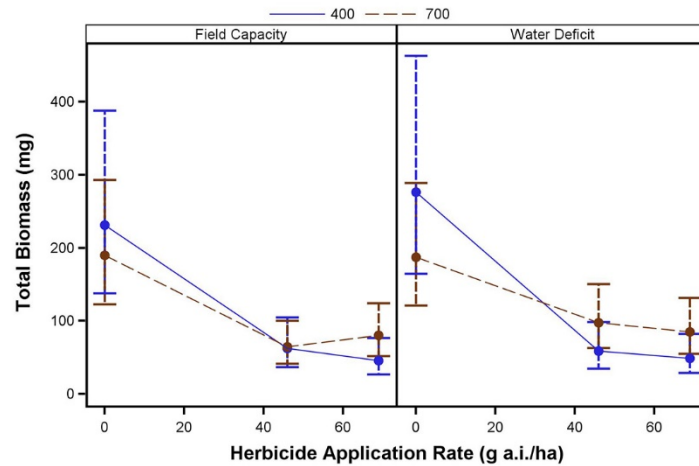


Figure 5. Weedy rice total biomass obtained 21 DAT. Error bars represent \pm standard errors of means.

CO₂ assimilation rate was significant for the [CO₂] x WL x herbicide rate interaction, $p=0.0220$ (Figure 6). While FC herbicide treated plants had higher CO₂ assimilation when compared to same treatments under aCO₂, the difference was not significant. A similar result was observed for herbicide treated plants under WD condition. In addition, higher [CO₂] had a positive effect on untreated plants under WD ($p=0.0009$). A significant reduction was found for *A* rate when increasing herbicide rate from 0 to ½ in both ambient and elevated [CO₂], at FC, $p=0.0008$ and $p=0.0232$, respectively.

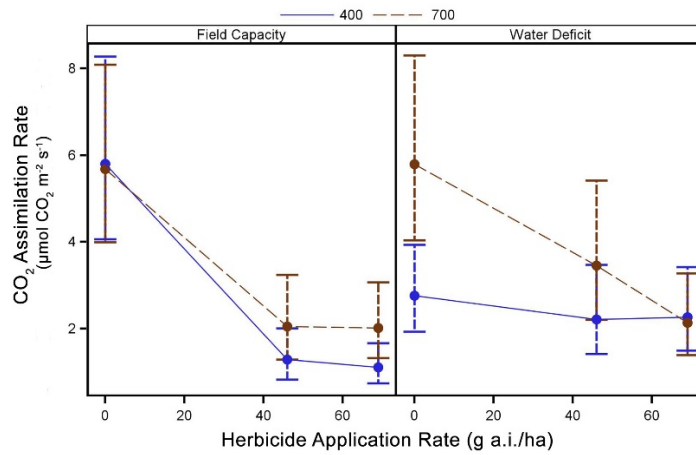


Figure 6. Weedy rice CO₂ assimilation rate measured 10 DAT. Error bars represent ± standard errors of means.

Although control and highest herbicide application rate plants presented a minor increase in g_s when compared to eCO_2 , these differences were not significant (Figure 7). The only statistical difference was found among the herbicide application rates, $\frac{1}{2}$ and $\frac{3}{4}$, at aCO_2 ($p=0.0177$).

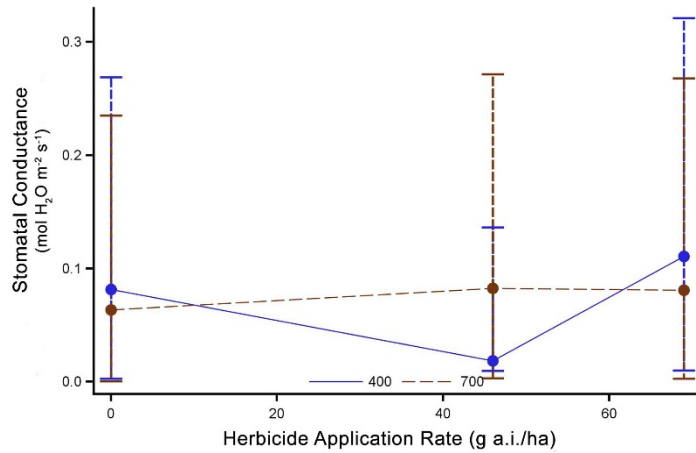


Figure 7. Weedy rice stomatal conductance response to the interaction of [CO₂] x herbicide rate measured 10 DAT. Error bars represent ± standard errors of means.

2.3.3 Palmer amaranth

At 10 DAT, plants showed higher herbicide injury under eCO₂ when compared to plants from aCO₂, at FC. The ½ application rate of tembotrione caused 18% more herbicide injury at eCO₂ when compared to the same herbicide rate at aCO₂, p=<0.0001 (Figure 8A). The same pattern was verified for the highest dose, where plants showed greater herbicide injury at eCO₂, p=0.0005. The contrary was observed when water restriction was imposed. Palmer amaranth plants at aCO₂ had a smaller but significant difference when compared to eCO₂ in control plants (p=0.0235), and in the highest applied rate (p=0.0188). Differences between herbicide application rates in each combination of WL and [CO₂] were not significant. At 21 DAT (Figure 8B), Palmer amaranth plants had more herbicide injury under eCO₂ independently of the WL treatment. Statistical

differences in injury between [CO₂] were observed at the lowest herbicide rate under both FC (p= 0.0009) and WD treatments (p=0.0068).

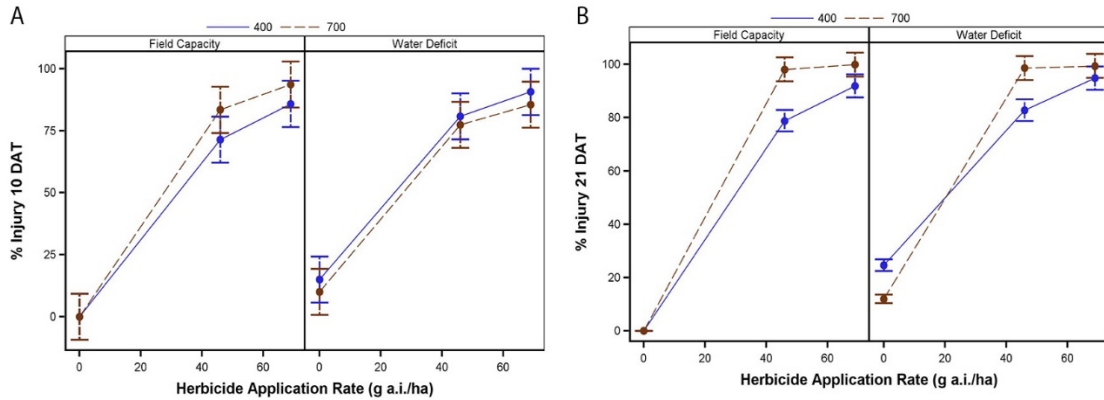


Figure 8. Palmer amaranth herbicide injury at 10 DAT (A) and 21 DAT (B) under different [CO₂] and water levels. Error bars represent ± standard errors of means.

Although herbicide injury was higher in eCO₂ treatments, plant total biomass was significant higher at the two applied herbicide rates when compared to plants under aCO₂ (Figure 9A). Even though herbicide treated plants developed greater root/shoot ratio at aCO₂ when compared to eCO₂, the difference was not significant (Figure 9B). Conversely, control plants developed higher root/shoot ratio at 700 ppm when compared to control plants at 400 ppm (p=0.0019).

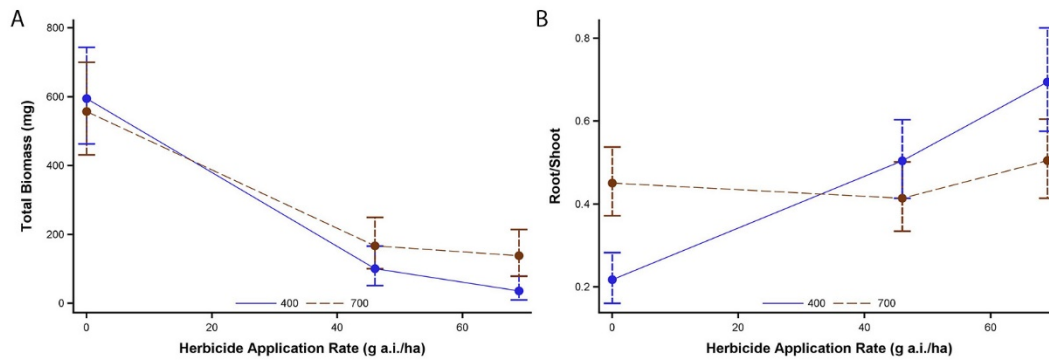


Figure 9. Palmer amaranth total biomass (A) and root/shoot ratio obtained 21 DAT (B). Error bars represent ± standard errors of means.

CO₂ assimilation rate was found not significant for Palmer amaranth. Although g_s was higher under aCO₂, there was no statistical difference when compared to plants grown under eCO₂. When comparing the effect of water condition on plants grown at eCO₂, WD caused a significant reduction in g_s compared to FC plants exposed to ½ herbicide rate ($p=0.0156$). Additionally, at FC, the g_s reduction caused by the application of ¾ herbicide rate was found to be significantly different from ½ application rate at eCO₂, $p=0.0027$ (Figure 10).

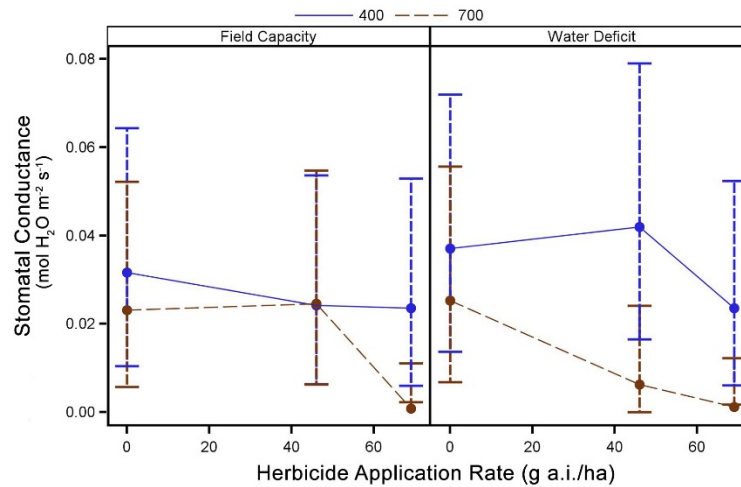


Figure 10. Palmer amaranth stomatal conductance (g_s) response to the interaction between $[CO_2]$ x water level x herbicide rate measured 10 DAT. Error bars represent \pm standard errors of means.

2.3.4 Velvetleaf

ANOVA test shows a significant three-way interaction was verified at 10 and 21 DAT (Figure 11). At 10 DAT herbicide injury was higher at FC with aCO₂ for both applied rates, compared to eCO₂. At 27 g a.i / ha, plant injury at FC was 35% higher at aCO₂ when compared to eCO₂, $p=0.0029$ (Figure 11A). When the higher herbicide rate was applied (46 g a.i. /ha), plants at aCO₂ presented 22% more injury than plants grown at eCO₂ ($p=0.0192$). On the other hand, plants exposed to WD treatments showed higher injury at eCO₂ (Figure 11A), however, none of these differences were statistically significant. In addition, the increased injury caused by the increase in herbicide rate from $\frac{1}{2}$ to $\frac{1}{3}$ was found significant at eCO₂ and FC. At 21 DAT, plants showed more injury on both FC and WD treatments at aCO₂. When $\frac{1}{3}$ herbicide rate was applied under FC,

plants had 50% more injury at aCO₂ when compared to same rate at eCO₂, p=<0.0001 (Figure 11B). Similar findings were found for the highest herbicide rate, which presented 27% more plant injury at aCO₂ compared to eCO₂ (p=<0.0001). While plants showed more injury at aCO₂, the increase in injury with herbicide rate was significant only for plants grown at eCO₂, for both FC and WD treatments (see table 10 in Appendix A).

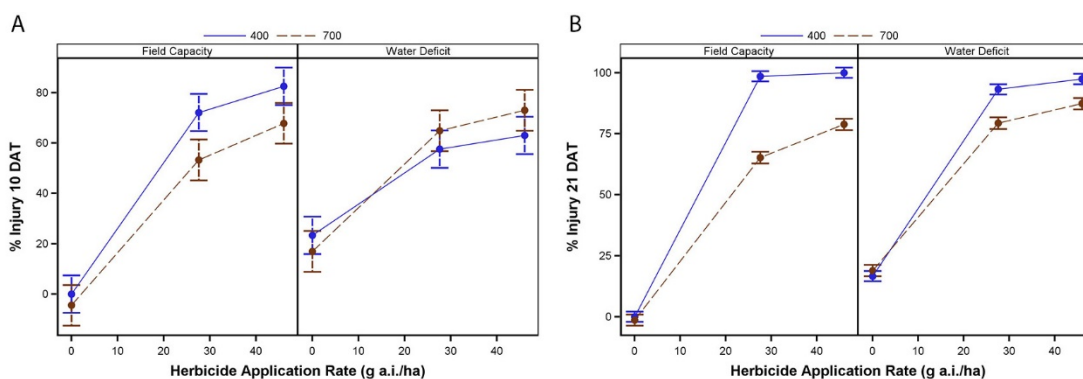


Figure 11. Velvetleaf herbicide injury at 10 DAT (A) and 21 DAT (B) under different [CO₂] and water levels. Error bars represent ± standard errors of means.

The results of the ANOVA test, as shown in Figure 12, indicate that there was a significant three-way interaction between CO₂, water level, and herbicide rate for velvetleaf total biomass (p=0.0065). Herbicide treated plants grown at eCO₂ produced more total biomass when compared to plants grown at aCO₂. However, untreated plants presented higher total biomass at aCO₂ on both water level treatments. Significant differences were found when comparing biomass at different herbicide rates between the two [CO₂] on both FC and WD treatments (Figure 12). Despite the fact that no changes

were observed when comparing water level treatments at aCO₂, significant differences were found between the two water levels at eCO₂. Although total biomass was smaller with herbicide treatment, at aCO₂ significant differences were observed when the herbicide rate was increased from 27 to 46 g a.i. /ha, independently of the water treatment (see table 15 in Appendix A).

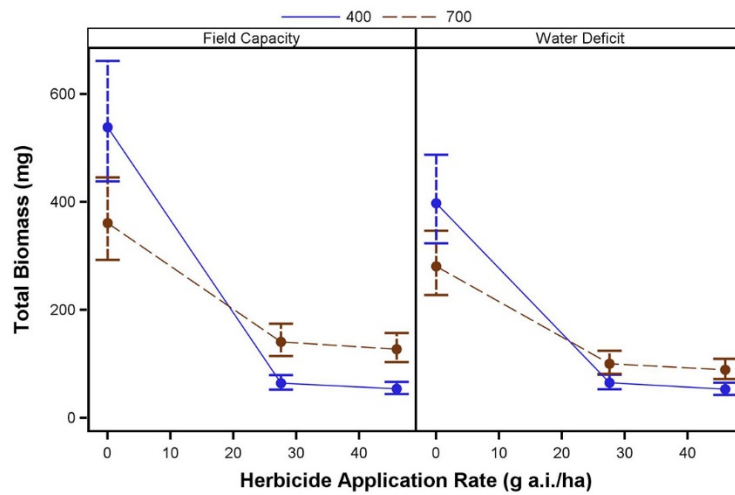


Figure 12. Velvetleaf total biomass obtained 21 DAT. Error bars represent \pm standard errors of means.

No statistical difference was observed for root shoot ratio in velvetleaf. However, plants under eCO₂ developed longer roots when compared to plants grown under aCO₂ in both FC and WD (see Appendices A Figure 1). Although plants exposed to WD treatments developed longer roots at aCO₂, a statistical difference was observed only in plants sprayed with $\frac{1}{3}$ herbicide rate when comparing WL treatments ($p=0.0038$).

Herbicide treated plants showed higher assimilation at aCO₂ when compared to their respective treatments at eCO₂, and a significant difference was found when comparing the application at ½ herbicide rate at both [CO₂], p=0.0212 (Figure 13). Though untreated plants had greater CO₂ assimilation rates at eCO₂, the difference was not significant.

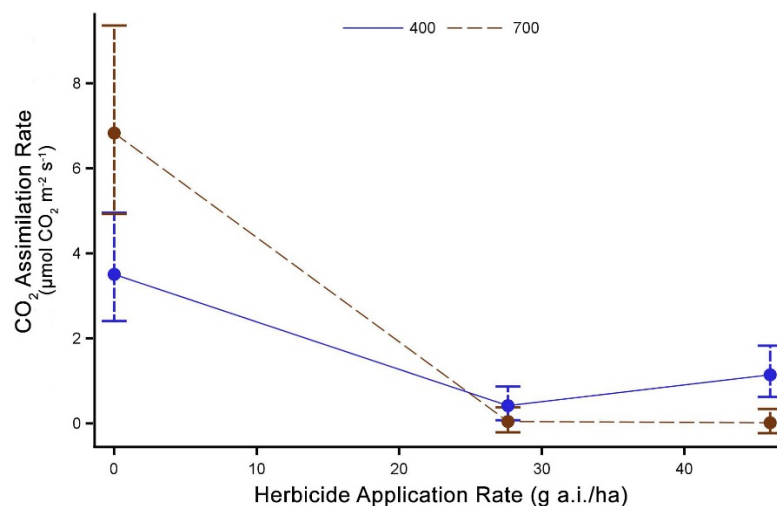


Figure 13. CO₂ assimilation rate in velvetleaf measured 10 DAT. Error bars represent ± standard errors of means.

The interaction WL x CO₂ was significant when evaluating g_s in velvetleaf (p-value 0.0086). Though g_s was higher at eCO₂, no significant difference was found when compared to aCO₂. When assessing the effect of water level, plants of FC treatments presented higher g_s when compared to WD treatments at aCO₂ (p-value 0.0381) (see table 26 in appendix A).

2.4 Discussion

2.4.1 Herbicide injury at 10 and 21 DAT

Overall, the results from the multifactor analysis show that for Johnsongrass, weedy rice, and Palmer amaranth, tembotrione had higher efficacy under eCO₂ after 21 days of herbicide application, in both FC and WD treatments. This would suggest future [CO₂] could increase HPPD herbicide efficacy.

ROS (reactive oxygen species; i.e. H₂O₂, O₂^{•-}, HO•, ¹O₂), occur constantly in plants as byproducts of different natural processes and metabolic pathways in the chloroplast, mitochondria, and peroxisomes. ROS can act as damaging, protecting, or signaling molecules based on the amount produced, and on how fast scavengers can eliminate or convert them into less reactive molecules (Gill & Tuteja, 2010). This equilibrium between ROS production and antioxidant scavenging is vital for continued plant growth. HPPD herbicides act on the tyrosine degradation pathway, by inhibiting the enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD; EC 1.13.11.2), which interrupts its conversion into homogentisate (HGA). The HPPD enzyme is critical for plant growth and development, since HGA is further converted into plastoquinone and tocopherols (Ahrens et al., 2013). This interruption results in loss of important antioxidant mechanisms, disrupting the plant's ROS balance, and allowing free oxygen radicals created by photodamage to disrupt cell membranes, degrade chlorophyll, and bleach plant tissue, which gives plants damaged by HPPD herbicides their characteristic symptom (van Almsick, 2009).

Light is a fundamental factor for the photosynthetic process. However, under abiotic stress conditions, such as that produced from herbicide application, the light-induced formation of ROS (even under low light), is aggravated due to photosynthetic inhibition, since the total energy absorbed cannot be used entirely in photosynthesis (Ksas et al., 2015). In a non-stress condition, plants have defense systems against photo damage that work by dissipating the energy through quenching, chlorophyll fluorescence, non-radiative decay (heat), and the Mehler reaction (Powles, 1984). All these responses help avoid photo oxidation and protect the D1 and D2 proteins in the reaction centers of photosystem II (PS II) and photosystem I (PS I), respectively. Plastoquinone is an important cofactor in the photosynthesis apparatus, as it is responsible for transferring electrons from PS II to PS I. Additionally, studies have shown that plastoquinone is also involved in energy dissipation, i.e. being able to dissipate energy back in the chlorophyll antenna (Samson & Bruce, 1996), and lipid membrane protection by quenching singlet oxygen (Yadav et al., 2010). Thus, considering the importance of plastoquinone, especially in protecting against photo damage, tembotrione application under eCO₂ can slow down antioxidant activity by several means (Farfan-Vignolo & Asard, 2012; Pérez-López et al., 2010) and reduce energy dissipation, since eCO₂ reduces g_s and transpiration rate (Bunce, 2001). Consequently due to the lower transpiration rate, decreased nitrogen assimilation has been observed in plants grown under eCO₂ because reduced transpiration can slow nitrogen absorption by decreasing the mass flow from the soil solution to the rhizosphere (McDonald et al., 2002), which could therefore lead plants to an increased susceptibility to tembotrione damage.

An additional abiotic stress known to cause ROS production in plants is water stress or deficit (Fan et al., 2014; Huang et al., 2013). Water stress has direct impacts on photosynthesis since it restricts CO₂ diffusion by closing stomata, which in turn impacts photo inhibition and photo oxidation (Chaves et al., 2008). Across levels of CO₂ and herbicide rates, when WD is imposed, a decrease in tembotrione efficacy is observed in Johnsongrass (Figure 1C and 1D). This can be due to the reduced soil moisture, which can decrease herbicide translocation, thus slowing herbicide action (Rocha-Pereira et al., 2012). Furthermore, reduced leaf area and increased cuticle thickness are some of the common features plant develop under water deficit conditions that can reduce herbicide action (Wang & Liu, 2007).

It has been proposed that under eCO₂, C3 species would show reduced photorespiration due to the higher Rubisco carboxylation versus oxygenation, and therefore would have less ROS production and more antioxidant molecules to protect cells against damage (AbdElgawad et al., 2016). However, Goufo et al. (2014) demonstrated that under eCO₂, rice reduced its production of total phenolics and total flavonoids, thus decreasing the effectiveness of the antioxidant system. C4 species, which can already suppress photorespiration through anatomical and biochemical features, would require the activation of different mechanisms to overcome stress. However, both C4 species, Johnsongrass and Palmer amaranth showed higher injury at eCO₂ when compared to plants grown at aCO₂. Therefore, photorespiration may be an essential feature to protect plants against photo inactivation, under stress conditions (Guidi et al., 2019).

Herbicide application and eCO₂ can show distinct independent responses, but their interaction appears more complex. In three of the four species tested, Johnsongrass, Weedy rice, and Palmer amaranth, it was clear that even under the positive effect of eCO₂, after herbicide application, plants may not be able to overcome the excess ROS production induced by it. Our results suggest that velvetleaf plants may have better antioxidant defense mechanisms under eCO₂ when compared to the other three species. Although, the plants presented some level of injury, the damage was reversible. Furthermore, it has been shown that certain anatomical features can also help plants to overcome stress. Velvetleaf has serrated leaves, which means they have higher heat dissipation capacity when compared to entire ones (Gottschlich & Smith, 1982; Vogel, 1970). Velvetleaf leaves have dense trichomes, which are specialized structures located on the epidermal surfaces of leaves, petioles, and stems. Since trichomes can assist plants in reducing leaf temperature and water loss, and increase light reflectance (Wagner et al., 2004), these specific features may play a role in protecting plant tissues against oxidative damage. Secondary metabolites such as terpenoids, which are volatile compounds closely associated with thermotolerance and tolerance to oxidative damage in plants (Peñuelas & Llusà, 2003) are often produced in trichome glands. Additionally, Sanyal et al. (2006) showed that the trichomes and glands on young velvetleaf leaves can impede herbicide absorption, and therefore herbicide efficacy, resulting in less herbicide damage (DiTomaso, 1999).

Our results show that plant response to tembotrione application under both concentrations of CO₂ and water deficit are dependent on species, rather than on a

generalized classification of the photosynthetic pathway. Furthermore, since CO₂ can cause variations in plant anatomy, morphology, and phenology (Kirkham, 2011), and such changes can drastically impact photosynthesis by changing CO₂ assimilation and sink capacity (Bowes, 1996), more research is needed to validate the mechanisms of our findings.

2.4.2 Total Biomass

It was expected that C3 plants grown under eCO₂ would produce more biomass than plants grown under aCO₂. On the other hand, differences in the growth of C4 plants under eCO₂ were expected to be minor. Unsurprisingly, our findings suggest that differences observed in total plant biomass can be influenced by the period plants were growing at different [CO₂] before herbicide application.

Although in this study the C3 plants, weedy rice and velvetleaf, were influenced by the interaction of [CO₂], water status, and herbicide, the responses varied. Velvetleaf showed lower herbicide injury and higher total biomass at eCO₂ in both FC and WD conditions when compared to plants grown at aCO₂. However, as described by (Bazzaz & Garbutt, 1988) and our results demonstrated, velvetleaf can present variations in growth response to eCO₂, since control plants had lower biomass under eCO₂.

Conversely, higher injury was not associated with lower biomass in weedy rice.

Tembotrione treated plants presented higher total biomass under WD than FC conditions at eCO₂, which may be explained by the stimulus of the higher [CO₂] before herbicide application, which allows greater CO₂ assimilation rates and growth (Carlson & Bazzaz,

1980). Moreover, we did observe that this weedy rice biotype presented higher tolerance to water deficit when compared to other biotypes of weedy rice exposed to drought. Differences were also observed for C4 plants. Johnsongrass plants had more biomass under aCO₂ and WD treatments, which corresponds with the observed injury results. Runion (2008) showed Johnsongrass biomass increased by 12.4% under eCO₂ while Tremmel & Patterson (1993) found no effect. Our results showed that aCO₂ promoted Johnsongrass growth. Similarly, Palmer amaranth biomass was increased by eCO₂ even though injury was also higher. According to Bernacchi et al. (2000), another Amaranthaceae species, redroot pigweed (*Amaranthus retroflexus*), presented faster growth in the earlier stages (10-20 days) at eCO₂ (700 ppm) than at aCO₂ (350 ppm). The effect of eCO₂ seems complex however, as redroot pigweed grown at FC showed decreased plant biomass as [CO₂] increased from 300 to 1200 ppm (Bazzaz & Carlson, 1984).

The outcomes of herbicide efficacy (mainly glyphosate) under eCO₂ are very diverse, which indicates responses are highly dependent on plant species (Waryszak et al., 2018). Jabran & Doğan (2018) showed that eCO₂ (800-900 ppm) promoted plant growth in all three species tested, (*Bromus tectorum*, *Hordeum murinum*, and *Lactuca serriola*) but glyphosate efficacy was not changed due to increased biomass. Conversely, Manea et al. (2011) showed three C4 grasses grown at eCO₂ (*Chloris gayana*, *Eragrostis curvula*, and *Paspalum dilatatum*), presented an increase in plant biomass, but glyphosate efficacy was reduced due to higher plant growth. Therefore, the results found in the literature are quite controversy. Hence, as stated by Fernando et al. (2016), morphological changes

(i.e. plant biomass) alone cannot explain which plants survive or resist to herbicide application under eCO₂ and more research is needed to clarify these findings.

It is important to point out that plants are subjected to a sudden increase in [CO₂] in experimental studies. However, in nature, the increase in [CO₂] is gradual, which gives a greater time-space for plants to adapt and acclimate, thus natural results might be different. Moreover, maternal effects can impact phytohormone status, water uptake and seed coat properties, which could therefore impact the results of these studies, since it is recognized that some traits can persist until maturity, and environmental maternal effects can provide higher plasticity to the offspring, especially if the conditions are similar to their maternal environment (Bischoff & Müller-Schärer, 2010).

Finally, it is important to comment that in this study C3 plants received herbicide application earlier at eCO₂ due to accelerated growth, while C4 plants were treated at the same time for both [CO₂].

2.4.3 Root to Shoot Ratio

Source-sink relations determine carbon allocation in plants, and are in turn influenced by species, plant phenology, and environmental conditions. To enable growth and development, plants must balance distribution of photosynthetic metabolites to both below and above ground to be able to survive and flourish. Therefore, changes in carbon partitioning can be vital for survival under changing environmental conditions like CO₂ elevation. At the same time variability found in R/S measurements may be influenced by

experimental conditions such as time of exposure to different [CO₂], water availability, pot size, light, and temperature (Rogers et al., 1996).

Water deficit has been shown to influence carbon allocation and R/S ratio (Huang & Fry, 1998). When water is limited, more carbon is allocated to the roots to allow them to explore greater soil volume and uptake more water and nutrients (Bazzaz, 1990). In our study, WD control plants did show reduced lateral root growth and increased root length, and herbicide treated plants reduced both lateral root growth and overall length.

Nevertheless, these changes were not enough to impact R/S, possibly due to re-watering, and herbicide application effects.

With regards to [CO₂], studies have shown eCO₂ increases root growth and development in a large number of species; however, responses can vary. According to Rogers et al. (1996), 262 observations from 62 reports showed that in 59.5% of the species root growth increased under eCO₂, 37.5% had reduced root growth, and 3% did not show any difference. Ziska et al. (2004) showed that in a field experiment, Canada thistle (*Cirsium arvense*), developed more roots, and consequently increased R/S ratio under eCO₂, which could have allowed it to survive and regrow after glyphosate application. Additionally, Manea et al. (2011) showed increased survival rate under eCO₂, justified by the increase in R/S in three of four species of their experiment. One theory on the mechanism that allows this increased survival rate posits that as higher [CO₂] stimulates belowground growth, it results in a dilution effect of the herbicide Patterson & Flint (1990). Overall in this study, neither CO₂, water level, nor herbicide rate affected R/S in three of four species tested, which means that carbon partitioning was evenly distributed among the

treatments, suggesting herbicide application resulted in uniform reduction of growth. These findings disagree with the herbicide dilution effect suggested by Patterson & Flint (1990). On the other hand, Palmer amaranth plants that received tembotrione application showed an increased R/S at aCO₂, which was followed by reduced plant injury, but not a smaller amount of biomass.

2.4.4 CO₂ Assimilation Rate (*A*)

Ziska et al. (1999) showed that at 7 DAT glyphosate application increased *A* in the C3 species *Chenopodium album*, but no significant changes were observed in the C4 species *Amaranthus retroflexus*, suggesting that C3 plants could have more impact on agronomy systems due to advantages such as reduced photorespiration, and transpiration rates. Our study shows *A* differs not just based on the carbon cycle but also by species. In addition, [CO₂] is not the only factor affecting *A*, as water level can also impact *A* after tembotrione application.

The responses of both C3 and C4 under eCO₂ and WD are diverse, and it is assumed that both stomatal and non-stomatal factors can influence these responses (Ghannoum, 2008). Although C4 species are expected to be less responsive to higher [CO₂] due to their carbon concentrating mechanisms, it has been shown that *A* can increase with eCO₂ under WD treatments (Leakey et al., 2006).

In this study, weedy rice showed higher *A* under WD when compared to FC/eCO₂. This is probably correlated with the diminished herbicide injury that resulted from reduced translocation of tembotrione in WD treatments when compared to FC in both [CO₂]. Lal

&Edwards (1996) showed that under aCO₂, even though plants suffered from water deficit (3-10 days), after re-watering, stressed plants showed *A* rates similar to FC plants. However, Loreto et al. (1995) found that sorghum *A* only partially recovered following rehydration, which corroborates our findings, since water deficit decreased *A* in Johnsongrass controls while no difference was observed among herbicide treated plants. Velvetleaf treated plants showed overall higher *A* when treated with herbicide under aCO₂. This can be related back to the increased defense mechanisms to overcome herbicide damage, since the metabolic changes to avoid photo damage to the photosynthetic apparatus under eCO₂ can reduce carbon assimilation (Fryer et al., 1998). Surprisingly, Palmer amaranth did not show significant differences in *A* among treatments, which agrees with Lal &Edwards (1996) since the C4 species *Amaranthus cruentus* and *Zea mays* recovered photosynthesis within 2-4 days when rewatered. In this study, the results show that even when herbicide injury was higher at eCO₂, some survivors (at 10 DAT) had similar *A* rates as plants under aCO₂, and neither water nor herbicide rate impacted CO₂ assimilation.

Finally, it has been discussed that a much higher increase in [CO₂] is necessary to overcome stomatal closure caused by water deficit (Ghannoum, 2008). Furthermore, the positive effect of eCO₂ on *A* can be explained by the impact of stomatal closure in reducing reduced plant transpiration rate, with consequently soil water conservation (Wall et al., 2001).

2.4.5 Stomatal Conductance (g_s)

Stomatal conductance g_s is another parameter that can be highly influenced by the abiotic stressors imposed in this experiment. eCO_2 and water deficit can cause stomatal closure, and the impact of herbicide application on these factors can be variable. As a general result, both C4 species, Johnsongrass and Palmer amaranth presented a higher g_s under aCO_2 , in both FC and WD treatments after tembotrione application. The same trend was observed for *Amaranthus retroflexus* after glyphosate application (Ziska et al., 1999). Likewise, the C3 species also showed similar results; both weedy rice and velvetleaf presented higher g_s under eCO_2 , but these results differs from what Ziska et al. (1999) presented, since in their study, *Chenopodium album*, a C3 species had a significant reduction in stomatal conductance, and the impact of higher CO_2 was greater in control than glyphosate treated plants.

2.5 Conclusion

Overall, tembotrione appears to be a good option for controlling Johnsongrass, weedy rice, and Palmer amaranth under future predicted elevated $[CO_2]$, under both FC and WD conditions. Although lower doses of tembotrione were applied to velvetleaf, this species showed higher tolerance to tembotrione application at eCO_2 , suggesting the need for further investigation to control this species in the future. In addition, the application of sublethal doses as tested in this experiment can contribute to the development of herbicide resistance. Therefore, the varying responses found in this study demonstrate a necessity for more research to explain the reasons behind the higher tembotrione

efficacy under eCO₂. Moreover, plant response to tembotrione appears to have a similar trend to that observed for glyphosate, in which responses depend on the species morphological and physiological characteristics rather than just a generalization based on the photosynthetic pathway.

2.6 References

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3. EFFECT OF ELEVATED CARBON DIOXIDE CONCENTRATION AND WATER DEFICIT ON RESPONSES OF TWO WEED SPECIES TO DICAMBA (XTENDIMAX®)

3.1 Introduction

The human population is expected to grow from 7.6 to 9 billion people by 2050 (DESA, 2017). This increase in population is source for extreme concern due to the necessity for higher food production under scenarios of a changing climate. Climate change, and its concomitant rise in temperatures and occurrence of extreme events (e.g. flooding, drought, heatwaves), can cause extreme pressure on ecosystems, by reducing biodiversity and provoking shifts in the communities, which in turn can threaten food productivity (Campbell et al., 2016).

The major factors of climatic change are rooted in anthropogenic activities, such as emissions of greenhouse gases, carbon dioxide (CO₂) and methane, and changes in land-use; additional contributions are also made from certain natural factors (IPCC, 2014). Since the beginning of the 21st century, the CO₂ concentration ([CO₂]) has increased about 20 ppm per decade, which represents an 10-fold increase when compared to any rate of rise in [CO₂] during the past 800,000 years (IPCC, 2018).

Several studies have quantified the impact of elevated CO₂ on crop yield, and have found overall positive effects, due to a ‘fertilization effect’, which could reduce the negative impacts of climate change (Bourgault et al., 2017; Högy et al., 2009; Kimball, 1983). However, the progressive increase in atmospheric [CO₂] is also predicted to

increase temperatures and change precipitation patterns (e.g. drought and flooding), which could outweigh any possible benefits of higher concentrations (Jin et al., 2018). Food production can also be impacted by the effects of climate change on agronomic pests (e.g. pathogens, insects, and weed species) (Ziska et al., 2011). Weed species are one of the major causes of yield loss in cropping systems (Bensch et al., 2003; Khan & Haq, 2002). The exact magnitude and nature of the impact of climate change on weed species is uncertain, however CO₂ enrichment can increase plant's competitiveness and impact weed control since weed species are highly adaptive to changes in the environment (Korres et al., 2016). Moreover, studies have shown herbicide efficacy can be reduced under elevated CO₂ (Matzrafi, 2019).

Chemical control is the most common method to control weed species in cropping systems due to its efficacy and relative low cost. However, effective weed control is highly dependent on environmental conditions. It is expected that C₃ weed species will benefit more from the rise in [CO₂], since higher concentrations of carbon dioxide are known to stimulate photosynthesis and reduce stomatal conductance and transpiration in C₃ species, as compared to C₄ species which are less sensitive to changes in [CO₂] (Long et al., 2006). Therefore, C₃ weed species could become more competitive in cropping systems, as opposed to the current situation where the most troublesome weeds species have a C₄ photosynthetic pathway.

In addition to CO₂ concentration, periods of water deficit can influence herbicide efficacy. Low soil moisture can reduce herbicide absorption, translocation, and metabolism (Zhou et al., 2007). In addition, drought can induce morphological and

physiological changes in plants, which can impact herbicide penetration and uptake (Fernando et al., 2016).

In spite of its vital importance in aiding yield increases, the use of chemical control tools for weed species has led to increasingly repeated applications which in turn create increasing selection pressures, and contribute in the selection of weed species resistant to herbicides (Nandula, 2019). Furthermore, the development of herbicide resistant crops, such as Roundup Ready (RR) has led to a drastic increase in glyphosate usage, since it allows farmers to apply a single broad-spectrum chemical, multiple times, during the growing season without concern for crop injury. Additionally, the expiration of the glyphosate patent in 2000 allowed the introduction of cheaper versions of the product into the market (Beckie et al., 2019), which contributed to make this chemical the most successful herbicide in the world (Duke, 2018). Glyphosate's commercial success emphasizes the impact of consecutive and intensive herbicide applications on the biodiversity, since it allows shifts in the weed populations and evolution of herbicide resistance (Owen & Zelaya, 2005).

As a consequence of the overapplication of glyphosate, the number of glyphosate resistant weed species has increased – to more than 45 species worldwide (Heap, 2018). In order to manage glyphosate resistance, companies have developed other technologies such as the glufosinate resistant trait (LibertyLink System®), introduced to the market in 2009 (Beckie et al., 2019). More recently (2015) dicamba-resistant soybean, Roundup Ready 2 Xtend® (RR2-Xtend), was approved (Nandula, 2019).

With the introduction of dicamba-resistant crops (e.g. soybean, corn, cotton), and the release of the over-the-top (OTT) dicamba products, dicamba use has increased substantially. The seasonal average of dicamba used, for cotton and soybeans from 2012 to 2016, was less than 800,000 pounds of equivalent acid (a.e.). However, after the introduction of OTT products in 2017, approximately 10 million pounds of dicamba a.e. were sprayed in these two crops (U.S.E.P.A, 2018).

Dicamba is an auxin herbicide that belongs to the group of growth regulator herbicides – Group O/4 or auxin herbicides (WSSA), and is classified as benzoic acid. Auxin herbicides overdose the production of the natural plant hormone indole-3-acetic acid (IAA). The development of auxin herbicides in the 1940's started a new era of weed control since the synthetic auxins were the first selective herbicide to be used in agriculture (Grossmann, 2003). The symptomology of dicamba application includes leaf epinasty, inhibition of root and shoot growth and stem curvature, ultimately leading to death (Grossmann, 2000). The exact mechanism of action for auxin herbicides is still unknown, with direct and indirect effects occurring, including hormone cross-talk between auxin, ethylene and ABA, and production of reactive oxygen species (Grossmann, 2010).

Dicamba has been used for more than 50 years on several crops, both pre and postemergence, and despite its long usage only two weed species, Kochia and prickly lettuce, have developed resistance to dicamba in the USA (Heap, 2018). Therefore, dicamba is still considered a useful tool for weed control in different crops. Companies are moving towards the introduction of herbicide resistance traits into crops (e.g.

Xtend®, Enlist™), and the use of ‘old-new chemicals’ - in the form of stacked chemicals – will only increase. Therefore, it is of extreme importance to study how these herbicides, in this case dicamba, will control weed species under a changing climate. Since there is no information about dicamba efficacy under changing climate, the aim of this study is to investigate dicamba efficacy when controlling C3 (*Abutilon theophrasti*) and C4 (*Amaranthus palmeri*) species under elevated CO₂ concentration and water deficit.

3.2 Material and Methods

The study was conducted in controlled environment chambers (CONVIRON, Model BDW40, Canada) at College Station, Texas, U.S.A., during 2017-2018. Seeds of velvetleaf and Palmer amaranth were obtained from Azlim® Seed Company. To obtain uniform germination, velvetleaf seeds were soaked in warm water (40°C) for one hour to break dormancy. For this multifactorial experiment, two [CO₂] were used, 400 ppm as control (aCO₂) and 700 ppm as an elevated concentration (eCO₂). Additionally, two water levels, field capacity and water deficit, as well as three herbicide doses were studied.

Seed of each weed species was sowed in a six-cell tray (5.4 cm x 6 cm x 5.7 cm deep). Each six-cell tray was considered as one experimental unit and contained only one of the weed species. Cells were filled with commercial growth medium (Sun Gro® Sunshine®LC1 Grower Mix with RESILIENCE). To minimize growth of fungus gnats, the growth mixture was saturated with a solution containing *B. thuringiensis* (0.38 g/L)

of Gnatrol®) before planting. Two to three seeds were planted in each cell and thinned to one plant per cell after germination. Following thinning, the growth mixture in the cells was saturated with Banrot® 40 WP fungicide (0.38 g/L). Plants were grown under a temperature of 30/26°C – day/night, and photoperiod of 14h light at 600 $\mu\text{mol m}^{-2}\text{s}^{-1}$ PPFD from incandescent and metal halide lamps. Humidity was set to 60%. Plants were fertilized with Miracle-Grow® Water Soluble All-purpose Plant Food 24-8-16, according to label instructions.

At the three to four expanded leaf stage, water deficit (WD) was simulated on half of the plants by withholding water for 3 days before herbicide application and was maintained for the next 6 hours after herbicide application. Field capacity (FC) treatments were watered daily by filling the trays to allow irrigation through capillary movement. When plants were acclimated to the water levels, herbicide application was made by an automated spray chamber calibrated to deliver 140 L ha⁻¹. An XR Teejet 8002 nozzle was used for application. Dicamba was applied with the addition of crop oil concentrate (COC) at 1%v/v. The recommended label dose of dicamba (XtendiMax®) is 560 grams per hectare. However, lower herbicide rates were chosen, given that our initial study suggested that using the labelled rate would kill all the plants (data not shown). Dicamba was therefore applied at 280 g a.e./ha (½ rate) and 420 g a.e./ha (¾ rate) of the recommended label rate for both species.

Eight replications of each treatment (CO₂ x WL x Herbicide rate) were used in the experiment. At 10 DAT (days after treatment), herbicide injury was evaluated on a visual score from 0-100, by taking the average of the six plants in the tray. A “0%” score

represented no visible herbicide injury, and a completely dead plant was considered as “100%” score.

For additional analyses, plants that did not present necrosis on their leaves were randomly selected for photosynthetic measurements. Photosynthetic parameters were analyzed using an infrared gas analyzer (model 6400XT, LI-COR Inc., Lincoln, NE, USA) with a CO₂ concentration of 400 μmol mol⁻¹ for aCO₂, and 700 μmol mol⁻¹ for eCO₂. The LED source light provided 1200 μmol m⁻² s⁻¹ PPD for C3 species and 1400 μmol m⁻² s⁻¹ PPD for C4 species. Block temperature control was set at 30°C and leaf size was adjusted according to each species. The variables analyzed were the carbon assimilation rate (*A*) and stomatal conductance (*g_s*). For these photosynthetic analyses, four different leaves from four different plants per treatment were analyzed.

At 21 DAT, herbicide injury was scored again; afterwards, all plants were collected and roots were separated from the shoots. Roots of 15 random plants per treatment were washed and dried together with their respective shoots in an oven at 60°C for 72 hours before determining their dry total biomass (shoot + root). Total biomass (mg) and R/S (root shoot ratio) were estimated based on the shoot and root dry biomass.

The experiment was repeated twice. The experimental design is a completely randomized design (CRD) with 3 factors ([CO₂], water level, and herbicide rate). All analyses were subjected to a three-way ANOVA (proc mixed SAS version 9.4), and means were separated by Fisher's Least Significant Difference (LSD), α= 0.05. Data was transformed when needed to achieve normality.

3.3 Results

3.3.1 Velvetleaf

The interactions between [CO₂] x herbicide rate, and water level x herbicide rate were statistically significant for injury at 10 and 21 DAT (Figure 14). At 10 DAT, herbicide injury was higher at aCO₂ (49.3%) when compared to the eCO₂ (38.9%), when ½ rate was applied (280 g a.e. / ha) (p= <.0001) (Figure 14A). On the other hand, no statistical difference was found when ¾ rate was applied (420 g a.e. / ha). For the interaction between water level and herbicide rate, injury was higher in the treatments exposed to water deficit conditions than in the ones under field capacity, at ¾ herbicide application (p= 0.0016) (Figure 14B). At 21 DAT, plants showed higher herbicide injury at aCO₂ (53.8%) when compared to eCO₂ (43.6%) (p=<.0001), when ½ herbicide rate was applied (Figure 14C). Water deficit conditions caused more injury across herbicide rates (½ at p= 0.0006, and ¾ at p= <0.0001), as compared to field capacity (Figure 14D).

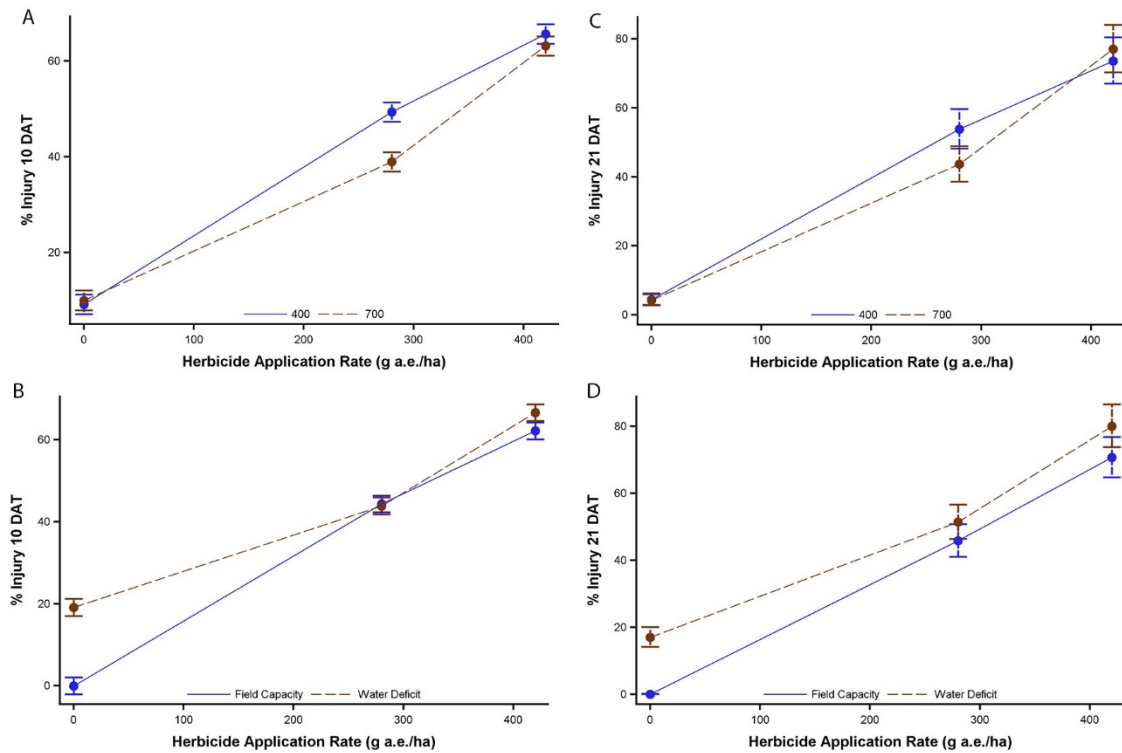


Figure 14. Velvetleaf herbicide injury at 10 DAT (A and B) and 21 DAT (C and D) under different [CO₂] and water levels. Error bars represent ± standard errors of means.

The interaction between [CO₂] x herbicide rate was found to be statistically significant for velvetleaf total biomass. Overall, plants grown under eCO₂ presented higher total biomass than plants grown at aCO₂ (p=0.0322) (Figure 15). Significant differences in biomass were found when ½ herbicide rate was applied (p=0.0091). The interaction of [CO₂] x water level was also significant (p=0.0137). Water deficit negatively impacted total plant biomass under both [CO₂]. However, biomass reduction was greater under eCO₂ than aCO₂ (see table 8 in appendix B).

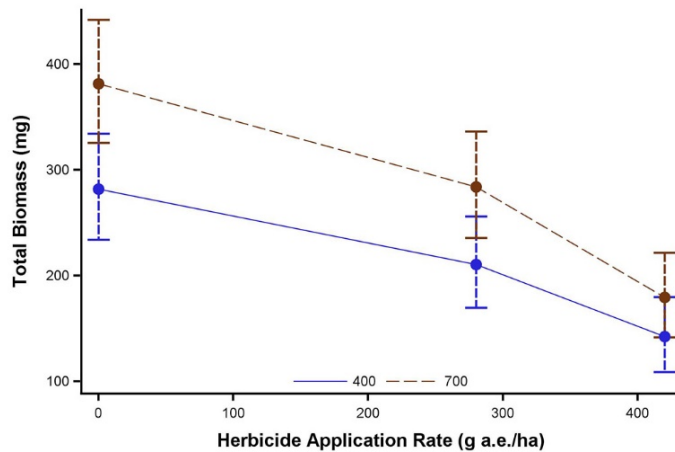


Figure 15. Velvetleaf total biomass and its interaction [CO₂] x Herbicide rate obtained 21 DAT. Error bars represent \pm standard errors of means.

As shown in Figure 16, two two-way interactions were found to be statistically significant, [CO₂] x herbicide rate ($p = <0.0001$) and water level x herbicide rate ($p = 0.0451$) for root: shoot. The rise in [CO₂] from 400 to 700 ppm increased root to shoot ratio when $\frac{3}{4}$ herbicide rate was applied, however, no statistical difference was found. In both [CO₂] tested, root to shoot ratio was greater as the herbicide rate increased (Figure 16A). The same trend was observed when testing different water conditions: the increase in herbicide rate produced R/S. Although, WD condition stimulated root growth (Figure 16B), statistical differences were found only when $\frac{1}{2}$ herbicide rate was applied ($p = 0.0027$).

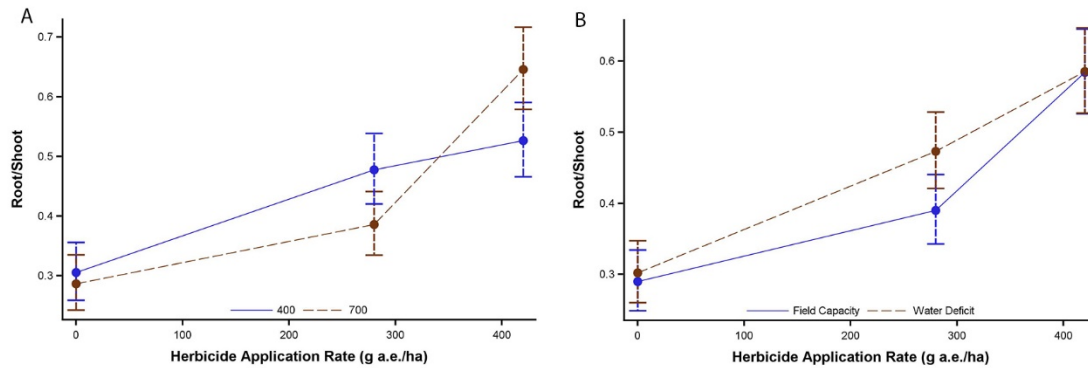


Figure 16. Velvetleaf root/shoot ratio interaction [CO₂] x Herbicide rate (A) and water level x Herbicide rate (B) obtained 21 DAT. Error bars represent ± standard errors of means.

ANOVA test, as shown in Figure 17, indicates that there was a significant three-way interaction between [CO₂], water level, and herbicide rate ($p=0.0078$). Under FC conditions, plants grown at eCO₂ presented higher carbon assimilation rates when compared to plants grown at aCO₂. Statistically significant differences were found for untreated plants ($p=0.0168$) and for the highest dicamba rate applied ($p=0.0140$). Under WD conditions, [CO₂] did not impact carbon assimilation rate at any of the three herbicide rates tested. The interaction between [CO₂] x WL was statistically significant for carbon assimilation rate in velvetleaf ($p=0.0223$). Although plants grown at 700 ppm presented higher assimilation rates in both FC and WD conditions, no significant differences were found (see table 20 in appendix B).

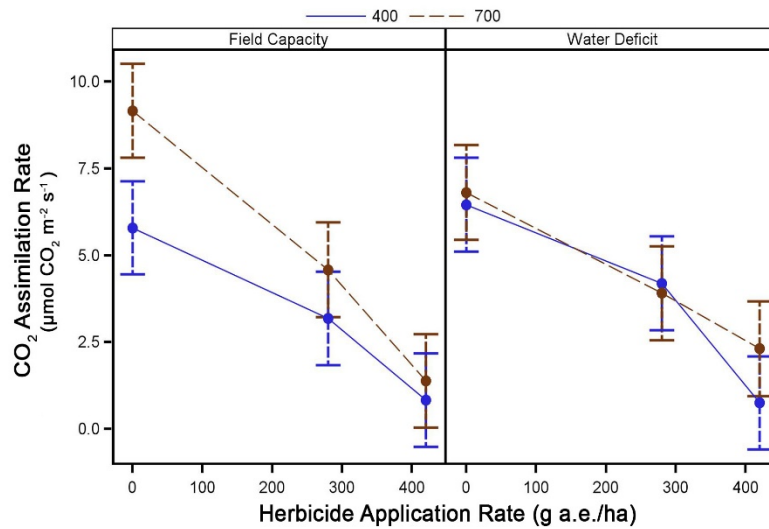


Figure 17. Velvetleaf CO₂ assimilation rate measured 10 DAT. Error bars represent ± standard errors of means.

The interaction between [CO₂] and herbicide rate was statistically significant ($p < 0.0001$) for stomatal conductance, g_s . As Figure 18 displays, [CO₂] did not impact herbicide treated plants, since a significant difference was found only in untreated plants. Plants grown at aCO₂ showed a significant reduction in g_s as the herbicide rate was increased (see table 23 in appendix B). No statistical differences were observed for plants grown at eCO₂.

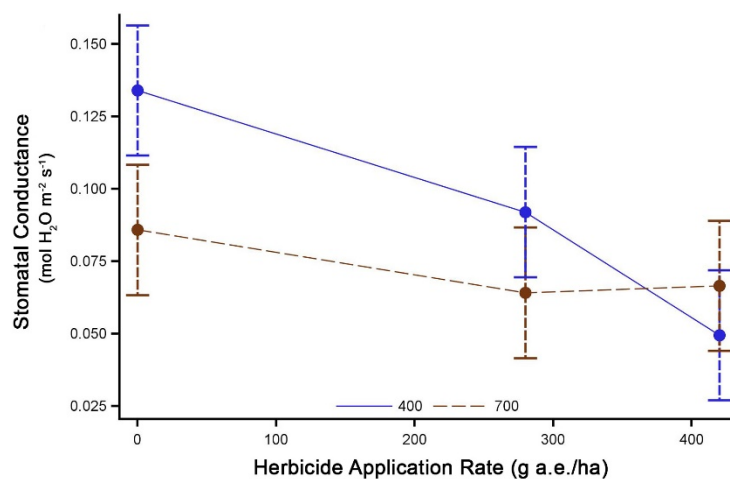


Figure 18. Velvetleaf stomatal conductance response to the interaction of [CO₂], water level and herbicide rate measured 10 DAT. Error bars represent ± standard errors of means.

3.3.2 Palmer amaranth

The interactions between [CO₂] and herbicide rate, as well as between water level and herbicide rate, were statistically significant for herbicide injury at 10 ($p < 0.0001$) and 21 DAT ($p < 0.0001$). At 10 DAT, herbicide injury was higher under FC conditions at aCO₂ when compared to eCO₂ (Figure 19A). Plants sprayed with ½ herbicide rate presented 10% greater herbicide injury at aCO₂ when compared to the same treatment at eCO₂ ($p = 0.0228$). The same trend was observed for the application of ¾ herbicide rate, for which herbicide injury was approximately 15% higher at aCO₂ ($p < 0.0001$). On the other hand, no statistical difference was found when WD was imposed to the treatments. At 21 DAT, herbicide injury was greater at aCO₂. The application of ½ herbicide rate caused an increase in herbicide injury from 65% at eCO₂, to approximately 82% at aCO₂

($p < 0.0001$), under FC conditions (Figure 19B). A similar pattern was observed when $\frac{3}{4}$ of herbicide rate was applied, where plants presented higher injury at aCO₂ when compared to same treatment at eCO₂ ($p = 0.0017$). Under WD conditions, a statistical difference was found for untreated plants ($p = 0.0063$) and $\frac{1}{2}$ herbicide rate application ($p < 0.0001$), when comparing both [CO₂]. While water level had no significant difference on the injury of herbicide treated plants at aCO₂, plants grown at eCO₂ (Figure 19B) presented higher herbicide injury at all three herbicide rates with WD treatments (see table 6 in appendix B).

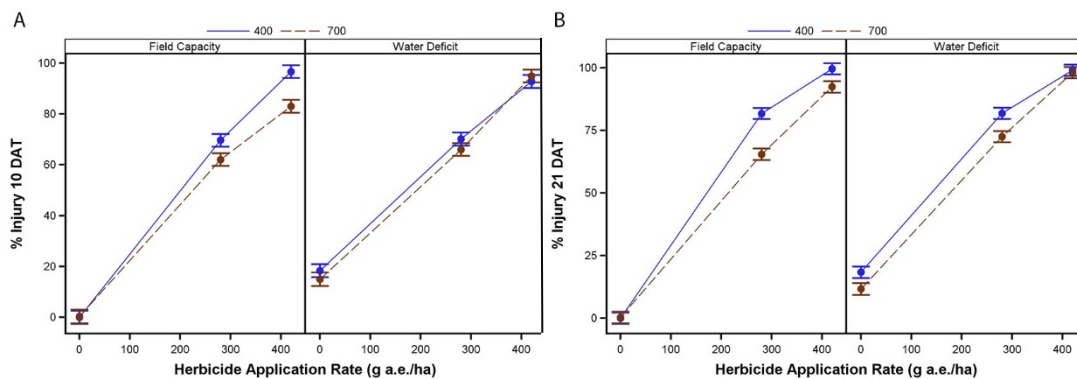


Figure 19. Palmer amaranth herbicide injury at 10 DAT (A) and 21 DAT (B) under different [CO₂] and water levels. Error bars represent \pm standard errors of means.

ANOVA test indicates the interaction of [CO₂] x water level x herbicide rate for total biomass (Figure 20). No statistical differences were found for untreated plants, or for plants that received $\frac{1}{2}$ herbicide rate, when comparing both [CO₂] under FC condition. Under WD condition, a significant difference was found only for untreated plants ($p = 0.0002$). At aCO₂, plants that received WD treatment showed a greater reduction in

total biomass when compared to plants grown at FC. A decrease of approximately 23% in total plant biomass was found for untreated plants ($p < 0.0001$) and plants receiving $\frac{1}{2}$ herbicide rate ($p = 0.0357$). At eCO_2 , statistical differences were found only for untreated plants, where this treatment had approximately 16% more total biomass under WD condition than at FC ($p = 0.0009$).

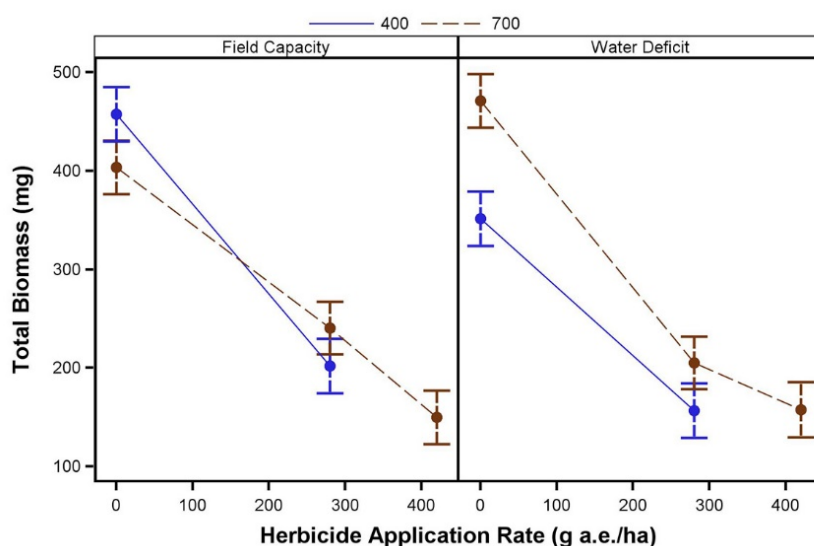


Figure 20. Palmer amaranth total biomass and its interaction [CO₂] x water level x Herbicide rate obtained 21 DAT. Error bars represent ± standard errors of means.

Since plants were greatly injured, we were not able to collect roots from the plants sprayed with $\frac{3}{4}$ of herbicide rate in both water levels at aCO_2 , we could not compare this treatment with the ones at eCO_2 . Therefore, we analyzed Palmer amaranth shoot biomass (Figure 21). Under FC conditions, plants grown at eCO_2 developed more shoot biomass when $\frac{1}{2}$ ($p = 0.0018$) and $\frac{3}{4}$ ($p = 0.0028$) herbicide rates were applied. The same pattern

was observed when plants were exposed to WD, where shoot biomass production was significantly greater with eCO₂ at all three herbicide rates tested when compared to their respective treatments at aCO₂ (see Table 11 in Appendices B). Water level had a greater impact under the application of ½ herbicide rate. WD reduced shoot biomass by approximately 17% (p=0.0230) and 21% (p=0.0008), at 400 and 700 ppm, respectively. However, no statistical difference was found when the highest herbicide rate was tested at either CO₂ concentration.

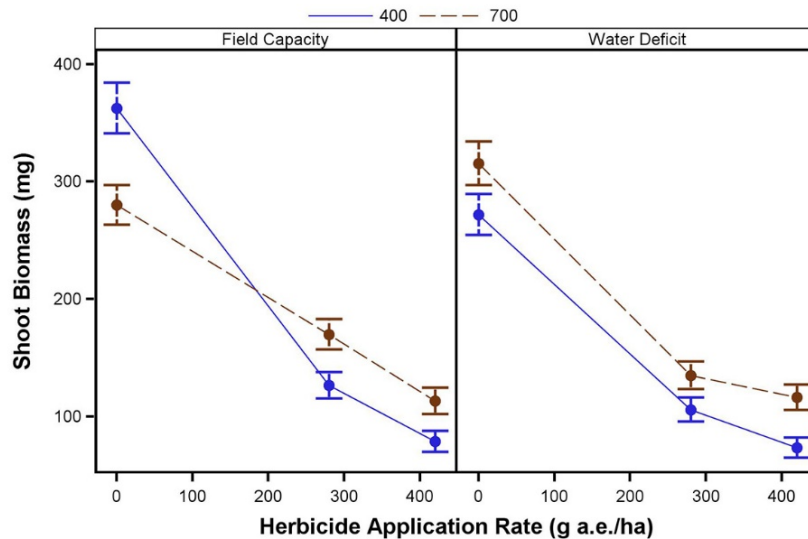


Figure 21. Palmer amaranth shoot biomass collected at 21 DAT. Error bars represent ± standard errors of means.

The interaction between [CO₂] x herbicide rate was found to be significant for root to shoot ratio (p=0.0155). The application of ½ herbicide rate increased the R/S ratio from 0.31 to 0.47 (p<0.0001) at aCO₂ (Figure 22). Even though plants sprayed with ½ herbicide rate produced higher root/shoot ratio at aCO₂ compared to plants at eCO₂, the difference was not found to be statistically significant. Additionally, no significant differences were found as herbicide rate increased under eCO₂.

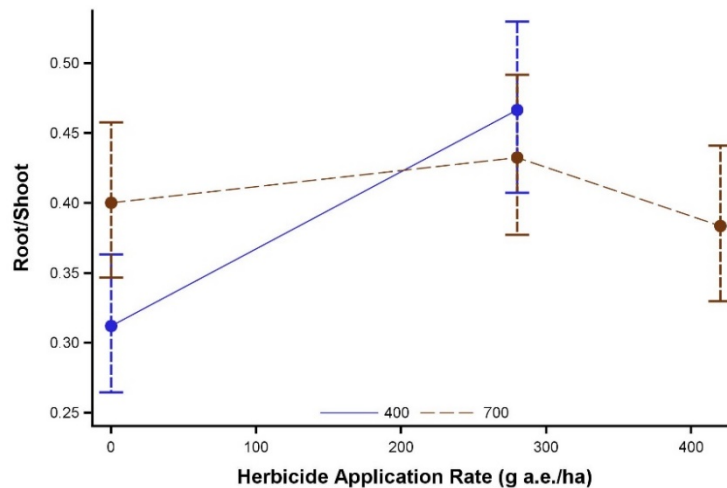


Figure 22. Palmer amaranth root/shoot ratio interaction [CO₂] x Herbicide rate obtained 21 DAT. Error bars represent ± standard errors of means.

CO₂ assimilation rate interactions were significant for [CO₂] x herbicide rate (p= 0.0090) and water level x herbicide rate (p=0.0372). Although CO₂ assimilation rates were numerically higher for plants at all three herbicides rates under aCO₂ than under eCO₂,

(Figure 23A), no statistical differences were found when comparing $[\text{CO}_2]$. Additionally, the increase in herbicide rate was followed by a significant decrease in CO_2 assimilation rate in both $[\text{CO}_2]$. Figure 23B shows that untreated and plants sprayed with $\frac{1}{2}$ herbicide rate presented significantly lower CO_2 assimilation rates under WD condition when compared to FC, $p=0.0137$ and $p=0.0128$, respectively. Moreover, the rise in herbicide rate triggered a reduction in CO_2 assimilation rate, independently of the water level.

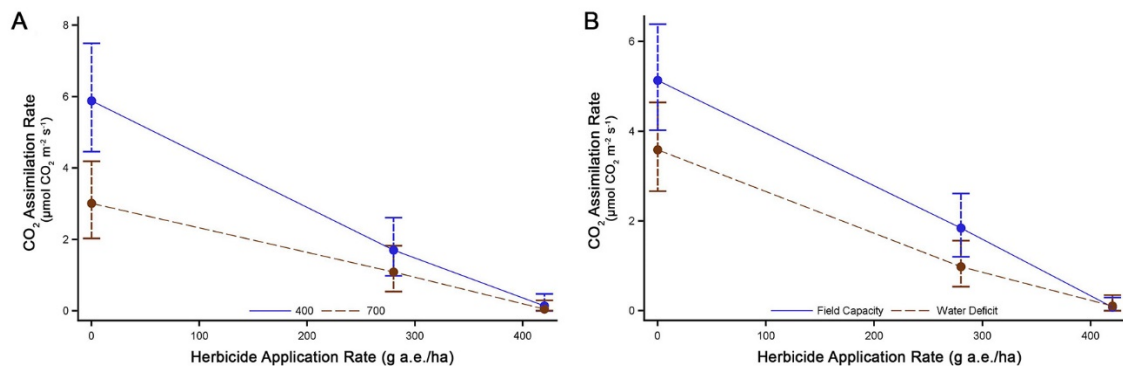


Figure 23. Palmer amaranth CO_2 assimilation rate interaction $[\text{CO}_2]$ x herbicide rate (A) and water level x herbicide rate (B) measured 10 DAT. Error bars represent \pm standard errors of means.

At 10 DAT none of the factors significantly influenced Palmer amaranth stomatal conductance (g_s).

Since plants sprayed with $\frac{3}{4}$ herbicide rate were severely injured we decided to collect Palmer amaranth photosynthetic data at 5 DAT as well. At 5 DAT the interaction between $[\text{CO}_2]$ x herbicide rate was found to be statistically significant ($p=0.0070$). At 5

DAT plants sprayed with $\frac{1}{2}$ and $\frac{3}{4}$ of the labelled herbicide rate presented higher carbon assimilation under aCO₂ concentration (Figure 24).

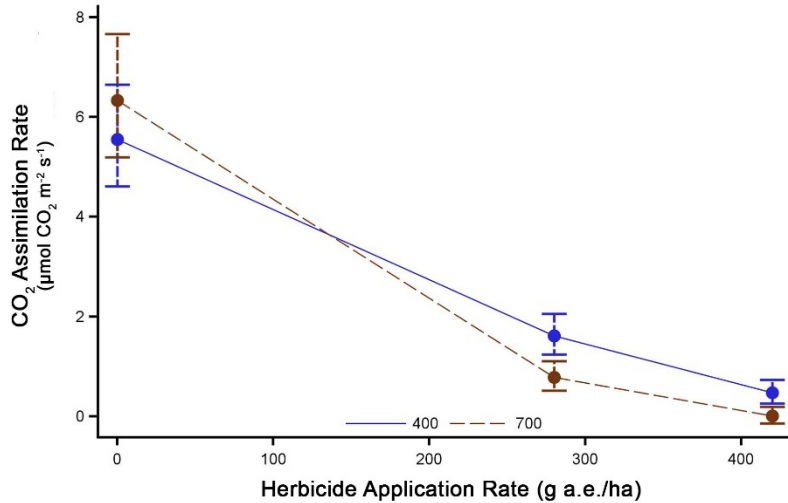


Figure 24. Palmer amaranth CO₂ Assimilation rate at 5 DAT. Error bars represent ± standard errors of means.

Moreover, the CO₂ assimilation rate was reduced as herbicide rate was increased.

Additionally, the interaction of CO₂ and herbicide rate was found to be significant for g_s ($p=0.0490$) at 5 DAT. Figure 25 shows that a significant difference was only observed when plants were sprayed with $\frac{3}{4}$ of the herbicide rate ($p=0.0451$). Under aCO₂ statistical differences were found between untreated plants and plants sprayed with $\frac{1}{2}$ ($p=0.0002$) and $\frac{3}{4}$ herbicide rate ($p<0.0001$). At eCO₂, untreated were found to be statistically different from plants received the application of $\frac{1}{2}$ herbicide rate ($p=0.0249$).

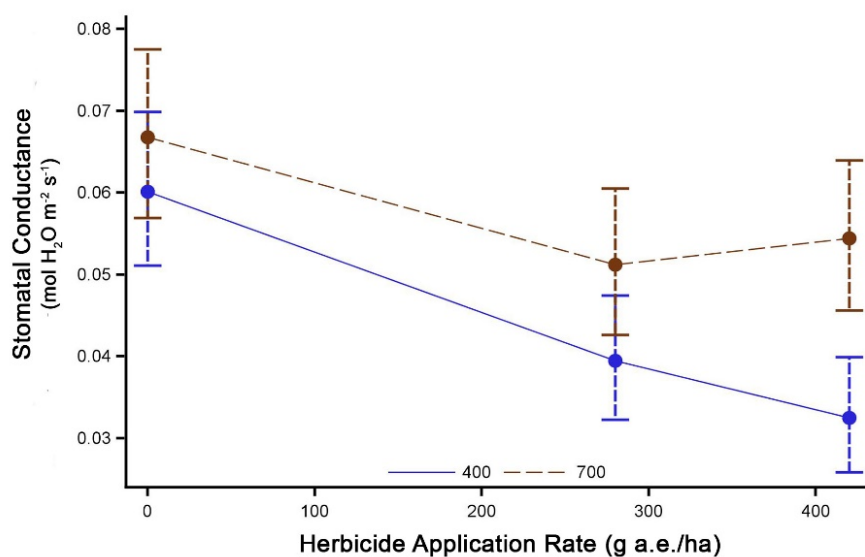


Figure 25. Palmer amaranth g_s at 5 DAT. Error bars represent \pm standard errors of means.

3.4 Discussion

3.4.1 Herbicide injury at 10 and 21 DAT

The multifactor analysis show that velvetleaf and Palmer amaranth responded quite differently to the three parameters tested. While both species showed a higher herbicide injury under aCO₂, the effects of water deficit were variable between species. Water deficit increased velvetleaf herbicide injury at both rates at 21 DAT, while in Palmer amaranth greater herbicide injury was seen at field capacity when $\frac{3}{4}$ herbicide rate was applied.

The increase in [CO₂] suppresses the oxygenation activity of Rubisco in C₃ species, which leads to an increase in the carbon assimilation rate, and consequently more plant

growth. Moreover, higher concentrations of CO₂ reduce stomatal conductance and transpiration which reduces water uptake. On the other hand, C₄ species are less impacted by the rise in [CO₂] since these plants already have anatomical and biochemical mechanisms to concentrate CO₂, which saves water (Ward et al., 1999). The natural auxin indole 3 -acetic acid (IAA) is vital for plant growth and development, and its closely associated with processes such as meristem differentiation, leaf initiation, root formation, and apical dominance (Grossmann, 2010). Dicamba is a synthetic auxin that in small amounts can stimulate plant growth and development. However, higher amounts of auxin induce anomalous growth and plants can be lethally damaged (Grossmann, 2003).

The mechanism of action for dicamba is still uncertain, however, the events that follow after herbicide application can be divided in three phases. The first phase, called the stimulation phase, starts a few hours after herbicide application and includes the stimulation of ethylene biosynthesis, the activation of membrane ion channels and H⁺-ATPases for cell elongation, as well as abscisic acid accumulation in the tissue.

Subsequently, the appearance of abnormal growth, such as leaf epinasty, tissue swelling, and stem bending becomes visible. The second phase is the inhibition phase, which consists of inhibition of root and shoot growth through reduced leaf area and internode elongation. Lastly, the third phase is the decay phase, where foliar senescence is accelerated due to destruction of membranes and vascular systems, leading to wilting, necrosis, and death. These three phases occur in a period of seventy-two hours; thus,

auxin herbicides are fast acting when compared to other herbicides such as glyphosate (Grossmann, 2003, 2010).

The increase in [CO₂] can alter plant redox homeostasis and hormone signaling since it can function as an environmental signal. However, the impact of the rise in CO₂ on hormone pathways and its effects on metabolic responses and growth are not completely understood. Therefore, the application of auxin herbicide under eCO₂ and water deficit can help to elucidate the efficacy of dicamba in the future.

Dicamba application induces the biosynthesis of ethylene. It would be useful to measure ethylene production in the current system since Sterling & Hall (1997) proposed that elevated CO₂ could delay or diminish the effects of auxin herbicides, because very high levels of CO₂ inhibit ethylene biosynthesis. Soybean plants grown under eCO₂ -550 μmol mol⁻¹ CO₂ – showed reduced expression of the ACC synthase gene, a key regulator in the biosynthesis of ethylene, which consequently reduced ethylene biosynthesis (Zavala et al., 2008). On the contrary, Woodrow & Grodzinski (1993), showed that tomato plants exposed to elevated [CO₂] had an increase in endogenous ethylene production when comparing to plants grown at lower [CO₂] and the same was observed for rice plants grown under eCO₂ (Seneweera et al., 2003). Moreover, auxin resistant biotypes produced less ethylene when compared to susceptible biotypes (Mithila et al., 2011). Therefore, the results found in the literature are controversial and more work on the effects of eCO₂ on ethylene biosynthesis may be necessary. Additionally, the increase in [CO₂] can alter plant redox homeostasis and hormone signaling since it can function as an environmental signal (Shi et al., 2015). However, the impact of the rise in

[CO₂] on hormone pathways and its consequences on metabolic responses and plant growth are not known (Xu et al., 2015).

Different responses to the application of auxin herbicides can be due to the diverse features of species, and can even be found between species in the same family (Hall & Swanton, 1988). Although differences in plant translocation and exudation can contribute to alter the movement of auxins in different species and biotypes, the major cause of selectivity is plant metabolism. Moreover, the chemical structure of the herbicide can also impact selectivity (Sterling & Hall, 1997).

While Palmer amaranth had increased herbicide injury under aCO₂, no statistical difference was found after the application of $\frac{3}{4}$ herbicide rate in velvetleaf. Therefore, when lower doses of dicamba are applied, [CO₂] may play a role in reducing the induction of ethylene production. However, as dicamba rate is increased, the auxin-ethylene induction is maintained. Nevertheless, more research is need to clarify the results found in this study.

Overall, our findings are in congruence with those of Refatti et al. (2019), where the C3 weed species *Echinochloa colona* showed reduced sensitive to the application of cyhalofop-butyl (ACCase inhibitor) under eCO₂. Reduced herbicide efficacy with eCO₂ was also observed when studying glyphosate control on *Elytrigia repens*, a C3 species (Ziska & Teasdale, 2000). In fact, it is important to highlight that no significant difference was found between [CO₂] when $\frac{3}{4}$ rate of dicamba was applied on velvetleaf. However, the reduced herbicide injury on velvetleaf sprayed with $\frac{1}{2}$ rate of dicamba at eCO₂ can serve as a warning, since sublethal doses of herbicides are often applied in the

field in an attempt to reduce costs. Moreover, the plant's phenological stage, weather conditions, and insufficient spray cover can result in the application of sublethal herbicide rates (Norsworthy et al., 2012). In fact, the application of sublethal doses of glyphosate on *Lolium rigidum* resulted in an increased level of resistance after three to four generations (Busi & Powles, 2009). The same was observed in Palmer amaranth treated with sublethal doses of dicamba, where the third generation of plants sprayed with sublethal doses of dicamba were 3-fold less susceptible than the first generation (Tehranchian et al., 2017). Therefore, the application of sublethal doses may be detrimental under future climate conditions, and could contribute to an increase in herbicide resistance cases.

Herbicide injury in Palmer amaranth was higher at aCO₂, which agrees with the outcomes found by Weller et al. (2019), since the rise in [CO₂] reduced glyphosate efficacy in *Chloris truncata*, a C₄ grass. Conversely, Ziska et al. (1999) showed that eCO₂ did not impact glyphosate control of *Amaranthus retroflexus*, also a C₄ weed species.

Water deficit can alter herbicide efficacy due to reduced absorption, translocation, and metabolism (Zhou et al., 2007). Additionally, a period of water restriction can alter plant morphology since plants can develop thicker cuticles, thus reducing herbicide uptake (Fernando et al., 2016). Although Skelton et al. (2016) showed that waterhemp (*Amaranthus tuberculatus*) plants had lower uptake of the auxin herbicide 2,4-D, translocation was not affected by drought stress and thus, herbicide efficacy was maintained even under water deficit conditions. These results match our findings since

dicamba efficacy was not changed by water conditions at aCO₂. Furthermore, although it has been shown that higher [CO₂] can minimize the impact of water scarcity in C₄ species, as demonstrated by Wall et al. (2001), Palmer amaranth plants sprayed with dicamba presented higher herbicide injury when water restriction was imposed at eCO₂. Velvetleaf plants also presented higher herbicide injury under WD. At 10 DAT, velvetleaf subjected to WD showed significant injury differences when $\frac{3}{4}$ herbicide rate was applied. Later, at 21 DAT, both herbicide rates applied showed higher herbicide injury with WD. One of the consequences of dicamba application is xylem obstruction (Peterson et al., 1974), thus the herbicide uptake by roots would at first be reduced due to the decrease in water movement imposed by the WD condition. However, since dicamba persists in the soil (Harp, 2010), when water is brought back to field capacity, roots could uptake herbicide from the soil again and increase herbicide injury. Moreover, Sterling & Hall (1997), stated that auxin herbicides such as dicamba are ambimobile because they can move from xylem to phloem and vice-versa, which can contribute to more effective control even under WD condition.

As mentioned earlier, dicamba induces the production of ethylene and ABA. However, it has been stated that WD may trigger the production of ABA, which induces the closure of stomata. Furthermore, ethylene production can also be activated under WD (Schachtman & Goodger, 2008). Therefore, a better understanding of the effects of application of dicamba on hormone homeostasis under WD, as well higher [CO₂] is needed.

3.4.2 Total Biomass

Elevated [CO₂] can impact both C₃ and C₄ species; however, since C₄ species have a saturation point at around 360 ppm, it is more likely they will be less affected by the rise in CO₂ (Leegood, 2002). However, C₄ plants can still benefit from higher [CO₂] by reducing stomatal conductance and thus, improving water use.

As a C₃ species, velvetleaf, had higher total biomass production when grown at eCO₂. In this study, the higher total biomass presented can be correlated to lower herbicide efficacy at eCO₂, but only when ½ herbicide rate was applied, since the application of ¾ dicamba rate did not result in any significant change in herbicide efficacy.

Although previous studies have correlated higher herbicide efficacy to reduced plant biomass (Manea et al., 2011; Ziska et al., 2004), recent studies have questioned this correlation. Jabran & Doğan (2018) showed that under eCO₂, three C₃ species (*Bromus tectorum*, *Hordeum murinum*, and *Lactuca serriola*) increased their growth rate and plant biomass, however, no difference in glyphosate efficacy was shown. Moreover, Waryszak et al. (2018), studied fourteen different weed species, including vines, herbs, shrubs, and grasses, and surprisingly only one species suggested that an increase in plant biomass could be related to lower herbicide efficacy.

Although the general consensus that C₄ plants would not benefit from the rise in [CO₂] is well established in the literature, our study shows the contrary. Palmer amaranth, a C₄ species presented higher total biomass at eCO₂, independently of the water condition.

While plants sprayed with ½ and ¾ herbicide rate were not affected by water condition at eCO₂, WD caused a significant reduction in total biomass when plants were sprayed

with ½ herbicide rate at aCO₂. The same pattern was found when analyzing shoot biomass in Palmer amaranth, where plants sprayed with dicamba had higher shoot biomass under eCO₂ than at aCO₂. Moreover, WD caused a reduction in shoot biomass in plants sprayed with ½ herbicide rate, independently of the [CO₂]. On the other hand, when the highest herbicide dose was applied, [CO₂] had no effect on shoot biomass. Thus, herbicide efficacy cannot always be related to plant biomass.

In addition to our findings, Ziska & Bunce (1997) verified that C4 weed species can still benefit from higher [CO₂], since C4 weeds species had a significant increase in total plant biomass under eCO₂. However, a resistant biotype of *Echinochloa colona*, another C4 weed species, showed reduced herbicide efficacy under eCO₂, while no difference was found in plant biomass when comparing [CO₂] (Refatti et al., 2019). Furthermore, Fernando et al. (2016) emphasized the importance of studying plant biochemistry and physiology as a way to better address plant responses to herbicides under changing climate rather than only considering morphological changes. Moreover, as shown by the author, correlations between plant biomass and herbicide efficacy under eCO₂, are not always accurate, since plants with higher plant biomass (fertilization effect) may present higher surface area, which would increase area of contact with the herbicide, increasing therefore herbicide efficacy.

3.4.3 Root to Shoot Ratio

In this study, the application of dicamba had varying impacts on root to shoot ratio. While no difference in root/shoot was found when comparing [CO₂], the increase in

herbicide rate in both [CO₂] was followed by an increase in R/S, which can be explained by the herbicide injury caused to the shoot, which may lead to an increased R/S as herbicide rate was increased. In addition, though Rogers et al. (1996) have shown the majority of the plants were positively impacted by eCO₂ since root growth was higher at elevated concentrations of CO₂, velvetleaf did not follow this pattern. In fact, it is important to mention that hormone crosstalk can influence the results observed here, since dicamba is an auxin herbicide. As stated earlier, dicamba induces the production of ethylene, and crosstalk between auxin and ethylene in root growth and development is still unclear (Qin & Huang, 2018).

Velvetleaf was also impacted by water level, since plants sprayed with ½ herbicide rate exposed to WD condition presented higher R/S comparing to plants at FC. Though the other treatments did not change R/S, Huang & Fry (1998) showed that under water scarcity, carbon partitioning can be altered, and consequently also the R/S. Previous studies showed water stress conditions can increase the R/S to enable roots to access deeper soils and uptake water and nutrients (Bazzaz, 1990). Additionally, carbon allocation and ABA content are involved in the process, since this hormone is linked to a decrease in stomatal conductance and wall extensibility of growing cells, which results in reduced leaf expansion, with a consequent increase in carbon allocation to the root system (Lambers et al., 2008).

In Palmer amaranth, an increase in R/S occurred in plants sprayed with ½ herbicide rate compared to untreated plants at aCO₂. However, no changes in R/S were observed in plants grown at eCO₂. Therefore, these findings suggest that even though higher

herbicide injury has occurred at aCO₂, plants were still able to increase R/S when a lower herbicide rate was applied. Even though it was not possible to estimate the R/S for ¾ herbicide rate at aCO₂, based on the herbicide injury, we can assume this value would be lower. Moreover, the higher shoot biomass production shown by Palmer amaranth under eCO₂ was not followed by an increase in the R/S. Hence, the question arises – What would be worse in future climate conditions? Lower herbicide injury at eCO₂ and no change in the R/S or higher injury at aCO₂, with a consequently higher R/S? According to (Ziska et al., 2004), the increase in R/S observed at eCO₂ could allow plants to survive and regrow after glyphosate application. Although some papers have related higher R/S to plant survival at eCO₂ (Manea et al., 2011), other studies showed that changes in plant growth cannot be related to herbicide efficacy (Marble et al., 2015). Moreover, Rogers et al. (1996) found great variability in the R/S regardless of the photosynthetic pathway.

Lastly, the application of an auxin herbicide involves changes in at least three hormones, auxin, ABA, and ethylene, and the additional effects of higher [CO₂] and water deficit to plants' metabolism need to be clarified with further research.

3.4.4 CO₂ Assimilation rate (*A*)

As expected for a C3 species, velvetleaf, presented higher *A* rate at FC compared to WD, except for plants sprayed with ½ herbicide rate. It was postulated that eCO₂ would lessen the stress triggered by water restriction, since higher [CO₂] provokes a reduction in stomatal conductance and transpiration (Wall, 2001), which would allow plants to

survive. However, in this study no difference in A was observed when comparing water conditions. Moreover, when comparing the effect of WD at each $[\text{CO}_2]$ on A , no difference was found between WD and FC. These results are contradictory to what was previously shown by Santesteban et al. (2009) and Souza et al. (2004), where it was shown that grapes and cowpea plants, respectively, had lower A rates after a period of water deficit.

For the C4 species, Palmer amaranth, both $[\text{CO}_2]$ and water level interacted with herbicide rate and impacted A rate. While no difference was found when comparing both $[\text{CO}_2]$, the reduction in A rate was much greater at a CO_2 than at e CO_2 , as herbicide rate was increased, which is in contrast to the results found by Ziska et al. (1999), where e CO_2 did not influence photosynthesis in *Amaranthus retroflexus*, another C4 weed species.

Although, Vanaja et al. (2011) displayed that A rate was not affected by water deficit in maize, a C4 crop, in this study Palmer amaranth showed an increased A rate at FC compared to WD. In addition, Lal & Edwards (1996) showed that C4 species exposed to WD were able to recover and maintain A at pre- stress levels.

Thus, the response of C3 and C4 plants exposed to e CO_2 and WD can vary, and stomatal and non-stomatal factors can impact the plant response (Ghannoum, 2008). Hence, dicamba mode of action can impact each species differentially, and generalizations made according to plant photosynthetic pathway should be avoided. Lastly, as an auxin herbicide, dicamba induces stomatal closure, reduces transpiration, carbon assimilation, and starch formation (Grossmann, 2003), and all these factors are closely linked to

higher [CO₂] and WD also, which makes this a complex process requiring further research.

3.4.5 Stomatal Conductance (g_s)

Although A rate was higher in some treatments under eCO₂, these results were not a consequence of higher g_s in velvetleaf, since no significant difference was found when comparing both [CO₂]. Moreover, while herbicide rate affected g_s at aCO₂, no difference was observed at eCO₂. This outcome shows that under eCO₂, velvetleaf plants reduced g_s , although no effect was seen in A rate. The same trend was observed for plants at aCO₂, where even though plants did not differ in A rate, an increase in g_s was observed. Indeed, no difference was found in g_s when comparing both [CO₂]. Reduction of g_s was also shown in *Chenopodium album* sprayed with glyphosate at eCO₂ (Ziska et al., 1999). At 10 DAT g_s was not impacted by herbicide, water condition, nor [CO₂]. However, at 5 DAT, plants presented lower g_s at aCO₂. This finding is contrary to what Ziska et al. (1999) observed for the C4 weed species, *Amaranthus retroflexus*, which reduced g_s at eCO₂ after treatment with glyphosate.

The reduction in g_s causes a drop in the C_i (internal carbon), and thus less carbon should be assimilated. Under well irrigated and stress-free conditions, this relationship holds true. However, the linear relationship between g_s and A rate tends to break down in an environment where water is restricted and source-sink carbon partitioning may be affected, as was reported by Damour et al. (2010). Moreover, ABA can negatively affect enzymes involved in the Calvin Cycle, hence impacting g_s and A rate (Damour et al.,

2010; Rook et al., 2006). Therefore, the application of dicamba may magnify the 'natural' responses to eCO₂ and WD, since the application of the herbicide interferes with several plant processes (e.g. photosynthesis, hormone balance, growth). Hence, additional research is needed to clarify the findings of this study and the potential involvement of eCO₂ and WD in g_s of plants sprayed with dicamba.

3.5 Conclusion

Dicamba is a good option to control velvetleaf and Palmer amaranth under aCO₂.

However, as [CO₂] rise dicamba efficacy may be reduced, especially when ½ rate herbicide is applied. Thus, adequate application is of extreme importance, since plants exposed to sublethal doses of dicamba can quickly evolve herbicide resistance.

Moreover, the WD effect varied by species; while WD increased injury in velvetleaf, in Palmer amaranth, herbicide injury was higher only at eCO₂. In addition, responses can vary due to the different plant features such as morphology, biochemistry, and physiology. Dicamba is a hormone herbicide that is connected to many processes in plants, and more research is needed to clarify the impact of dicamba applications under a changing climate.

3.6 References

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4. CONCLUSIONS

These studies have provided insights into the efficacy of tembotrione and dicamba under elevated [CO₂], water deficit, and the interactions between these different parameters. Previous studies suggested that under future [CO₂], herbicides could be less effective in controlling C3 species due to the direct impact high [CO₂] concentrations have on their growth and consequently on their biomass. Conversely, no such changes in growth and biomass are expected for C4 species since they already have anatomical and biochemical mechanisms to concentrate CO₂. It is expected that water deficit would cause a reduction of herbicide efficacy since both herbicides are systemic and need to be translocated into the plant to ensure high activity. However, responses to the combination of the three factors are harder to speculate on based on previous generalizations about herbicide efficacy. These studies show that herbicide efficacy under eCO₂ and water deficit can vary according to plant species rather than according to plant photosynthetic system. Plant-specific features such as anatomy, morphology, biochemistry, and physiology seem to have influenced the plant responses observed here. Moreover, as shown in previous studies, the correlation between plant biomass and less herbicide efficacy was not always apparent, possibly since higher surface area means more area of contact with the herbicide. Thus, the dilution effect theories are not the best approach to explain herbicide efficacy under climate change conditions. Furthermore, it is important to emphasize that these plants were subjected to higher [CO₂] abruptly, while in nature, the increase in [CO₂] is gradual which could give plants time to acclimate, and thus plant

responses to herbicide application in future climate conditions could differ from the results found here. Ultimately, since each herbicide affected plant physiology in a different way due to their different modes of action, more research is necessary to clarify the findings of these studies, and different herbicide modes of action should be investigated to ensure accurate chemical control in the future.

APPENDIX A
TEMBOTRIONE HERBICIDE

Table A1. Analysis of variance (ANOVA) for % of injury at 10 DAT for all four studied species.

Sources of Variance	Johnsongrass		Palmer		Weedy rice		Velvetleaf	
	F- value	P- value	F- value	P- value	F- value	P- value	F- value	P- value
CO ₂ Concentration ([CO ₂])	1.57	0.4285	1.36	0.4512	1.57	0.4287	0.58	0.5863
Water Level (WL)	0.86	0.5235	9.56	0.1991	1.75	0.3168	3.18	0.2167
[CO ₂] x WL	3.53	0.0619	39.08	<.0001	1.31	0.2542	65.82	<.0001
Herbicide rate (H_rate)	16.55	0.0570	52.57	0.0187	23.95	0.0059	29.79	0.0040
[CO ₂] x H_rate	18.57	<.0001	4.87	0.0087	7.26	0.0009	1.03	0.3578
WL x H_rate	35.66	<.0001	22.80	<.0001	28.59	<.0001	92.92	<.0001
[CO ₂] x WL x H_rate	2.23	0.1109	3.14	0.0456	6.52	0.0018	24.05	<.0001

Table A2: Johnsongrass least square means of the interactions CO₂ x Herbicide rate and Water level x Herbicide rate for % of injury at 10 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	46	-28.688	9.236	168	-3.11	0.0022	0.05
CO2*H_rate	-	400	0	-	400	69	-42.508	9.244	168	-4.6	<.0001	0.05
CO2*H_rate	-	400	0	-	700	0	1.795	8.370	168	0.21	0.8304	0.05
CO2*H_rate	-	400	0	-	700	46	-51.563	12.108	168	-4.26	<.0001	0.05
CO2*H_rate	-	400	0	-	700	69	-51.531	12.108	168	-4.26	<.0001	0.05
CO2*H_rate	-	400	46	-	400	69	-13.820	9.208	168	-1.5	0.1353	0.05
CO2*H_rate	-	400	46	-	700	0	30.483	12.094	168	2.52	0.0126	0.05
CO2*H_rate	-	400	46	-	700	46	-22.875	8.313	168	-2.75	0.0066	0.05
CO2*H_rate	-	400	46	-	700	69	-22.844	12.081	168	-1.89	0.0604	0.05
CO2*H_rate	-	400	69	-	700	0	44.303	12.099	168	3.66	0.0003	0.05
CO2*H_rate	-	400	69	-	700	46	-9.055	12.087	168	-0.75	0.4548	0.05
CO2*H_rate	-	400	69	-	700	69	-9.023	8.321	168	-1.08	0.2798	0.05
CO2*H_rate	-	700	0	-	700	46	-53.358	9.217	168	-5.79	<.0001	0.05
CO2*H_rate	-	700	0	-	700	69	-53.327	9.217	168	-5.79	<.0001	0.05
CO2*H_rate	-	700	46	-	700	69	0.031	9.201	168	0	0.9973	0.05
WL*H_rate	FC	-	0	FC	-	46	-54.031	9.201	168	-5.87	<.0001	0.05
WL*H_rate	FC	-	0	FC	-	69	-64.383	9.208	168	-6.99	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	0	-23.205	4.548	168	-5.1	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	46	-51.219	9.828	168	-5.21	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	69	-54.656	9.828	168	-5.56	<.0001	0.05
WL*H_rate	FC	-	46	FC	-	69	-10.352	9.208	168	-1.12	0.2625	0.05

Table A2: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
WL*H_rate	FC	-	46	WD	-	0	30.827	9.876	168	3.12	0.0021	0.05
WL*H_rate	FC	-	46	WD	-	46	2.813	4.443	168	0.63	0.5276	0.05
WL*H_rate	FC	-	46	WD	-	69	-0.625	9.828	168	-0.06	0.9494	0.05
WL*H_rate	FC	-	69	WD	-	0	41.178	9.883	168	4.17	<.0001	0.05
WL*H_rate	FC	-	69	WD	-	46	13.164	9.834	168	1.34	0.1825	0.05
WL*H_rate	FC	-	69	WD	-	69	9.727	4.458	168	2.18	0.0305	0.05
WL*H_rate	WD	-	0	WD	-	46	-28.014	9.252	168	-3.03	0.0029	0.05
WL*H_rate	WD	-	0	WD	-	69	-31.452	9.252	168	-3.4	0.0008	0.05
WL*H_rate	WD	-	46	WD	-	69	-3.438	9.201	168	-0.37	0.7092	0.05

- * WL= Water level; FC= Field capacity and WD= Water deficit;
- * CO₂ = CO₂ concentrations; 400 and 700 ppm;
- * H_rate = Herbicide rate; 0, 46, and 69 g a.i. /ha.

Table A3: Weedy rice least square means of the interaction CO₂ x Water level x Herbicide rate for % of injury at 10 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	46	-55.59	10.26	213	-5.42	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	69	-75.67	10.26	213	-7.38	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-14.57	4.27	213	-3.41	0.0008	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	-54.61	10.40	213	-5.25	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	69	-58.71	10.42	213	-5.63	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	-3.53	5.80	213	-0.61	0.5431	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	-77.12	11.09	213	-6.95	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	69	-73.68	11.08	213	-6.65	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-13.27	5.97	213	-2.22	0.0273	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	-61.39	11.19	213	-5.49	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	69	-66.31	11.19	213	-5.93	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	400	69	-20.08	10.26	213	-1.96	0.0516	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	41.02	10.48	213	3.91	0.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	46	0.98	4.07	213	0.24	0.8094	0.05
CO2*WL*H_rate	FC	400	46	WD	400	69	-3.12	10.42	213	-0.3	0.7651	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	52.06	11.09	213	4.69	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	-21.53	5.80	213	-3.71	0.0003	0.05
CO2*WL*H_rate	FC	400	46	FC	700	69	-18.09	11.08	213	-1.63	0.104	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	42.32	11.19	213	3.78	0.0002	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	-5.80	5.97	213	-0.97	0.3324	0.05
CO2*WL*H_rate	FC	400	46	WD	700	69	-10.72	11.19	213	-0.96	0.3391	0.05
CO2*WL*H_rate	FC	400	69	WD	400	0	61.10	10.48	213	5.83	<.0001	0.05

Table A3: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr t	Alpha
CO2*WL*H_rate	FC	400	69	WD	400	46	21.06	10.40	213	2.02	0.0441	0.05
CO2*WL*H_rate	FC	400	69	WD	400	69	16.96	4.12	213	4.11	<.0001	0.05
CO2*WL*H_rate	FC	400	69	FC	700	0	72.14	11.09	213	6.5	<.0001	0.05
CO2*WL*H_rate	FC	400	69	FC	700	46	-1.45	11.09	213	-0.13	0.8963	0.05
CO2*WL*H_rate	FC	400	69	FC	700	69	1.99	5.77	213	0.34	0.7309	0.05
CO2*WL*H_rate	FC	400	69	WD	700	0	62.40	11.19	213	5.58	<.0001	0.05
CO2*WL*H_rate	FC	400	69	WD	700	46	14.28	11.19	213	1.28	0.2032	0.05
CO2*WL*H_rate	FC	400	69	WD	700	69	9.36	5.97	213	1.57	0.1183	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	-40.04	10.34	213	-3.87	0.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	69	-44.14	10.36	213	-4.26	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	11.04	6.13	213	1.8	0.0732	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	-62.55	11.27	213	-5.55	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	69	-59.11	11.26	213	-5.25	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	1.31	5.92	213	0.22	0.8256	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	-46.82	11.16	213	-4.2	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	69	-51.74	11.16	213	-4.64	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	400	69	-4.10	10.28	213	-0.4	0.6903	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	51.08	11.19	213	4.56	<.0001	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	-22.51	5.99	213	-3.76	0.0002	0.05
CO2*WL*H_rate	WD	400	46	FC	700	69	-19.07	11.19	213	-1.71	0.0896	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	41.34	11.08	213	3.73	0.0002	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	-6.78	5.77	213	-1.18	0.2413	0.05
CO2*WL*H_rate	WD	400	46	WD	700	69	-11.70	11.08	213	-1.06	0.2923	0.05

Table A3: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr t	Alpha
CO2*WL*H_rate	WD	400	69	FC	700	0	55.18	11.22	213	4.92	<.0001	0.05
CO2*WL*H_rate	WD	400	69	FC	700	46	-18.41	11.22	213	-1.64	0.1022	0.05
CO2*WL*H_rate	WD	400	69	FC	700	69	-14.97	6.01	213	-2.49	0.0135	0.05
CO2*WL*H_rate	WD	400	69	WD	700	0	45.44	11.10	213	4.09	<.0001	0.05
CO2*WL*H_rate	WD	400	69	WD	700	46	-2.68	11.10	213	-0.24	0.8094	0.05
CO2*WL*H_rate	WD	400	69	WD	700	69	-7.60	5.81	213	-1.31	0.1925	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	-73.59	9.95	213	-7.4	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	69	-70.15	9.94	213	-7.06	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-9.74	3.40	213	-2.86	0.0046	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	-57.86	10.05	213	-5.76	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	69	-62.78	10.05	213	-6.24	<.0001	0.05
CO2*WL*H_rate	FC	700	46	FC	700	69	3.43	9.94	213	0.35	0.7299	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	63.85	10.05	213	6.35	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	15.73	3.40	213	4.62	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	69	10.81	10.05	213	1.08	0.2835	0.05
CO2*WL*H_rate	FC	700	69	WD	700	0	60.42	10.04	213	6.02	<.0001	0.05
CO2*WL*H_rate	FC	700	69	WD	700	46	12.29	10.04	213	1.22	0.2223	0.05
CO2*WL*H_rate	FC	700	69	WD	700	69	7.38	3.37	213	2.19	0.0298	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	-48.13	9.93	213	-4.85	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	69	-53.04	9.93	213	-5.34	<.0001	0.05
CO2*WL*H_rate	WD	700	46	WD	700	69	-4.92	9.93	213	-0.5	0.6209	0.05

Table A4: Palmer amaranth least square means of the interaction CO₂ x Water level x Herbicide rate for % of injury at 10 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr > t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	46	-71.38	8.97	175	-7.96	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	69	-85.81	8.97	175	-9.56	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-15.00	2.41	175	-6.22	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	-80.81	9.03	175	-8.95	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	69	-90.69	9.03	175	-10.04	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	0.00	2.19	175	0	1	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	-83.44	8.97	175	-9.3	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	69	-93.63	8.97	175	-10.43	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-10.00	2.41	175	-4.14	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	-77.38	9.03	175	-8.57	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	69	-85.50	9.03	175	-9.47	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	400	69	-14.44	8.97	175	-1.61	0.1094	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	56.38	9.03	175	6.24	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	46	-9.44	2.41	175	-3.91	0.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	69	-19.31	9.03	175	-2.14	0.0338	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	71.38	8.97	175	7.96	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	-12.06	2.19	175	-5.51	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	69	-22.25	8.97	175	-2.48	0.0141	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	61.38	9.03	175	6.8	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	-6.00	2.41	175	-2.49	0.0138	0.05
CO2*WL*H_rate	FC	400	46	WD	700	69	-14.13	9.03	175	-1.56	0.1196	0.05
CO2*WL*H_rate	FC	400	69	WD	400	0	70.81	9.03	175	7.84	<.0001	0.05

Table A4: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr t	Alpha
CO2*WL*H_rate	FC	400	69	WD	400	46	5.00	9.03	175	0.55	0.5805	0.05
CO2*WL*H_rate	FC	400	69	WD	400	69	-4.88	2.41	175	-2.02	0.0449	0.05
CO2*WL*H_rate	FC	400	69	FC	700	0	85.81	8.97	175	9.56	<.0001	0.05
CO2*WL*H_rate	FC	400	69	FC	700	46	2.38	8.97	175	0.26	0.7915	0.05
CO2*WL*H_rate	FC	400	69	FC	700	69	-7.81	2.19	175	-3.57	0.0005	0.05
CO2*WL*H_rate	FC	400	69	WD	700	0	75.81	9.03	175	8.4	<.0001	0.05
CO2*WL*H_rate	FC	400	69	WD	700	46	8.44	9.03	175	0.93	0.3514	0.05
CO2*WL*H_rate	FC	400	69	WD	700	69	0.31	2.41	175	0.13	0.8971	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	-65.81	8.97	175	-7.34	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	69	-75.69	8.97	175	-8.44	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	15.00	2.41	175	6.22	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	-68.44	9.03	175	-7.58	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	69	-78.63	9.03	175	-8.71	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	5.00	2.19	175	2.29	0.0235	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	-62.38	8.97	175	-6.95	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	69	-70.50	8.97	175	-7.86	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	400	69	-9.88	8.97	175	-1.1	0.2726	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	80.81	9.03	175	8.95	<.0001	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	-2.63	2.41	175	-1.09	0.2781	0.05
CO2*WL*H_rate	WD	400	46	FC	700	69	-12.81	9.03	175	-1.42	0.1577	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	70.81	8.97	175	7.89	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	3.44	2.19	175	1.57	0.1179	0.05
CO2*WL*H_rate	WD	400	46	WD	700	69	-4.69	8.97	175	-0.52	0.602	0.05

Table A4: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr t	Alpha
CO2*WL*H_rate	WD	400	69	FC	700	0	90.69	9.03	175	10.04	<.0001	0.05
CO2*WL*H_rate	WD	400	69	FC	700	46	7.25	9.03	175	0.8	0.4231	0.05
CO2*WL*H_rate	WD	400	69	FC	700	69	-2.94	2.41	175	-1.22	0.2251	0.05
CO2*WL*H_rate	WD	400	69	WD	700	0	80.69	8.97	175	8.99	<.0001	0.05
CO2*WL*H_rate	WD	400	69	WD	700	46	13.31	8.97	175	1.48	0.1397	0.05
CO2*WL*H_rate	WD	400	69	WD	700	69	5.19	2.19	175	2.37	0.0188	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	-83.44	8.97	175	-9.3	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	69	-93.63	8.97	175	-10.43	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-10.00	2.41	175	-4.14	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	-77.38	9.03	175	-8.57	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	69	-85.50	9.03	175	-9.47	<.0001	0.05
CO2*WL*H_rate	FC	700	46	FC	700	69	-10.19	8.97	175	-1.14	0.2577	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	73.44	9.03	175	8.13	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	6.06	2.41	175	2.51	0.0129	0.05
CO2*WL*H_rate	FC	700	46	WD	700	69	-2.06	9.03	175	-0.23	0.8196	0.05
CO2*WL*H_rate	FC	700	69	WD	700	0	83.63	9.03	175	9.26	<.0001	0.05
CO2*WL*H_rate	FC	700	69	WD	700	46	16.25	9.03	175	1.8	0.0736	0.05
CO2*WL*H_rate	FC	700	69	WD	700	69	8.13	2.41	175	3.37	0.0009	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	-67.38	8.97	175	-7.51	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	69	-75.50	8.97	175	-8.41	<.0001	0.05
CO2*WL*H_rate	WD	700	46	WD	700	69	-8.13	8.97	175	-0.91	0.3664	0.05

Table A5: Velvetleaf least square means of the interaction CO₂ x Water level x Herbicide rate for % of injury at 10 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	27.6	-72.13	8.89	227	-8.11	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	46	-82.54	8.89	227	-9.28	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-23.33	3.21	227	-7.27	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	27.6	-57.61	9.22	227	-6.25	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	-63.02	9.22	227	-6.83	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	4.43	6.25	227	0.71	0.4793	0.05
CO2*WL*H_rate	FC	400	0	FC	700	27.6	-53.28	10.67	227	-5	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	-67.81	10.67	227	-6.36	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-16.98	6.73	227	-2.52	0.0123	0.05
CO2*WL*H_rate	FC	400	0	WD	700	27.6	-64.87	10.94	227	-5.93	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	-73.01	10.94	227	-6.67	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	FC	400	46	-10.42	8.89	227	-1.17	0.2427	0.05
CO2*WL*H_rate	FC	400	27.6	WD	400	0	48.79	9.22	227	5.29	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	400	27.6	14.52	3.22	227	4.5	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	400	46	9.11	9.22	227	0.99	0.3246	0.05
CO2*WL*H_rate	FC	400	27.6	FC	700	0	76.55	10.67	227	7.18	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	FC	700	27.6	18.85	6.25	227	3.02	0.0029	0.05
CO2*WL*H_rate	FC	400	27.6	FC	700	46	4.32	10.67	227	0.4	0.6859	0.05
CO2*WL*H_rate	FC	400	27.6	WD	700	0	55.15	10.96	227	5.03	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	700	27.6	7.25	6.71	227	1.08	0.2807	0.05
CO2*WL*H_rate	FC	400	27.6	WD	700	46	-0.89	10.94	227	-0.08	0.9355	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	59.21	9.22	227	6.42	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	27.6	24.93	9.22	227	2.7	0.0074	0.05

Table A5: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	46	WD	400	46	19.52	3.22	227	6.06	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	86.97	10.67	227	8.15	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	27.6	29.26	10.67	227	2.74	0.0066	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	14.74	6.25	227	2.36	0.0192	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	65.56	10.96	227	5.98	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	27.6	17.67	10.94	227	1.62	0.1077	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	9.53	6.71	227	1.42	0.1567	0.05
CO2*WL*H_rate	WD	400	0	WD	400	27.6	-34.27	8.90	227	-3.85	0.0002	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	-39.69	8.90	227	-4.46	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	27.76	6.71	227	4.14	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	27.6	-29.95	10.94	227	-2.74	0.0067	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	-44.47	10.94	227	-4.07	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	6.35	6.28	227	1.01	0.3125	0.05
CO2*WL*H_rate	WD	400	0	WD	700	27.6	-41.54	10.67	227	-3.89	0.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	-49.68	10.67	227	-4.66	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	WD	400	46	-5.41	8.90	227	-0.61	0.5439	0.05
CO2*WL*H_rate	WD	400	27.6	FC	700	0	62.04	10.94	227	5.67	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	FC	700	27.6	4.33	6.72	227	0.64	0.5199	0.05
CO2*WL*H_rate	WD	400	27.6	FC	700	46	-10.20	10.94	227	-0.93	0.3523	0.05
CO2*WL*H_rate	WD	400	27.6	WD	700	0	40.63	10.69	227	3.8	0.0002	0.05
CO2*WL*H_rate	WD	400	27.6	WD	700	27.6	-7.27	6.26	227	-1.16	0.247	0.05
CO2*WL*H_rate	WD	400	27.6	WD	700	46	-15.40	10.67	227	-1.44	0.1502	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	67.45	10.94	227	6.16	<.0001	0.05

Table A5: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	46	FC	700	27.6	9.74	10.94	227	0.89	0.3744	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	-4.79	6.72	227	-0.71	0.4766	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	46.04	10.69	227	4.31	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	27.6	-1.86	10.67	227	-0.17	0.8621	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	-9.99	6.26	227	-1.6	0.1119	0.05
CO2*WL*H_rate	FC	700	0	FC	700	27.6	-57.71	9.03	227	-6.39	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	-72.23	9.03	227	-8	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-21.41	3.57	227	-6	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	27.6	-69.30	9.39	227	-7.38	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	-77.44	9.39	227	-8.25	<.0001	0.05
CO2*WL*H_rate	FC	700	27.6	FC	700	46	-14.53	9.03	227	-1.61	0.1093	0.05
CO2*WL*H_rate	FC	700	27.6	WD	700	0	36.30	9.41	227	3.86	0.0001	0.05
CO2*WL*H_rate	FC	700	27.6	WD	700	27.6	-11.60	3.51	227	-3.3	0.0011	0.05
CO2*WL*H_rate	FC	700	27.6	WD	700	46	-19.73	9.39	227	-2.1	0.0367	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	50.83	9.41	227	5.4	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	27.6	2.93	9.39	227	0.31	0.7551	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	-5.21	3.51	227	-1.48	0.1399	0.05
CO2*WL*H_rate	WD	700	0	WD	700	27.6	-47.90	9.05	227	-5.29	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	-56.03	9.05	227	-6.19	<.0001	0.05
CO2*WL*H_rate	WD	700	27.6	WD	700	46	-8.14	9.03	227	-0.9	0.3687	0.05

Table A6. Analysis of variance (ANOVA) for % of injury at 21 DAT for all four studied species.

Sources of Variance	Johnsongrass		Palmer		Weedy rice		Velvetleaf	
	F- value	P-value	F- value	P- value	F- value	P- value	F- value	P- value
CO ₂ Concentration	30.13	0.1147	2.75	0.3457	2.49	0.3594	202.49	0.0447
Water Level (WL)	0.01	0.9256	54.29	0.0859	13.90	0.0650	22.90	0.0410
CO ₂ x WL	3.24	0.0737	9.01	0.0031	1.61	0.2061	40.18	<.0001
Herbicide rate (H_rate)	426.22	0.0023	1426.69	0.0007	131.42	0.0002	1002.94	<.0001
CO ₂ x H_rate	39.43	<.0001	54.65	<.0001	12.44	<.0001	64.43	<.0001
WL x H_rate	21.87	<.0001	37.70	<.0001	26.43	<.0001	36.13	<.0001
CO ₂ x WL x H_rate	3.12	0.0499	3.23	0.0418	3.31	0.0385	7.68	0.0006

Table A7: Johnsongrass least square means of the interactions CO₂ x Herbicide rate and Water level x Herbicide rate for % of injury at 21 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	46	-26.17	3.29	166	-7.96	<.0001	0.05
CO2*H_rate	-	400	0	-	400	69	-56.96	3.31	166	-17.19	<.0001	0.05
CO2*H_rate	-	400	0	-	700	0	1.22	4.47	166	0.27	0.7846	0.05
CO2*H_rate	-	400	0	-	700	46	-66.54	4.46	166	-14.91	<.0001	0.05
CO2*H_rate	-	400	0	-	700	69	-75.84	4.46	166	-16.99	<.0001	0.05
CO2*H_rate	-	400	46	-	400	69	-30.78	3.25	166	-9.46	<.0001	0.05
CO2*H_rate	-	400	46	-	700	0	27.39	4.42	166	6.19	<.0001	0.05
CO2*H_rate	-	400	46	-	700	46	-40.37	4.42	166	-9.13	<.0001	0.05
CO2*H_rate	-	400	46	-	700	69	-49.67	4.42	166	-11.24	<.0001	0.05
CO2*H_rate	-	400	69	-	700	0	58.18	4.44	166	13.09	<.0001	0.05
CO2*H_rate	-	400	69	-	700	46	-9.59	4.44	166	-2.16	0.0323	0.05
CO2*H_rate	-	400	69	-	700	69	-18.89	4.44	166	-4.25	<.0001	0.05
CO2*H_rate	-	700	0	-	700	46	-67.77	3.34	166	-20.28	<.0001	0.05
CO2*H_rate	-	700	0	-	700	69	-77.07	3.34	166	-23.06	<.0001	0.05
CO2*H_rate	-	700	46	-	700	69	-9.30	3.33	166	-2.79	0.0059	0.05
WL*H_rate	FC	-	0	FC	-	46	-58.29	3.25	166	-17.91	<.0001	0.05
WL*H_rate	FC	-	0	FC	-	69	-81.94	3.28	166	-24.97	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	0	-18.12	5.91	166	-3.07	0.0025	0.05
WL*H_rate	FC	-	0	WD	-	46	-53.76	5.86	166	-9.17	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	69	-70.20	5.86	166	-11.98	<.0001	0.05
WL*H_rate	FC	-	46	FC	-	69	-23.65	3.31	166	-7.15	<.0001	0.05
WL*H_rate	FC	-	46	WD	-	0	40.17	5.93	166	6.78	<.0001	0.05

Table A7: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
WL*H_rate	FC	-	46	WD	-	46	4.53	5.88	166	0.77	0.4417	0.05
WL*H_rate	FC	-	46	WD	-	69	-11.90	5.88	166	-2.03	0.0443	0.05
WL*H_rate	FC	-	69	WD	-	0	63.82	5.94	166	10.74	<.0001	0.05
WL*H_rate	FC	-	69	WD	-	46	28.18	5.89	166	4.78	<.0001	0.05
WL*H_rate	FC	-	69	WD	-	69	11.74	5.89	166	1.99	0.0478	0.05
WL*H_rate	WD	-	0	WD	-	46	-35.64	3.37	166	-10.56	<.0001	0.05
WL*H_rate	WD	-	0	WD	-	69	-52.08	3.37	166	-15.43	<.0001	0.05
WL*H_rate	WD	-	46	WD	-	69	-16.44	3.28	166	-5.01	<.0001	0.05

Table A8: Weedy rice least square means of the interaction CO₂ x Water level x Herbicide rate for % of injury at 21 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	46	-64.21	6.16	206	-10.42	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	69	-82.08	6.16	206	-13.32	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-9.60	3.92	206	-2.45	0.0152	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	-62.28	6.16	206	-10.11	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	69	-63.87	6.21	206	-10.29	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	0.30	6.32	206	0.05	0.9627	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	-88.59	7.86	206	-11.27	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	69	-89.26	7.86	206	-11.36	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-7.63	6.30	206	-1.21	0.2274	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	-73.25	7.86	206	-9.32	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	69	-75.26	7.86	206	-9.57	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	400	69	-17.87	6.20	206	-2.88	0.0044	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	54.61	6.30	206	8.67	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	46	1.93	3.82	206	0.51	0.6134	0.05
CO2*WL*H_rate	FC	400	46	WD	400	69	0.35	6.24	206	0.06	0.9558	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	64.51	7.89	206	8.18	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	-24.38	6.36	206	-3.83	0.0002	0.05
CO2*WL*H_rate	FC	400	46	FC	700	69	-25.05	7.89	206	-3.17	0.0017	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	56.58	7.88	206	7.18	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	-9.03	6.36	206	-1.42	0.1571	0.05
CO2*WL*H_rate	FC	400	46	WD	700	69	-11.05	7.89	206	-1.4	0.1629	0.05
CO2*WL*H_rate	FC	400	69	WD	400	0	72.48	6.30	206	11.51	<.0001	0.05

Table A8: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	69	WD	400	46	19.80	6.20	206	3.19	0.0016	0.05
CO2*WL*H_rate	FC	400	69	WD	400	69	18.21	3.89	206	4.68	<.0001	0.05
CO2*WL*H_rate	FC	400	69	FC	700	0	82.37	7.89	206	10.44	<.0001	0.05
CO2*WL*H_rate	FC	400	69	FC	700	46	-6.52	7.89	206	-0.83	0.4098	0.05
CO2*WL*H_rate	FC	400	69	FC	700	69	-7.18	6.36	206	-1.13	0.26	0.05
CO2*WL*H_rate	FC	400	69	WD	700	0	74.45	7.88	206	9.45	<.0001	0.05
CO2*WL*H_rate	FC	400	69	WD	700	46	8.83	7.89	206	1.12	0.2643	0.05
CO2*WL*H_rate	FC	400	69	WD	700	69	6.82	6.36	206	1.07	0.2853	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	-52.68	6.30	206	-8.37	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	69	-54.27	6.34	206	-8.56	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	9.90	6.45	206	1.53	0.1268	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	-78.99	7.97	206	-9.91	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	69	-79.66	7.97	206	-10	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	1.97	6.44	206	0.31	0.7598	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	-63.65	7.97	206	-7.99	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	69	-65.66	7.97	206	-8.24	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	400	69	-1.59	6.24	206	-0.25	0.7997	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	62.58	7.89	206	7.93	<.0001	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	-26.32	6.36	206	-4.14	<.0001	0.05
CO2*WL*H_rate	WD	400	46	FC	700	69	-26.98	7.89	206	-3.42	0.0008	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	54.65	7.88	206	6.94	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	-10.97	6.36	206	-1.72	0.0862	0.05
CO2*WL*H_rate	WD	400	46	WD	700	69	-12.98	7.89	206	-1.65	0.1014	0.05

Table A8: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	69	FC	700	0	64.16	7.93	206	8.09	<.0001	0.05
CO2*WL*H_rate	WD	400	69	FC	700	46	-24.73	7.93	206	-3.12	0.0021	0.05
CO2*WL*H_rate	WD	400	69	FC	700	69	-25.40	6.41	206	-3.96	0.0001	0.05
CO2*WL*H_rate	WD	400	69	WD	700	0	56.24	7.91	206	7.11	<.0001	0.05
CO2*WL*H_rate	WD	400	69	WD	700	46	-9.38	7.93	206	-1.18	0.2379	0.05
CO2*WL*H_rate	WD	400	69	WD	700	69	-11.40	6.41	206	-1.78	0.0767	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	-88.89	5.61	206	-15.86	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	69	-89.56	5.61	206	-15.98	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-7.93	3.05	206	-2.6	0.0101	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	-73.54	5.61	206	-13.12	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	69	-75.56	5.61	206	-13.48	<.0001	0.05
CO2*WL*H_rate	FC	700	46	FC	700	69	-0.67	5.60	206	-0.12	0.9052	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	80.96	5.59	206	14.49	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	15.35	3.09	206	4.97	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	69	13.33	5.60	206	2.38	0.0183	0.05
CO2*WL*H_rate	FC	700	69	WD	700	0	81.63	5.59	206	14.61	<.0001	0.05
CO2*WL*H_rate	FC	700	69	WD	700	46	16.02	5.60	206	2.86	0.0047	0.05
CO2*WL*H_rate	FC	700	69	WD	700	69	14.00	3.09	206	4.54	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	-65.62	5.59	206	-11.74	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	69	-67.63	5.59	206	-12.11	<.0001	0.05
CO2*WL*H_rate	WD	700	46	WD	700	69	-2.02	5.60	206	-0.36	0.7194	0.05

Table A9: Palmer amaranth least square means of the interaction CO₂ x Water level x Herbicide rate for % of injury at 21 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	46	-8.88	0.18	174	-50.37	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	69	-9.59	0.18	174	-54.39	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-4.96	0.15	174	-33.77	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	-9.10	0.18	174	-51.02	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	69	-9.74	0.18	174	-55.25	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	0.00	0.30	174	0	1	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	-9.90	0.32	174	-31.18	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	69	-9.99	0.32	174	-31.46	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-3.46	0.30	174	-11.43	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	-9.93	0.32	174	-31.25	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	69	-9.97	0.32	174	-31.38	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	400	69	-0.71	0.18	174	-4.02	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	3.92	0.18	174	22.22	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	46	-0.22	0.15	174	-1.48	0.1418	0.05
CO2*WL*H_rate	FC	400	46	WD	400	69	-0.86	0.18	174	-4.88	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	8.88	0.32	174	27.94	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	-1.03	0.30	174	-3.39	0.0009	0.05
CO2*WL*H_rate	FC	400	46	FC	700	69	-1.12	0.32	174	-3.51	0.0006	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	5.42	0.32	174	17.06	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	-1.05	0.30	174	-3.48	0.0006	0.05
CO2*WL*H_rate	FC	400	46	WD	700	69	-1.09	0.32	174	-3.43	0.0007	0.05
CO2*WL*H_rate	FC	400	69	WD	400	0	4.63	0.18	174	26.24	<.0001	0.05
CO2*WL*H_rate	FC	400	69	WD	400	46	0.49	0.18	174	2.74	0.0068	0.05

Table A9: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	69	WD	400	69	-0.15	0.15	174	-1.03	0.3038	0.05
CO2*WL*H_rate	FC	400	69	FC	700	0	9.59	0.32	174	30.18	<.0001	0.05
CO2*WL*H_rate	FC	400	69	FC	700	46	-0.32	0.32	174	-1	0.3191	0.05
CO2*WL*H_rate	FC	400	69	FC	700	69	-0.41	0.30	174	-1.35	0.1796	0.05
CO2*WL*H_rate	FC	400	69	WD	700	0	6.13	0.32	174	19.3	<.0001	0.05
CO2*WL*H_rate	FC	400	69	WD	700	46	-0.34	0.32	174	-1.08	0.282	0.05
CO2*WL*H_rate	FC	400	69	WD	700	69	-0.38	0.30	174	-1.26	0.2087	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	-4.14	0.18	174	-23.2	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	69	-4.78	0.18	174	-27.1	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	4.96	0.30	174	16.41	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	-4.94	0.32	174	-15.56	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	69	-5.03	0.32	174	-15.84	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	1.50	0.30	174	4.98	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	-4.97	0.32	174	-15.64	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	69	-5.01	0.32	174	-15.76	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	400	69	-0.64	0.18	174	-3.59	0.0004	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	9.10	0.32	174	28.53	<.0001	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	-0.81	0.30	174	-2.66	0.0087	0.05
CO2*WL*H_rate	WD	400	46	FC	700	69	-0.90	0.32	174	-2.81	0.0055	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	5.64	0.32	174	17.69	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	-0.83	0.30	174	-2.74	0.0068	0.05
CO2*WL*H_rate	WD	400	46	WD	700	69	-0.87	0.32	174	-2.73	0.007	0.05
CO2*WL*H_rate	WD	400	69	FC	700	0	9.74	0.32	174	30.65	<.0001	0.05

Table A9: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	69	FC	700	46	-0.17	0.32	174	-0.52	0.6022	0.05
CO2*WL*H_rate	WD	400	69	FC	700	69	-0.26	0.30	174	-0.85	0.3986	0.05
CO2*WL*H_rate	WD	400	69	WD	700	0	6.28	0.32	174	19.77	<.0001	0.05
CO2*WL*H_rate	WD	400	69	WD	700	46	-0.19	0.32	174	-0.6	0.5478	0.05
CO2*WL*H_rate	WD	400	69	WD	700	69	-0.23	0.30	174	-0.76	0.4477	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	-9.90	0.18	174	-56.19	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	69	-9.99	0.18	174	-56.7	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-3.46	0.15	174	-23.53	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	-9.93	0.18	174	-56.33	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	69	-9.97	0.18	174	-56.55	<.0001	0.05
CO2*WL*H_rate	FC	700	46	FC	700	69	-0.09	0.18	174	-0.51	0.6103	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	6.45	0.18	174	36.58	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	-0.03	0.15	174	-0.17	0.863	0.05
CO2*WL*H_rate	FC	700	46	WD	700	69	-0.06	0.18	174	-0.36	0.7162	0.05
CO2*WL*H_rate	FC	700	69	WD	700	0	6.54	0.18	174	37.09	<.0001	0.05
CO2*WL*H_rate	FC	700	69	WD	700	46	0.06	0.18	174	0.37	0.7144	0.05
CO2*WL*H_rate	FC	700	69	WD	700	69	0.03	0.15	174	0.18	0.8608	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	-6.47	0.18	174	-36.72	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	69	-6.51	0.18	174	-36.94	<.0001	0.05
CO2*WL*H_rate	WD	700	46	WD	700	69	-0.04	0.18	174	-0.22	0.826	0.05

Table A10: Velvetleaf least square means of the interaction CO₂ x Water level x Herbicide rate for % of injury at 21 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	27.6	-98.52	2.52	214	-39.02	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	46	-99.99	2.52	214	-39.6	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-16.67	2.40	214	-6.96	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	27.6	-93.19	2.99	214	-31.2	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	-97.45	3.00	214	-32.45	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	1.33	2.05	214	0.65	0.5193	0.05
CO2*WL*H_rate	FC	400	0	FC	700	27.6	-65.24	2.74	214	-23.78	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	-78.82	2.74	214	-28.73	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-18.94	2.64	214	-7.19	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	27.6	-79.36	3.16	214	-25.11	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	-87.32	3.16	214	-27.62	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	FC	400	46	-1.47	2.54	214	-0.58	0.564	0.05
CO2*WL*H_rate	FC	400	27.6	WD	400	0	81.86	2.97	214	27.54	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	400	27.6	5.34	2.44	214	2.18	0.03	0.05
CO2*WL*H_rate	FC	400	27.6	WD	400	46	1.08	3.02	214	0.36	0.7216	0.05
CO2*WL*H_rate	FC	400	27.6	FC	700	0	99.85	2.71	214	36.89	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	FC	700	27.6	33.28	2.15	214	15.49	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	FC	700	46	19.70	2.76	214	7.14	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	700	0	79.58	3.17	214	25.13	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	700	27.6	19.17	2.66	214	7.21	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	700	46	11.21	3.17	214	3.53	0.0005	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	83.32	2.97	214	28.03	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	27.6	6.80	3.00	214	2.27	0.0243	0.05

Table A10: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	46	WD	400	46	2.54	2.46	214	1.03	0.3029	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	101.32	2.71	214	37.43	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	27.6	34.75	2.76	214	12.6	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	21.17	2.15	214	9.85	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	81.05	3.17	214	25.59	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	27.6	20.63	3.17	214	6.51	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	12.67	2.66	214	4.76	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	27.6	-76.52	2.54	214	-30.11	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	-80.78	2.56	214	-31.54	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	17.99	2.58	214	6.96	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	27.6	-48.57	3.16	214	-15.37	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	-62.16	3.16	214	-19.67	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	-2.27	2.12	214	-1.07	0.2843	0.05
CO2*WL*H_rate	WD	400	0	WD	700	27.6	-62.69	2.74	214	-22.85	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	-70.65	2.75	214	-25.73	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	WD	400	46	-4.26	2.59	214	-1.64	0.1015	0.05
CO2*WL*H_rate	WD	400	27.6	FC	700	0	94.51	3.14	214	30.13	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	FC	700	27.6	27.95	2.68	214	10.44	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	FC	700	46	14.36	3.18	214	4.51	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	WD	700	0	74.25	2.77	214	26.78	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	WD	700	27.6	13.83	2.18	214	6.34	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	WD	700	46	5.87	2.78	214	2.11	0.0359	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	98.77	3.15	214	31.34	<.0001	0.05

Table A10: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	46	FC	700	27.6	32.21	3.20	214	10.08	<.0001	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	18.62	2.70	214	6.89	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	78.51	2.79	214	28.11	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	27.6	18.09	2.80	214	6.46	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	10.13	2.22	214	4.56	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	27.6	-66.57	2.87	214	-23.21	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	-80.15	2.87	214	-27.94	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-20.27	2.74	214	-7.4	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	27.6	-80.68	3.33	214	-24.23	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	-88.64	3.33	214	-26.61	<.0001	0.05
CO2*WL*H_rate	FC	700	27.6	FC	700	46	-13.58	2.92	214	-4.66	<.0001	0.05
CO2*WL*H_rate	FC	700	27.6	WD	700	0	46.30	3.37	214	13.75	<.0001	0.05
CO2*WL*H_rate	FC	700	27.6	WD	700	27.6	-14.12	2.79	214	-5.06	<.0001	0.05
CO2*WL*H_rate	FC	700	27.6	WD	700	46	-22.08	3.37	214	-6.55	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	59.88	3.37	214	17.79	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	27.6	-0.53	3.37	214	-0.16	0.8743	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	-8.49	2.79	214	-3.05	0.0026	0.05
CO2*WL*H_rate	WD	700	0	WD	700	27.6	-60.42	2.91	214	-20.73	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	-68.38	2.92	214	-23.46	<.0001	0.05
CO2*WL*H_rate	WD	700	27.6	WD	700	46	-7.96	2.92	214	-2.73	0.0069	0.05

Table A11. Analysis of variance (ANOVA) for total biomass for all four studied species.

Sources of Variance	Johnsongrass		Palmer		Weedy rice		Velvetleaf	
	F- value	P- value	F- value	P- value	F- value	P- value	F- value	P- value
CO ₂ Concentration (CO ₂)	4.93	0.2693	22.20	0.1331	0.10	0.8011	13.82	0.1673
Water Level (WL)	0.03	0.8925	4.15	0.2905	2.74	0.2395	36.53	0.0263
CO ₂ x WL	1.54	0.2175	0.26	0.6108	1.27	0.2616	13.27	0.0004
Herbicide rate (H_rate)	152.04	0.0065	82.79	0.0119	23.09	0.0064	623.31	<.0001
CO ₂ x H_rate	22.91	<.0001	11.51	<.0001	37.64	<.0001	124.84	<.0001
WL x H_rate	5.31	0.0064	1.06	0.3506	0.95	0.3909	1.42	0.2460
CO ₂ x WL x H_rate	0.70	0.4995	0.79	0.4562	7.57	0.0008	5.23	0.0065

Table A12. Johnsongrass least square means of the interactions CO₂ x Herbicide rate and Water level x Herbicide for total biomass.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	46	767.35	54.91	103	13.97	<.0001	0.05
CO2*H_rate	-	400	0	-	400	69	909.40	55.80	103	16.3	<.0001	0.05
CO2*H_rate	-	400	0	-	700	0	376.69	71.54	103	5.27	<.0001	0.05
CO2*H_rate	-	400	0	-	700	46	840.23	78.15	103	10.75	<.0001	0.05
CO2*H_rate	-	400	0	-	700	69	864.29	78.15	103	11.06	<.0001	0.05
CO2*H_rate	-	400	46	-	400	69	142.05	52.69	103	2.7	0.0082	0.05
CO2*H_rate	-	400	46	-	700	0	-390.65	75.46	103	-5.18	<.0001	0.05
CO2*H_rate	-	400	46	-	700	46	72.89	70.10	103	1.04	0.3009	0.05
CO2*H_rate	-	400	46	-	700	69	96.94	76.25	103	1.27	0.2065	0.05
CO2*H_rate	-	400	69	-	700	0	-532.70	76.11	103	-7	<.0001	0.05
CO2*H_rate	-	400	69	-	700	46	-69.16	76.90	103	-0.9	0.3705	0.05
CO2*H_rate	-	400	69	-	700	69	-45.11	70.77	103	-0.64	0.5253	0.05
CO2*H_rate	-	700	0	-	700	46	463.54	52.56	103	8.82	<.0001	0.05
CO2*H_rate	-	700	0	-	700	69	487.59	52.57	103	9.28	<.0001	0.05
CO2*H_rate	-	700	46	-	700	69	24.06	53.54	103	0.45	0.6542	0.05
WL*H_rate	FC	-	0	FC	-	46	701.44	54.23	103	12.93	<.0001	0.05
WL*H_rate	FC	-	0	FC	-	69	792.61	54.59	103	14.52	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	0	124.47	45.90	103	2.71	0.0078	0.05
WL*H_rate	FC	-	0	WD	-	46	653.91	53.17	103	12.3	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	69	728.84	53.70	103	13.57	<.0001	0.05
WL*H_rate	FC	-	46	FC	-	69	91.17	54.11	103	1.68	0.0951	0.05
WL*H_rate	FC	-	46	WD	-	0	-576.98	54.23	103	-10.64	<.0001	0.05

Table A12: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t 	Alpha
WL*H_rate	FC	-	46	WD	-	46	-47.53	43.35	103	-1.1	0.2755	0.05
WL*H_rate	FC	-	46	WD	-	69	27.40	53.22	103	0.51	0.6078	0.05
WL*H_rate	FC	-	69	WD	-	0	-668.15	54.59	103	-12.24	<.0001	0.05
WL*H_rate	FC	-	69	WD	-	46	-138.70	53.10	103	-2.61	0.0103	0.05
WL*H_rate	FC	-	69	WD	-	69	-63.77	44.37	103	-1.44	0.1537	0.05
WL*H_rate	WD	-	0	WD	-	46	529.44	53.17	103	9.96	<.0001	0.05
WL*H_rate	WD	-	0	WD	-	69	604.38	53.70	103	11.25	<.0001	0.05
WL*H_rate	WD	-	46	WD	-	69	74.93	52.12	103	1.44	0.1535	0.05

Table A13. Weedy rice least square means of the interaction CO₂ x Water level x Herbicide rate for total biomass.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	46	1.30	0.22	141	5.84	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	69	1.61	0.22	141	7.17	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-0.18	0.12	141	-1.47	0.1434	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	1.36	0.23	141	5.91	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	69	1.54	0.23	141	6.69	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	0.20	0.55	141	0.36	0.7225	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	1.26	0.59	141	2.15	0.0329	0.05
CO2*WL*H_rate	FC	400	0	FC	700	69	1.05	0.59	141	1.79	0.0759	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	0.21	0.56	141	0.38	0.7042	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	0.86	0.59	141	1.46	0.1471	0.05
CO2*WL*H_rate	FC	400	0	WD	700	69	0.99	0.59	141	1.68	0.0944	0.05
CO2*WL*H_rate	FC	400	46	FC	400	69	0.31	0.22	141	1.4	0.1636	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	-1.48	0.23	141	-6.41	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	46	0.06	0.11	141	0.55	0.5864	0.05
CO2*WL*H_rate	FC	400	46	WD	400	69	0.24	0.23	141	1.05	0.2976	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	-1.10	0.59	141	-1.88	0.0618	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	-0.04	0.55	141	-0.07	0.9475	0.05
CO2*WL*H_rate	FC	400	46	FC	700	69	-0.25	0.59	141	-0.43	0.6688	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	-1.09	0.59	141	-1.85	0.0661	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	-0.44	0.55	141	-0.8	0.4267	0.05
CO2*WL*H_rate	FC	400	46	WD	700	69	-0.31	0.59	141	-0.52	0.6007	0.05

Table A13: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	69	WD	400	0	-1.79	0.23	141	-7.69	<.0001	0.05
CO2*WL*H_rate	FC	400	69	WD	400	46	-0.25	0.23	141	-1.1	0.2731	0.05
CO2*WL*H_rate	FC	400	69	WD	400	69	-0.07	0.11	141	-0.66	0.5129	0.05
CO2*WL*H_rate	FC	400	69	FC	700	0	-1.41	0.59	141	-2.41	0.0173	0.05
CO2*WL*H_rate	FC	400	69	FC	700	46	-0.35	0.59	141	-0.59	0.5558	0.05
CO2*WL*H_rate	FC	400	69	FC	700	69	-0.56	0.55	141	-1.02	0.3118	0.05
CO2*WL*H_rate	FC	400	69	WD	700	0	-1.40	0.59	141	-2.38	0.0189	0.05
CO2*WL*H_rate	FC	400	69	WD	700	46	-0.75	0.59	141	-1.28	0.2038	0.05
CO2*WL*H_rate	FC	400	69	WD	700	69	-0.62	0.56	141	-1.11	0.2672	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	1.54	0.22	141	6.88	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	69	1.71	0.22	141	7.68	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	0.37	0.56	141	0.67	0.5034	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	1.44	0.59	141	2.44	0.0158	0.05
CO2*WL*H_rate	WD	400	0	FC	700	69	1.23	0.59	141	2.08	0.0393	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	0.39	0.55	141	0.7	0.4848	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	1.04	0.59	141	1.76	0.0801	0.05
CO2*WL*H_rate	WD	400	0	WD	700	69	1.17	0.59	141	1.99	0.0485	0.05
CO2*WL*H_rate	WD	400	46	WD	400	69	0.18	0.22	141	0.81	0.4167	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	-1.16	0.59	141	-1.97	0.0503	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	-0.09	0.55	141	-0.17	0.8644	0.05
CO2*WL*H_rate	WD	400	46	FC	700	69	-0.31	0.59	141	-0.53	0.5995	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	-1.15	0.59	141	-1.96	0.0521	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	-0.50	0.55	141	-0.91	0.3663	0.05

Table A13: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	46	WD	700	69	-0.37	0.59	141	-0.63	0.5321	0.05
CO2*WL*H_rate	WD	400	69	FC	700	0	-1.34	0.59	141	-2.28	0.0242	0.05
CO2*WL*H_rate	WD	400	69	FC	700	46	-0.27	0.59	141	-0.47	0.6426	0.05
CO2*WL*H_rate	WD	400	69	FC	700	69	-0.49	0.55	141	-0.88	0.38	0.05
CO2*WL*H_rate	WD	400	69	WD	700	0	-1.33	0.59	141	-2.26	0.0251	0.05
CO2*WL*H_rate	WD	400	69	WD	700	46	-0.68	0.59	141	-1.16	0.2484	0.05
CO2*WL*H_rate	WD	400	69	WD	700	69	-0.55	0.55	141	-0.99	0.3246	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	1.07	0.21	141	5.03	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	69	0.85	0.21	141	4.02	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	0.01	0.10	141	0.15	0.882	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	0.66	0.22	141	3.03	0.0029	0.05
CO2*WL*H_rate	FC	700	0	WD	700	69	0.80	0.22	141	3.64	0.0004	0.05
CO2*WL*H_rate	FC	700	46	FC	700	69	-0.21	0.21	141	-1.02	0.3099	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	-1.05	0.22	141	-4.84	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	-0.41	0.09	141	-4.4	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	69	-0.27	0.22	141	-1.25	0.2121	0.05
CO2*WL*H_rate	FC	700	69	WD	700	0	-0.84	0.22	141	-3.85	0.0002	0.05
CO2*WL*H_rate	FC	700	69	WD	700	46	-0.19	0.22	141	-0.88	0.3811	0.05
CO2*WL*H_rate	FC	700	69	WD	700	69	-0.06	0.09	141	-0.62	0.5346	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	0.65	0.21	141	3.07	0.0026	0.05
CO2*WL*H_rate	WD	700	0	WD	700	69	0.78	0.21	141	3.7	0.0003	0.05
CO2*WL*H_rate	WD	700	46	WD	700	69	0.13	0.21	141	0.63	0.5278	0.05

Table A14. Palmer amaranth least square means of the interaction CO₂ x Herbicide rate for total biomass.

Differences of Least Squares Means												
Effect	CO2	H_rate	CO2	H_rate	Estimate	Error	DF	tValue	Pr> t	Alpha	Lower	Upper
CO2*H_rate	400	0	400	46	14.36	1.45	94	9.92	<.0001	0.05	11.48	17.23
CO2*H_rate	400	0	400	69	18.38	1.48	94	12.42	<.0001	0.05	15.44	21.32
CO2*H_rate	400	0	700	0	0.78	0.94	94	0.84	0.4056	0.05	-1.08	2.64
CO2*H_rate	400	0	700	46	11.47	1.44	94	7.94	<.0001	0.05	8.60	14.34
CO2*H_rate	400	0	700	69	12.63	1.47	94	8.62	<.0001	0.05	9.72	15.54
CO2*H_rate	400	46	400	69	4.02	1.46	94	2.76	0.0069	0.05	1.13	6.91
CO2*H_rate	400	46	700	0	-13.57	1.38	94	-9.82	<.0001	0.05	-16.3	-10.8
CO2*H_rate	400	46	700	46	-2.89	0.95	94	-3.03	0.0031	0.05	-4.78	-1.00
CO2*H_rate	400	46	700	69	-1.73	1.44	94	-1.2	0.2342	0.05	-4.59	1.14
CO2*H_rate	400	69	700	0	-17.60	1.42	94	-12.43	<.0001	0.05	-20.4	-14.8
CO2*H_rate	400	69	700	46	-6.91	1.45	94	-4.77	<.0001	0.05	-9.79	-4.03
CO2*H_rate	400	69	700	69	-5.75	1.00	94	-5.76	<.0001	0.05	-7.73	-3.77
CO2*H_rate	700	0	700	46	10.69	1.38	94	7.75	<.0001	0.05	7.95	13.42
CO2*H_rate	700	0	700	69	11.85	1.40	94	8.46	<.0001	0.05	9.07	14.63
CO2*H_rate	700	46	700	69	1.16	1.44	94	0.81	0.42	0.05	-1.69	4.01

Table A15. Velvetleaf least square means of the interaction CO₂ x Herbicide rate for total biomass.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	27.6	2.11	0.08	133	26.67	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	46	2.28	0.08	133	28.56	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	0.30	0.08	133	3.95	0.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	27.6	2.09	0.08	133	25.41	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	2.30	0.08	133	28.31	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	0.40	0.11	133	3.69	0.0003	0.05
CO2*WL*H_rate	FC	400	0	FC	700	27.6	1.33	0.11	133	11.82	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	1.43	0.11	133	12.72	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	0.65	0.11	133	5.92	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	27.6	1.67	0.11	133	14.61	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	1.79	0.11	133	15.88	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	FC	400	46	0.17	0.07	133	2.35	0.0205	0.05
CO2*WL*H_rate	FC	400	27.6	WD	400	0	-1.81	0.08	133	-23.09	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	400	27.6	-0.01	0.07	133	-0.2	0.841	0.05
CO2*WL*H_rate	FC	400	27.6	WD	400	46	0.20	0.07	133	2.61	0.01	0.05
CO2*WL*H_rate	FC	400	27.6	FC	700	0	-1.71	0.11	133	-15.8	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	FC	700	27.6	-0.77	0.10	133	-7.58	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	FC	700	46	-0.67	0.11	133	-6.23	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	700	0	-1.46	0.11	133	-13.28	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	700	27.6	-0.44	0.10	133	-4.2	<.0001	0.05
CO2*WL*H_rate	FC	400	27.6	WD	700	46	-0.32	0.11	133	-2.92	0.0042	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	-1.98	0.08	133	-25.02	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	400	27.6	-0.19	0.08	133	-2.41	0.0173	0.05

Table A15: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	46	WD	400	46	0.02	0.07	133	0.35	0.7234	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	-1.88	0.11	133	-17.28	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	27.6	-0.95	0.11	133	-8.7	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	-0.85	0.10	133	-8.2	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	-1.63	0.11	133	-14.76	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	27.6	-0.61	0.11	133	-5.52	<.0001	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	-0.49	0.10	133	-4.72	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	27.6	1.79	0.08	133	23.32	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	2.00	0.08	133	26.43	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	0.10	0.11	133	0.9	0.3724	0.05
CO2*WL*H_rate	WD	400	0	FC	700	27.6	1.03	0.11	133	9.21	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	1.13	0.11	133	10.11	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	0.34	0.10	133	3.29	0.0013	0.05
CO2*WL*H_rate	WD	400	0	WD	700	27.6	1.37	0.11	133	12.41	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	1.49	0.11	133	13.71	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	WD	400	46	0.21	0.07	133	2.85	0.0051	0.05
CO2*WL*H_rate	WD	400	27.6	FC	700	0	-1.70	0.11	133	-15.31	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	FC	700	27.6	-0.76	0.11	133	-7.24	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	FC	700	46	-0.66	0.11	133	-5.96	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	WD	700	0	-1.45	0.11	133	-13.26	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	WD	700	27.6	-0.42	0.10	133	-4.1	<.0001	0.05
CO2*WL*H_rate	WD	400	27.6	WD	700	46	-0.30	0.11	133	-2.81	0.0057	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	-1.91	0.11	133	-17.33	<.0001	0.05

Table A15: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	46	FC	700	27.6	-0.97	0.11	133	-8.84	<.0001	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	-0.87	0.10	133	-8.35	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	-1.66	0.11	133	-15.31	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	27.6	-0.63	0.11	133	-5.86	<.0001	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	-0.51	0.10	133	-5.08	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	27.6	0.94	0.08	133	11.5	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	1.04	0.08	133	12.74	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	0.25	0.07	133	3.36	0.001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	27.6	1.27	0.08	133	15.05	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	1.39	0.08	133	16.9	<.0001	0.05
CO2*WL*H_rate	FC	700	27.6	FC	700	46	0.10	0.08	133	1.25	0.2137	0.05
CO2*WL*H_rate	FC	700	27.6	WD	700	0	-0.69	0.08	133	-8.11	<.0001	0.05
CO2*WL*H_rate	FC	700	27.6	WD	700	27.6	0.34	0.07	133	4.54	<.0001	0.05
CO2*WL*H_rate	FC	700	27.6	WD	700	46	0.46	0.08	133	5.57	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	-0.79	0.08	133	-9.31	<.0001	0.05
CO2*WL*H_rate	FC	700	46	WD	700	27.6	0.24	0.08	133	2.79	0.006	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	0.36	0.07	133	4.97	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	27.6	1.02	0.08	133	12.57	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	1.15	0.08	133	14.45	<.0001	0.05
CO2*WL*H_rate	WD	700	27.6	WD	700	46	0.12	0.08	133	1.54	0.1251	0.05

Table A16. Analysis of variance (ANOVA) for root/shoot ratio for all four studied species

Sources of Variance	Johnsongrass		Palmer		Weedy rice		Velvetleaf	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
CO ₂ Concentration(CO ₂)	1.68	0.4185	0.02	0.9185	0.03	0.8902	0.80	0.5352
Water Level (WL)	2.60	0.3537	1.42	0.4446	0.01	0.9399	5.15	0.1513
CO ₂ x WL	0.06	0.8112	0.05	0.8184	0.30	0.5866	1.74	0.1893
Herbicide rate (H _{rate})	0.59	0.6270	3.14	0.2415	0.39	0.6989	5.43	0.0724
CO ₂ x H _{rate}	3.11	0.0493	6.73	0.0019	0.49	0.6109	0.03	0.9685
WL x H _{rate}	0.84	0.4364	1.03	0.3597	1.79	0.1701	0.46	0.6312
CO ₂ x WL x H _{rate}	0.99	0.3762	0.12	0.8874	1.12	0.3288	2.47	0.0882

Table A17. Palmer amaranth least square means of the interaction CO₂ x Herbicide rate for root/shoot ratio.

Differences of Least Squares Means												
Effect	CO2	H_rate	CO2	H_rate	Estimate	Error	DF	tValue	Pr> t	Alpha	Lower	Upper
CO2*H_rate	400	0	400	46	-0.2437	0.0935	89	-2.61	0.011	0.05	-0.43	-0.06
CO2*H_rate	400	0	400	69	-0.3669	0.09959	89	-3.68	0.000	0.05	-0.56	-0.17
CO2*H_rate	400	0	700	0	-0.205	0.06387	89	-3.21	0.002	0.05	-0.33	-0.08
CO2*H_rate	400	0	700	46	-0.177	0.0924	89	-1.92	0.059	0.05	-0.36	0.01
CO2*H_rate	400	0	700	69	-0.2443	0.09393	89	-2.6	0.011	0.05	-0.43	-0.06
CO2*H_rate	400	46	400	69	-0.1232	0.1002	89	-1.23	0.222	0.05	-0.32	0.08
CO2*H_rate	400	46	700	0	0.03877	0.09069	89	0.43	0.670	0.05	-0.14	0.22
CO2*H_rate	400	46	700	46	0.06679	0.06784	89	0.98	0.328	0.05	-0.07	0.20
CO2*H_rate	400	46	700	69	-0.00052	0.09455	89	-0.01	0.996	0.05	-0.19	0.19
CO2*H_rate	400	69	700	0	0.1619	0.09696	89	1.67	0.098	0.05	-0.03	0.35
CO2*H_rate	400	69	700	46	0.1899	0.09915	89	1.92	0.059	0.05	-0.01	0.39
CO2*H_rate	400	69	700	69	0.1226	0.07691	89	1.59	0.114	0.05	-0.03	0.28
CO2*H_rate	700	0	700	46	0.02802	0.08956	89	0.31	0.755	0.05	-0.15	0.21
CO2*H_rate	700	0	700	69	-0.03929	0.09114	89	-0.43	0.667	0.05	-0.22	0.14
CO2*H_rate	700	46	700	69	-0.06731	0.09346	89	-0.72	0.473	0.05	-0.25	0.12

Table A18. Analysis of variance (ANOVA) for CO₂ Assimilation rate (A) for all four studied species.

Sources of Variance	Johnsongrass		Palmer		Weedy rice		Velvetleaf	
	F-value	P-value	F-value	P-value	F-value	P-value	F-value	P-value
CO ₂ Concentration(CO ₂)	4.06	0.2931	0.00	0.9748	10.89	0.1873	0.31	0.6753
Water Level (WL)	1.76	0.4116	0.00	0.9845	0.21	0.7281	0.01	0.9468
CO ₂ x WL	0.38	0.5403	0.84	0.3617	0.02	0.8951	0.03	0.8570
Herbicide rate (H_rate)	2.65	0.2740	7.40	0.1191	5.30	0.2937	48.45	0.0202
CO ₂ x H_rate	0.02	0.9775	0.31	0.7375	0.20	0.8232	28.09	<.0001
WL x H_rate	5.76	0.0044	0.45	0.6407	5.93	0.0050	1.32	0.2729
CO ₂ x WL x H_rate	0.32	0.7265	2.18	0.1190	4.13	0.0220	2.91	0.0602

Table A19. Johnsongrass least square means for CO₂ assimilation rate.

Differences of Least Squares Means														
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha	Lower	Upper
WL*H_rate	FC	-	0	FC	-	46	1.12	0.40	91	2.78	0.007	0.05	0.32	1.92
WL*H_rate	FC	-	0	FC	-	69	1.27	0.41	91	3.12	0.002	0.05	0.46	2.07
WL*H_rate	FC	-	0	WD	-	0	0.77	0.23	91	3.4	0.001	0.05	0.32	1.23
WL*H_rate	FC	-	0	WD	-	46	0.92	0.41	91	2.27	0.026	0.05	0.11	1.73
WL*H_rate	FC	-	0	WD	-	69	1.18	0.40	91	2.96	0.004	0.05	0.39	1.97
WL*H_rate	FC	-	46	FC	-	69	0.15	0.41	91	0.37	0.715	0.05	-0.66	0.95
WL*H_rate	FC	-	46	WD	-	0	-0.34	0.41	91	-0.83	0.407	0.05	-1.17	0.48
WL*H_rate	FC	-	46	WD	-	46	-0.20	0.21	91	-0.92	0.359	0.05	-0.62	0.23
WL*H_rate	FC	-	46	WD	-	69	0.06	0.40	91	0.15	0.880	0.05	-0.73	0.85
WL*H_rate	FC	-	69	WD	-	0	-0.49	0.42	91	-1.18	0.240	0.05	-1.32	0.33
WL*H_rate	FC	-	69	WD	-	46	-0.34	0.41	91	-0.84	0.403	0.05	-1.16	0.47
WL*H_rate	FC	-	69	WD	-	69	-0.09	0.20	91	-0.43	0.665	0.05	-0.49	0.31
WL*H_rate	WD	-	0	WD	-	46	0.15	0.42	91	0.36	0.722	0.05	-0.68	0.98
WL*H_rate	WD	-	0	WD	-	69	0.40	0.41	91	0.99	0.326	0.05	-0.41	1.22
WL*H_rate	WD	-	46	WD	-	69	0.26	0.40	91	0.64	0.527	0.05	-0.54	1.06

Table A20. Weedy rice least square means for CO₂ assimilation rate.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	46	0.653	0.183	48	3.57	0.001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	69	0.718	0.164	48	4.37	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	0.323	0.188	48	1.72	0.093	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	0.417	0.249	48	1.68	0.100	0.05
CO2*WL*H_rate	FC	400	0	WD	400	69	0.408	0.237	48	1.73	0.091	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	0.008	0.085	48	0.1	0.921	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	0.452	0.190	48	2.38	0.021	0.05
CO2*WL*H_rate	FC	400	0	FC	700	69	0.459	0.171	48	2.68	0.010	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	0.000	0.190	48	0	0.999	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	0.224	0.249	48	0.9	0.371	0.05
CO2*WL*H_rate	FC	400	0	WD	700	69	0.434	0.242	48	1.79	0.079	0.05
CO2*WL*H_rate	FC	400	46	FC	400	69	0.064	0.197	48	0.33	0.745	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	-0.331	0.247	48	-1.34	0.187	0.05
CO2*WL*H_rate	FC	400	46	WD	400	46	-0.236	0.215	48	-1.1	0.278	0.05
CO2*WL*H_rate	FC	400	46	WD	400	69	-0.245	0.260	48	-0.94	0.351	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	-0.645	0.182	48	-3.54	0.001	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	-0.201	0.134	48	-1.51	0.138	0.05
CO2*WL*H_rate	FC	400	46	FC	700	69	-0.195	0.196	48	-1	0.324	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	-0.653	0.249	48	-2.63	0.012	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	-0.429	0.215	48	-1.99	0.052	0.05
CO2*WL*H_rate	FC	400	46	WD	700	69	-0.219	0.265	48	-0.83	0.412	0.05
CO2*WL*H_rate	FC	400	69	WD	400	0	-0.395	0.234	48	-1.69	0.098	0.05
CO2*WL*H_rate	FC	400	69	WD	400	46	-0.300	0.260	48	-1.15	0.254	0.05

Table A20: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	69	WD	400	69	-0.309	0.218	48	-1.42	0.163	0.05
CO2*WL*H_rate	FC	400	69	FC	700	0	-0.709	0.163	48	-4.35	<.0001	0.05
CO2*WL*H_rate	FC	400	69	FC	700	46	-0.266	0.204	48	-1.31	0.198	0.05
CO2*WL*H_rate	FC	400	69	FC	700	69	-0.259	0.142	48	-1.82	0.075	0.05
CO2*WL*H_rate	FC	400	69	WD	700	0	-0.717	0.235	48	-3.05	0.004	0.05
CO2*WL*H_rate	FC	400	69	WD	700	46	-0.494	0.260	48	-1.9	0.064	0.05
CO2*WL*H_rate	FC	400	69	WD	700	69	-0.284	0.224	48	-1.27	0.211	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	0.095	0.185	48	0.51	0.610	0.05
CO2*WL*H_rate	WD	400	0	WD	400	69	0.086	0.168	48	0.51	0.612	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	-0.314	0.187	48	-1.68	0.099	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	0.129	0.252	48	0.51	0.611	0.05
CO2*WL*H_rate	WD	400	0	FC	700	69	0.136	0.239	48	0.57	0.572	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	-0.322	0.091	48	-3.55	0.001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	-0.098	0.185	48	-0.53	0.597	0.05
CO2*WL*H_rate	WD	400	0	WD	700	69	0.111	0.176	48	0.63	0.529	0.05
CO2*WL*H_rate	WD	400	46	WD	400	69	-0.009	0.189	48	-0.05	0.962	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	-0.409	0.248	48	-1.65	0.105	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	0.035	0.221	48	0.16	0.876	0.05
CO2*WL*H_rate	WD	400	46	FC	700	69	0.041	0.261	48	0.16	0.875	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	-0.417	0.185	48	-2.25	0.029	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	-0.193	0.124	48	-1.56	0.125	0.05
CO2*WL*H_rate	WD	400	46	WD	700	69	0.017	0.196	48	0.08	0.933	0.05
CO2*WL*H_rate	WD	400	69	FC	700	0	-0.400	0.235	48	-1.7	0.096	0.05

Table A20: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	69	FC	700	46	0.044	0.265	48	0.16	0.870	0.05
CO2*WL*H_rate	WD	400	69	FC	700	69	0.050	0.209	48	0.24	0.811	0.05
CO2*WL*H_rate	WD	400	69	WD	700	0	-0.408	0.169	48	-2.42	0.019	0.05
CO2*WL*H_rate	WD	400	69	WD	700	46	-0.184	0.189	48	-0.97	0.335	0.05
CO2*WL*H_rate	WD	400	69	WD	700	69	0.026	0.113	48	0.23	0.822	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	0.444	0.189	48	2.34	0.023	0.05
CO2*WL*H_rate	FC	700	0	FC	700	69	0.450	0.170	48	2.64	0.011	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-0.008	0.189	48	-0.04	0.966	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	0.216	0.248	48	0.87	0.388	0.05
CO2*WL*H_rate	FC	700	0	WD	700	69	0.425	0.241	48	1.77	0.084	0.05
CO2*WL*H_rate	FC	700	46	FC	700	69	0.007	0.202	48	0.03	0.974	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	-0.452	0.254	48	-1.78	0.081	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	-0.228	0.221	48	-1.03	0.308	0.05
CO2*WL*H_rate	FC	700	46	WD	700	69	-0.018	0.270	48	-0.07	0.947	0.05
CO2*WL*H_rate	FC	700	69	WD	700	0	-0.458	0.240	48	-1.91	0.062	0.05
CO2*WL*H_rate	FC	700	69	WD	700	46	-0.234	0.261	48	-0.9	0.374	0.05
CO2*WL*H_rate	FC	700	69	WD	700	69	-0.025	0.215	48	-0.11	0.909	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	0.224	0.185	48	1.21	0.232	0.05
CO2*WL*H_rate	WD	700	0	WD	700	69	0.434	0.176	48	2.46	0.017	0.05
CO2*WL*H_rate	WD	700	46	WD	700	69	0.210	0.196	48	1.07	0.289	0.05

Table A21. Velvetleaf least square means for CO₂ assimilation rate.

Differences of Least Squares Means												
Effect	CO2	H_rate	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha	Lower	Upper
CO2*H_rate	400	0	400	27.6	1.155	0.197	83	5.87	<.0001	0.05	0.76	1.55
CO2*H_rate	400	0	400	46	0.695	0.197	83	3.52	0.0007	0.05	0.30	1.09
CO2*H_rate	400	0	700	0	-0.558	0.345	83	-1.62	0.11	0.05	-1.24	0.13
CO2*H_rate	400	0	700	27.6	1.460	0.373	83	3.91	0.0002	0.05	0.72	2.20
CO2*H_rate	400	0	700	46	1.496	0.373	83	4.01	0.0001	0.05	0.75	2.24
CO2*H_rate	400	27.6	400	46	-0.460	0.194	83	-2.37	0.0199	0.05	-0.85	-0.07
CO2*H_rate	400	27.6	700	0	-1.713	0.374	83	-4.58	<.0001	0.05	-2.46	-0.97
CO2*H_rate	400	27.6	700	27.6	0.305	0.340	83	0.89	0.3734	0.05	-0.37	0.98
CO2*H_rate	400	27.6	700	46	0.340	0.371	83	0.92	0.3625	0.05	-0.40	1.08
CO2*H_rate	400	46	700	0	-1.253	0.374	83	-3.35	0.0012	0.05	-2.00	-0.51
CO2*H_rate	400	46	700	27.6	0.765	0.372	83	2.06	0.0427	0.05	0.03	1.50
CO2*H_rate	400	46	700	46	0.800	0.341	83	2.35	0.0212	0.05	0.12	1.48
CO2*H_rate	700	0	700	27.6	2.018	0.196	83	10.29	<.0001	0.05	1.63	2.41
CO2*H_rate	700	0	700	46	2.053	0.196	83	10.47	<.0001	0.05	1.66	2.44
CO2*H_rate	700	27.6	700	46	0.035	0.191	83	0.19	0.8533	0.05	-0.34	0.42

Table A22. Analysis of variance (ANOVA) for Stomatal Conductance (g_s) for all four studied species.

Sources of Variance	Johnsongrass		Palmer		Weedy rice		Velvetleaf	
	F- value	P- value	F- value	P- value	F- value	P- value	F- value	P- value
CO ₂ Concentration	2.74	0.3457	0.61	0.5790	0.14	0.7750	0.51	0.6058
Water Level (WL)	1.19	0.4727	0.00	0.9939	5.62	0.2541	0.27	0.6939
CO ₂ x WL	2.35	0.1286	3.56	0.0623	0.75	0.3891	7.23	0.0086
Herbicide rate	6.17	0.1395	3.43	0.2259	0.91	0.5223	3.06	0.2465
CO ₂ x H_rate	0.84	0.4341	6.00	0.0036	4.76	0.0112	1.60	0.2079
WL x H_rate	2.98	0.0558	0.40	0.6687	1.38	0.2589	0.54	0.5851
CO ₂ x WL x H_rate	3.51	0.0339	3.30	0.0413	1.75	0.1797	0.19	0.8257

Table A23. Johnsongrass least square means for g_s

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	46	0.148	0.035	90	4.2	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	69	0.127	0.039	90	3.27	0.0015	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	0.098	0.039	90	2.52	0.0134	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	0.074	0.039	90	1.9	0.0601	0.05
CO2*WL*H_rate	FC	400	0	WD	400	69	0.109	0.039	90	2.82	0.0059	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	0.105	0.048	90	2.19	0.0314	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	0.131	0.051	90	2.58	0.0116	0.05
CO2*WL*H_rate	FC	400	0	FC	700	69	0.158	0.049	90	3.19	0.002	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	0.137	0.056	90	2.45	0.0164	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	0.163	0.055	90	2.95	0.004	0.05
CO2*WL*H_rate	FC	400	0	WD	700	69	0.229	0.052	90	4.38	<.0001	0.05
CO2*WL*H_rate	FC	400	46	FC	400	69	-0.021	0.035	90	-0.6	0.5533	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	-0.050	0.037	90	-1.36	0.1758	0.05
CO2*WL*H_rate	FC	400	46	WD	400	46	-0.074	0.034	90	-2.17	0.0324	0.05
CO2*WL*H_rate	FC	400	46	WD	400	69	-0.040	0.035	90	-1.13	0.2631	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	-0.044	0.047	90	-0.93	0.3524	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	-0.018	0.047	90	-0.38	0.7064	0.05
CO2*WL*H_rate	FC	400	46	FC	700	69	0.009	0.047	90	0.2	0.8431	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	-0.011	0.055	90	-0.2	0.8416	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	0.015	0.052	90	0.29	0.7758	0.05
CO2*WL*H_rate	FC	400	46	WD	700	69	0.080	0.050	90	1.61	0.1102	0.05
CO2*WL*H_rate	FC	400	69	WD	400	0	-0.029	0.040	90	-0.73	0.4695	0.05
CO2*WL*H_rate	FC	400	69	WD	400	46	-0.053	0.039	90	-1.35	0.1813	0.05

Table A23: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	69	WD	400	69	-0.019	0.038	90	-0.5	0.6215	0.05
CO2*WL*H_rate	FC	400	69	FC	700	0	-0.022	0.049	90	-0.45	0.6508	0.05
CO2*WL*H_rate	FC	400	69	FC	700	46	0.003	0.051	90	0.07	0.9482	0.05
CO2*WL*H_rate	FC	400	69	FC	700	69	0.030	0.049	90	0.63	0.533	0.05
CO2*WL*H_rate	FC	400	69	WD	700	0	0.010	0.057	90	0.18	0.8598	0.05
CO2*WL*H_rate	FC	400	69	WD	700	46	0.036	0.055	90	0.65	0.518	0.05
CO2*WL*H_rate	FC	400	69	WD	700	69	0.102	0.051	90	1.98	0.0513	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	-0.024	0.033	90	-0.71	0.4795	0.05
CO2*WL*H_rate	WD	400	0	WD	400	69	0.011	0.033	90	0.33	0.7414	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	0.007	0.049	90	0.14	0.8875	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	0.033	0.052	90	0.63	0.5294	0.05
CO2*WL*H_rate	WD	400	0	FC	700	69	0.060	0.051	90	1.18	0.2405	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	0.040	0.052	90	0.75	0.4522	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	0.065	0.051	90	1.27	0.2064	0.05
CO2*WL*H_rate	WD	400	0	WD	700	69	0.131	0.048	90	2.73	0.0076	0.05
CO2*WL*H_rate	WD	400	46	WD	400	69	0.034	0.031	90	1.1	0.2755	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	0.030	0.049	90	0.62	0.5389	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	0.056	0.050	90	1.13	0.2623	0.05
CO2*WL*H_rate	WD	400	46	FC	700	69	0.083	0.050	90	1.68	0.0973	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	0.063	0.053	90	1.2	0.2333	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	0.089	0.049	90	1.8	0.0758	0.05
CO2*WL*H_rate	WD	400	46	WD	700	69	0.154	0.047	90	3.29	0.0014	0.05
CO2*WL*H_rate	WD	400	69	FC	700	0	-0.004	0.049	90	-0.08	0.938	0.05

Table A23: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	69	FC	700	46	0.022	0.051	90	0.43	0.6653	0.05
CO2*WL*H_rate	WD	400	69	FC	700	69	0.049	0.048	90	1.02	0.3114	0.05
CO2*WL*H_rate	WD	400	69	WD	700	0	0.029	0.052	90	0.55	0.5821	0.05
CO2*WL*H_rate	WD	400	69	WD	700	46	0.055	0.050	90	1.09	0.2781	0.05
CO2*WL*H_rate	WD	400	69	WD	700	69	0.120	0.045	90	2.65	0.0096	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	0.026	0.034	90	0.75	0.4535	0.05
CO2*WL*H_rate	FC	700	0	FC	700	69	0.053	0.032	90	1.63	0.106	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	0.033	0.042	90	0.77	0.4434	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	0.058	0.041	90	1.43	0.1565	0.05
CO2*WL*H_rate	FC	700	0	WD	700	69	0.124	0.036	90	3.42	0.001	0.05
CO2*WL*H_rate	FC	700	46	FC	700	69	0.027	0.035	90	0.78	0.4364	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	0.007	0.045	90	0.15	0.8801	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	0.033	0.041	90	0.79	0.4311	0.05
CO2*WL*H_rate	FC	700	46	WD	700	69	0.098	0.038	90	2.55	0.0123	0.05
CO2*WL*H_rate	FC	700	69	WD	700	0	-0.020	0.044	90	-0.47	0.6421	0.05
CO2*WL*H_rate	FC	700	69	WD	700	46	0.006	0.041	90	0.13	0.8933	0.05
CO2*WL*H_rate	FC	700	69	WD	700	69	0.071	0.035	90	2.01	0.047	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	0.026	0.044	90	0.59	0.5566	0.05
CO2*WL*H_rate	WD	700	0	WD	700	69	0.091	0.041	90	2.24	0.0274	0.05
CO2*WL*H_rate	WD	700	46	WD	700	69	0.066	0.038	90	1.72	0.0881	0.05

Table A24. Weedy rice least square means table for g_s

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	46	0.150	0.080	78	1.88	0.0644	0.05
CO2*H_rate	-	400	0	-	400	69	-0.048	0.080	78	-0.59	0.5537	0.05
CO2*H_rate	-	400	0	-	700	0	0.034	0.073	78	0.46	0.6475	0.05
CO2*H_rate	-	400	0	-	700	46	-0.002	0.098	78	-0.02	0.9846	0.05
CO2*H_rate	-	400	0	-	700	69	0.001	0.097	78	0.01	0.9918	0.05
CO2*H_rate	-	400	46	-	400	69	-0.198	0.082	78	-2.42	0.0177	0.05
CO2*H_rate	-	400	46	-	700	0	-0.117	0.097	78	-1.2	0.2351	0.05
CO2*H_rate	-	400	46	-	700	46	-0.152	0.077	78	-1.98	0.0509	0.05
CO2*H_rate	-	400	46	-	700	69	-0.149	0.098	78	-1.52	0.1325	0.05
CO2*H_rate	-	400	69	-	700	0	0.081	0.097	78	0.83	0.4078	0.05
CO2*H_rate	-	400	69	-	700	46	0.046	0.099	78	0.46	0.6456	0.05
CO2*H_rate	-	400	69	-	700	69	0.049	0.076	78	0.64	0.5222	0.05
CO2*H_rate	-	700	0	-	700	46	-0.035	0.080	78	-0.44	0.6578	0.05
CO2*H_rate	-	700	0	-	700	69	-0.033	0.078	78	-0.41	0.6795	0.05
CO2*H_rate	-	700	46	-	700	69	0.003	0.080	78	0.04	0.9714	0.05

Table A25. Palmer amaranth least square means table for g_s

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	46	0.022	0.038	89	0.59	0.560	0.05
CO2*WL*H_rate	FC	400	0	FC	400	69	0.024	0.039	89	0.62	0.534	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-0.015	0.026	89	-0.57	0.572	0.05
CO2*WL*H_rate	FC	400	0	WD	400	46	-0.027	0.039	89	-0.68	0.496	0.05
CO2*WL*H_rate	FC	400	0	WD	400	69	0.024	0.038	89	0.65	0.518	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	0.026	0.095	89	0.27	0.787	0.05
CO2*WL*H_rate	FC	400	0	FC	700	46	0.021	0.099	89	0.21	0.832	0.05
CO2*WL*H_rate	FC	400	0	FC	700	69	0.149	0.099	89	1.51	0.135	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	0.019	0.095	89	0.2	0.845	0.05
CO2*WL*H_rate	FC	400	0	WD	700	46	0.099	0.099	89	1	0.321	0.05
CO2*WL*H_rate	FC	400	0	WD	700	69	0.143	0.099	89	1.45	0.150	0.05
CO2*WL*H_rate	FC	400	46	FC	400	69	0.002	0.039	89	0.05	0.962	0.05
CO2*WL*H_rate	FC	400	46	WD	400	0	-0.037	0.038	89	-0.96	0.338	0.05
CO2*WL*H_rate	FC	400	46	WD	400	46	-0.049	0.029	89	-1.71	0.090	0.05
CO2*WL*H_rate	FC	400	46	WD	400	69	0.002	0.038	89	0.06	0.956	0.05
CO2*WL*H_rate	FC	400	46	FC	700	0	0.003	0.099	89	0.03	0.972	0.05
CO2*WL*H_rate	FC	400	46	FC	700	46	-0.001	0.096	89	-0.01	0.991	0.05
CO2*WL*H_rate	FC	400	46	FC	700	69	0.127	0.099	89	1.28	0.203	0.05
CO2*WL*H_rate	FC	400	46	WD	700	0	-0.004	0.099	89	-0.04	0.971	0.05
CO2*WL*H_rate	FC	400	46	WD	700	46	0.076	0.095	89	0.8	0.424	0.05
CO2*WL*H_rate	FC	400	46	WD	700	69	0.121	0.099	89	1.22	0.225	0.05
CO2*WL*H_rate	FC	400	69	WD	400	0	-0.039	0.039	89	-1	0.321	0.05

Table A25: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	69	WD	400	46	-0.051	0.040	89	-1.27	0.209	0.05
CO2*WL*H_rate	FC	400	69	WD	400	69	0.000	0.027	89	0.01	0.994	0.05
CO2*WL*H_rate	FC	400	69	FC	700	0	0.002	0.099	89	0.02	0.988	0.05
CO2*WL*H_rate	FC	400	69	FC	700	46	-0.003	0.100	89	-0.03	0.976	0.05
CO2*WL*H_rate	FC	400	69	FC	700	69	0.125	0.095	89	1.31	0.193	0.05
CO2*WL*H_rate	FC	400	69	WD	700	0	-0.005	0.100	89	-0.06	0.956	0.05
CO2*WL*H_rate	FC	400	69	WD	700	46	0.074	0.099	89	0.75	0.455	0.05
CO2*WL*H_rate	FC	400	69	WD	700	69	0.119	0.095	89	1.25	0.214	0.05
CO2*WL*H_rate	WD	400	0	WD	400	46	-0.012	0.038	89	-0.32	0.751	0.05
CO2*WL*H_rate	WD	400	0	WD	400	69	0.039	0.036	89	1.08	0.284	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	0.040	0.095	89	0.43	0.671	0.05
CO2*WL*H_rate	WD	400	0	FC	700	46	0.036	0.100	89	0.36	0.719	0.05
CO2*WL*H_rate	WD	400	0	FC	700	69	0.164	0.099	89	1.66	0.101	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	0.033	0.095	89	0.35	0.726	0.05
CO2*WL*H_rate	WD	400	0	WD	700	46	0.113	0.098	89	1.15	0.252	0.05
CO2*WL*H_rate	WD	400	0	WD	700	69	0.158	0.098	89	1.61	0.111	0.05
CO2*WL*H_rate	WD	400	46	WD	400	69	0.051	0.038	89	1.36	0.178	0.05
CO2*WL*H_rate	WD	400	46	FC	700	0	0.053	0.099	89	0.53	0.598	0.05
CO2*WL*H_rate	WD	400	46	FC	700	46	0.048	0.096	89	0.5	0.618	0.05
CO2*WL*H_rate	WD	400	46	FC	700	69	0.176	0.099	89	1.77	0.080	0.05
CO2*WL*H_rate	WD	400	46	WD	700	0	0.046	0.099	89	0.46	0.648	0.05
CO2*WL*H_rate	WD	400	46	WD	700	46	0.126	0.095	89	1.32	0.190	0.05
CO2*WL*H_rate	WD	400	46	WD	700	69	0.170	0.099	89	1.72	0.089	0.05

Table A25: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	69	FC	700	0	0.001	0.099	89	0.01	0.989	0.05
CO2*WL*H_rate	WD	400	69	FC	700	46	-0.003	0.099	89	-0.03	0.975	0.05
CO2*WL*H_rate	WD	400	69	FC	700	69	0.125	0.095	89	1.32	0.192	0.05
CO2*WL*H_rate	WD	400	69	WD	700	0	-0.006	0.099	89	-0.06	0.954	0.05
CO2*WL*H_rate	WD	400	69	WD	700	46	0.074	0.098	89	0.76	0.452	0.05
CO2*WL*H_rate	WD	400	69	WD	700	69	0.119	0.094	89	1.26	0.210	0.05
CO2*WL*H_rate	FC	700	0	FC	700	46	-0.005	0.041	89	-0.11	0.914	0.05
CO2*WL*H_rate	FC	700	0	FC	700	69	0.123	0.040	89	3.11	0.003	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-0.007	0.030	89	-0.23	0.818	0.05
CO2*WL*H_rate	FC	700	0	WD	700	46	0.073	0.040	89	1.83	0.071	0.05
CO2*WL*H_rate	FC	700	0	WD	700	69	0.118	0.040	89	2.95	0.004	0.05
CO2*WL*H_rate	FC	700	46	FC	700	69	0.128	0.041	89	3.08	0.003	0.05
CO2*WL*H_rate	FC	700	46	WD	700	0	-0.003	0.043	89	-0.06	0.953	0.05
CO2*WL*H_rate	FC	700	46	WD	700	46	0.077	0.031	89	2.47	0.016	0.05
CO2*WL*H_rate	FC	700	46	WD	700	69	0.122	0.042	89	2.93	0.004	0.05
CO2*WL*H_rate	FC	700	69	WD	700	0	-0.130	0.041	89	-3.17	0.002	0.05
CO2*WL*H_rate	FC	700	69	WD	700	46	-0.051	0.040	89	-1.27	0.208	0.05
CO2*WL*H_rate	FC	700	69	WD	700	69	-0.006	0.029	89	-0.2	0.841	0.05
CO2*WL*H_rate	WD	700	0	WD	700	46	0.080	0.040	89	2	0.048	0.05
CO2*WL*H_rate	WD	700	0	WD	700	69	0.125	0.040	89	3.13	0.002	0.05
CO2*WL*H_rate	WD	700	46	WD	700	69	0.045	0.039	89	1.16	0.248	0.05

Table A26. Velvetleaf least square means table for g_s

Differences of Least Squares Means												
Effect	WL	CO2	WL	CO2	Estimate	S.Error	DF	tValue	Pr> t	Alpha	Lower	Upper
CO2*WL	FC	400	WD	400	0.179	0.085	89	2.11	0.038	0.05	0.01	0.35
CO2*WL	FC	400	FC	700	-0.081	0.322	89	-0.25	0.802	0.05	-0.72	0.56
CO2*WL	FC	400	WD	700	-0.192	0.324	89	-0.59	0.555	0.05	-0.84	0.45
CO2*WL	WD	400	FC	700	-0.260	0.323	89	-0.8	0.424	0.05	-0.90	0.38
CO2*WL	WD	400	WD	700	-0.371	0.321	89	-1.15	0.252	0.05	-1.01	0.27
CO2*WL	FC	700	WD	700	-0.111	0.084	89	-1.31	0.192	0.05	-0.28	0.06

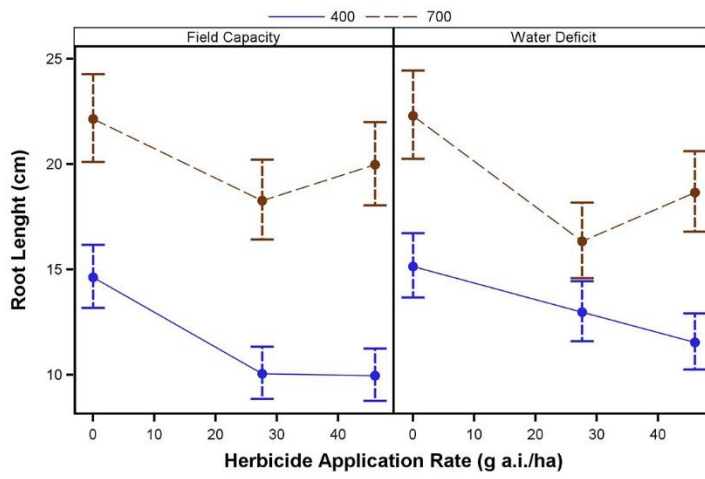


Figure A1. Velvetleaf root length measured at 21 DAT. Error bars represent \pm standard errors of means.

APPENDIX B
DICAMBA HERBICIDE

Table B1. Analysis of variance (ANOVA) for % of injury at 10 DAT for the two studied species.

Sources of Variance	Velvetleaf		Palmer amaranth	
	F- value	P- value	F- value	P- value
CO ₂ Concentration ([CO ₂])	25.47	0.0371	2.47	0.2564
Water Level (WL)	81.83	0.0120	74.08	0.0132
[CO ₂] x WL	7.99	0.0051	8.57	0.0037
Herbicide rate (H_rate)	208.79	<.0001	1328.01	<.0001
[CO ₂] x H_rate	17.50	<.0001	2.43	0.0895
WL x H_rate	52.22	<.0001	24.16	<.0001
[CO ₂] x WL x H_rate	1.22	0.2972	10.22	<.0001

Table B2: Palmer amaranth least square means of the interactions CO₂ x Herbicide rate, Water level x Herbicide rate and CO₂ x water level for % of injury at 10 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	280	-40.188	2.874	255	-13.98	<.0001	0.05
CO2*H_rate	-	400	0	-	400	420	-56.503	2.883	255	-19.6	<.0001	0.05
CO2*H_rate	-	400	0	-	700	0	-0.832	1.451	255	-0.57	0.5668	0.05
CO2*H_rate	-	400	0	-	700	280	-29.772	2.879	255	-10.34	<.0001	0.05
CO2*H_rate	-	400	0	-	700	420	-53.993	2.873	255	-18.79	<.0001	0.05
CO2*H_rate	-	400	280	-	400	420	-16.315	2.869	255	-5.69	<.0001	0.05
CO2*H_rate	-	400	280	-	700	0	39.356	2.893	255	13.6	<.0001	0.05
CO2*H_rate	-	400	280	-	700	280	10.416	1.344	255	7.75	<.0001	0.05
CO2*H_rate	-	400	280	-	700	420	-13.805	2.859	255	-4.83	<.0001	0.05
CO2*H_rate	-	400	420	-	700	0	55.671	2.902	255	19.18	<.0001	0.05
CO2*H_rate	-	400	420	-	700	280	26.731	2.874	255	9.3	<.0001	0.05
CO2*H_rate	-	400	420	-	700	420	2.509	1.353	255	1.85	0.0648	0.05
CO2*H_rate	-	700	0	-	700	280	-28.940	2.898	255	-9.98	<.0001	0.05
CO2*H_rate	-	700	0	-	700	420	-53.161	2.893	255	-18.38	<.0001	0.05
CO2*H_rate	-	700	280	-	700	420	-24.221	2.864	255	-8.46	<.0001	0.05
WL*H_rate	FC	-	0	FC	-	280	-44.370	2.878	255	-15.42	<.0001	0.05
WL*H_rate	FC	-	0	FC	-	420	-62.185	2.881	255	-21.58	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	0	-19.129	1.479	255	-12.93	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	280	-43.888	2.892	255	-15.18	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	420	-66.608	2.892	255	-23.03	<.0001	0.05
WL*H_rate	FC	-	280	FC	-	420	-17.815	2.869	255	-6.21	<.0001	0.05
WL*H_rate	FC	-	280	WD	-	0	25.241	2.910	255	8.67	<.0001	0.05

Table B2: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
WL*H_rate	FC	-	280	WD	-	280	0.482	1.376	255	0.35	0.7264	0.05
WL*H_rate	FC	-	280	WD	-	420	-22.239	2.880	255	-7.72	<.0001	0.05
WL*H_rate	FC	-	420	WD	-	0	43.056	2.913	255	14.78	<.0001	0.05
WL*H_rate	FC	-	420	WD	-	280	18.297	2.883	255	6.35	<.0001	0.05
WL*H_rate	FC	-	420	WD	-	420	-4.423	1.384	255	-3.2	0.0016	0.05
WL*H_rate	WD	-	0	WD	-	280	-24.759	2.895	255	-8.55	<.0001	0.05
WL*H_rate	WD	-	0	WD	-	420	-47.479	2.895	255	-16.4	<.0001	0.05
WL*H_rate	WD	-	280	WD	-	420	-22.720	2.864	255	-7.93	<.0001	0.05
CO2*WL	FC	400	-	WD	400	-	-5.432	1.161	255	-4.68	<.0001	0.05
CO2*WL	FC	400	-	FC	700	-	6.289	1.125	255	5.59	<.0001	0.05
CO2*WL	FC	400	-	WD	700	-	-3.659	1.182	255	-3.1	0.0022	0.05
CO2*WL	WD	400	-	FC	700	-	11.721	1.151	255	10.19	<.0001	0.05
CO2*WL	WD	400	-	WD	700	-	1.773	1.134	255	1.56	0.1191	0.05
CO2*WL	FC	700	-	WD	700	-	-9.948	1.172	255	-8.49	<.0001	0.05

Table B3: Palmer amaranth least square means of the interactions CO₂ x Water level x Herbicide rate for % of injury at 10 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	280	-69.629	2.423	273	-28.74	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	420	-96.639	2.435	273	-39.69	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-18.346	2.211	273	-8.3	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	280	-70.149	2.477	273	-28.33	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	420	-92.771	2.482	273	-37.37	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	-0.377	3.463	273	-0.11	0.9133	0.05
CO2*WL*H_rate	FC	400	0	FC	700	280	-62.075	3.563	273	-17.42	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	420	-83.032	3.610	273	-23	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-15.000	3.506	273	-4.28	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	280	-66.017	3.576	273	-18.46	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	420	-94.932	3.576	273	-26.55	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	400	420	-27.011	2.369	273	-11.4	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	400	0	51.283	2.464	273	20.81	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	400	280	-0.520	2.060	273	-0.25	0.8008	0.05
CO2*WL*H_rate	FC	400	280	WD	400	420	-23.143	2.411	273	-9.6	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	700	0	69.251	3.633	273	19.06	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	700	280	7.553	3.300	273	2.29	0.0228	0.05
CO2*WL*H_rate	FC	400	280	FC	700	420	-13.404	3.571	273	-3.75	0.0002	0.05
CO2*WL*H_rate	FC	400	280	WD	700	0	54.629	3.674	273	14.87	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	700	280	3.612	3.313	273	1.09	0.2766	0.05
CO2*WL*H_rate	FC	400	280	WD	700	420	-25.303	3.537	273	-7.15	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	400	0	78.294	2.478	273	31.59	<.0001	0.05

Table B3: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	420	WD	400	280	26.490	2.422	273	10.94	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	400	420	3.868	2.084	273	1.86	0.0645	0.05
CO2*WL*H_rate	FC	400	420	FC	700	0	96.262	3.641	273	26.44	<.0001	0.05
CO2*WL*H_rate	FC	400	420	FC	700	280	34.564	3.532	273	9.79	<.0001	0.05
CO2*WL*H_rate	FC	400	420	FC	700	420	13.607	3.362	273	4.05	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	700	0	81.639	3.682	273	22.17	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	700	280	30.622	3.545	273	8.64	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	700	420	1.707	3.323	273	0.51	0.6078	0.05
CO2*WL*H_rate	WD	400	0	WD	400	280	-51.803	2.517	273	-20.58	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	420	-74.425	2.522	273	-29.52	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	17.969	3.494	273	5.14	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	280	-43.729	3.595	273	-12.16	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	420	-64.686	3.641	273	-17.76	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	3.346	3.537	273	0.95	0.3451	0.05
CO2*WL*H_rate	WD	400	0	WD	700	280	-47.671	3.607	273	-13.22	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	420	-76.586	3.608	273	-21.23	<.0001	0.05
CO2*WL*H_rate	WD	400	280	WD	400	420	-22.622	2.463	273	-9.18	<.0001	0.05
CO2*WL*H_rate	WD	400	280	FC	700	0	69.772	3.669	273	19.02	<.0001	0.05
CO2*WL*H_rate	WD	400	280	FC	700	280	8.074	3.339	273	2.42	0.0163	0.05
CO2*WL*H_rate	WD	400	280	FC	700	420	-12.883	3.608	273	-3.57	0.0004	0.05
CO2*WL*H_rate	WD	400	280	WD	700	0	55.149	3.710	273	14.87	<.0001	0.05
CO2*WL*H_rate	WD	400	280	WD	700	280	4.132	3.351	273	1.23	0.2187	0.05
CO2*WL*H_rate	WD	400	280	WD	700	420	-24.783	3.575	273	-6.93	<.0001	0.05
CO2*WL*H_rate	WD	400	420	FC	700	0	92.394	3.673	273	25.15	<.0001	0.05

Table B3: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	420	FC	700	280	30.696	3.566	273	8.61	<.0001	0.05
CO2*WL*H_rate	WD	400	420	FC	700	420	9.739	3.394	273	2.87	0.0044	0.05
CO2*WL*H_rate	WD	400	420	WD	700	0	77.771	3.713	273	20.94	<.0001	0.05
CO2*WL*H_rate	WD	400	420	WD	700	280	26.754	3.579	273	7.48	<.0001	0.05
CO2*WL*H_rate	WD	400	420	WD	700	420	-2.161	3.354	273	-0.64	0.52	0.05
CO2*WL*H_rate	FC	700	0	FC	700	280	-61.698	2.514	273	-24.54	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	420	-82.655	2.579	273	-32.05	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-14.623	2.433	273	-6.01	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	280	-65.640	2.531	273	-25.93	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	420	-94.555	2.531	273	-37.35	<.0001	0.05
CO2*WL*H_rate	FC	700	280	FC	700	420	-20.957	2.420	273	-8.66	<.0001	0.05
CO2*WL*H_rate	FC	700	280	WD	700	0	47.075	2.573	273	18.29	<.0001	0.05
CO2*WL*H_rate	FC	700	280	WD	700	280	-3.942	2.018	273	-1.95	0.0518	0.05
CO2*WL*H_rate	FC	700	280	WD	700	420	-32.857	2.366	273	-13.89	<.0001	0.05
CO2*WL*H_rate	FC	700	420	WD	700	0	68.032	2.638	273	25.79	<.0001	0.05
CO2*WL*H_rate	FC	700	420	WD	700	280	17.015	2.437	273	6.98	<.0001	0.05
CO2*WL*H_rate	FC	700	420	WD	700	420	-11.900	2.102	273	-5.66	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	280	-51.017	2.591	273	-19.69	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	420	-79.932	2.591	273	-30.85	<.0001	0.05
CO2*WL*H_rate	WD	700	280	WD	700	420	-28.915	2.383	273	-12.13	<.0001	0.05

Table B4. Analysis of variance (ANOVA) for % of injury at 21 DAT for the two studied species.

Sources of Variance	Velvetleaf		Palmer amaranth	
	F- value	P- value	F- value	P- value
CO ₂ Concentration	0.35	0.6121	14.33	0.0632
Water Level (WL)	448.80	0.0022	66.34	0.0147
[CO ₂] x WL	0.04	0.8447	2.40	0.1223
Herbicide rate (H_rate)	120.48	0.0003	831.32	<.0001
[CO ₂] x H_rate	21.69	<.0001	21.54	<.0001
WL x H_rate	394.54	<.0001	32.08	<.0001
[CO ₂] x WL x H_rate	1.77	0.1733	10.73	<.0001

Table B5. Velvetleaf least square means of the interactions CO₂ x Herbicide rate and Water level x Herbicide rate for % of injury at 21 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	280	-5.2285	0.4473	242	-11.69	<.0001	0.05
CO2*H_rate	-	400	0	-	400	420	-6.4736	0.4473	242	-14.47	<.0001	0.05
CO2*H_rate	-	400	0	-	700	0	0.0564	0.3408	242	0.17	0.8687	0.05
CO2*H_rate	-	400	0	-	700	280	-4.4992	0.5521	242	-8.15	<.0001	0.05
CO2*H_rate	-	400	0	-	700	420	-6.6712	0.552	242	-12.08	<.0001	0.05
CO2*H_rate	-	400	280	-	400	420	-1.245	0.4474	242	-2.78	0.0058	0.05
CO2*H_rate	-	400	280	-	700	0	5.2849	0.5527	242	9.56	<.0001	0.05
CO2*H_rate	-	400	280	-	700	280	0.7293	0.3398	242	2.15	0.0328	0.05
CO2*H_rate	-	400	280	-	700	420	-1.4427	0.5522	242	-2.61	0.0095	0.05
CO2*H_rate	-	400	420	-	700	0	6.53	0.5527	242	11.82	<.0001	0.05
CO2*H_rate	-	400	420	-	700	280	1.9744	0.5522	242	3.58	0.0004	0.05
CO2*H_rate	-	400	420	-	700	420	-0.1976	0.3398	242	-0.58	0.5614	0.05
CO2*H_rate	-	700	0	-	700	280	-4.5556	0.4478	242	-10.17	<.0001	0.05
CO2*H_rate	-	700	0	-	700	420	-6.7276	0.4478	242	-15.02	<.0001	0.05
CO2*H_rate	-	700	280	-	700	420	-2.172	0.447	242	-4.86	<.0001	0.05
CO2*H_rate	FC	-	0	FC	-	280	-6.7387	0.4474	242	-15.06	<.0001	0.05
WL*H_rate	FC	-	0	FC	-	420	-8.3774	0.4474	242	-18.72	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	0	-4.0932	0.1182	242	-34.63	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	280	-7.1385	0.45	242	-15.86	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	420	-8.917	0.45	242	-19.82	<.0001	0.05
WL*H_rate	FC	-	280	FC	-	420	-1.6386	0.4476	242	-3.66	0.0003	0.05
WL*H_rate	FC	-	280	WD	-	0	2.6456	0.4509	242	5.87	<.0001	0.05

Table B5: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
WL*H_rate	FC	-	280	WD	-	280	-0.3998	0.1152	242	-3.47	0.0006	0.05
WL*H_rate	FC	-	280	WD	-	420	-2.1782	0.4502	242	-4.84	<.0001	0.05
WL*H_rate	FC	-	420	WD	-	0	4.2842	0.4509	242	9.5	<.0001	0.05
WL*H_rate	FC	-	420	WD	-	280	1.2388	0.4502	242	2.75	0.0064	0.05
WL*H_rate	FC	-	420	WD	-	420	-0.5396	0.1151	242	-4.69	<.0001	0.05
WL*H_rate	WD	-	0	WD	-	280	-3.0454	0.4477	242	-6.8	<.0001	0.05
WL*H_rate	WD	-	0	WD	-	420	-4.8238	0.4476	242	-10.78	<.0001	0.05
WL*H_rate	WD	-	280	WD	-	420	-1.7784	0.4468	242	-3.98	<.0001	0.05

Table 6B. Palmer amaranth least square means of the interactions CO₂ x Water level x Herbicide rate for % of injury at 21 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	280	-81.793	2.665	270	-30.7	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	420	-99.684	2.663	270	-37.44	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-18.376	1.759	270	-10.45	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	280	-81.907	2.721	270	-30.1	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	420	-99.106	2.729	270	-36.31	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	-0.311	2.373	270	-0.13	0.8959	0.05
CO2*WL*H_rate	FC	400	0	FC	700	280	-65.507	3.143	270	-20.84	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	420	-92.419	3.148	270	-29.36	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-11.667	2.467	270	-4.73	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	280	-72.558	3.185	270	-22.78	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	420	-98.158	3.171	270	-30.95	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	400	420	-17.891	2.638	270	-6.78	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	400	0	63.417	2.741	270	23.14	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	400	280	-0.114	1.639	270	-0.07	0.9446	0.05
CO2*WL*H_rate	FC	400	280	WD	400	420	-17.314	2.706	270	-6.4	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	700	0	81.482	3.171	270	25.7	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	700	280	16.286	2.278	270	7.15	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	700	420	-10.626	3.130	270	-3.39	0.0008	0.05
CO2*WL*H_rate	FC	400	280	WD	700	0	70.126	3.242	270	21.63	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	700	280	9.234	2.340	270	3.95	0.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	700	420	-16.365	3.156	270	-5.19	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	400	0	81.308	2.739	270	29.68	<.0001	0.05

Table B6: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	420	WD	400	280	17.777	2.697	270	6.59	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	400	420	0.578	1.654	270	0.35	0.7272	0.05
CO2*WL*H_rate	FC	400	420	FC	700	0	99.373	3.169	270	31.36	<.0001	0.05
CO2*WL*H_rate	FC	400	420	FC	700	280	34.177	3.124	270	10.94	<.0001	0.05
CO2*WL*H_rate	FC	400	420	FC	700	420	7.265	2.289	270	3.17	0.0017	0.05
CO2*WL*H_rate	FC	400	420	WD	700	0	88.017	3.240	270	27.17	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	700	280	27.126	3.166	270	8.57	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	700	420	1.526	2.319	270	0.66	0.5111	0.05
CO2*WL*H_rate	WD	400	0	WD	400	280	-63.531	2.690	270	-23.62	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	420	-80.731	2.698	270	-29.92	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	18.065	2.459	270	7.35	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	280	-47.131	3.210	270	-14.68	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	420	-74.043	3.215	270	-23.03	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	6.709	2.435	270	2.75	0.0063	0.05
CO2*WL*H_rate	WD	400	0	WD	700	280	-54.183	3.161	270	-17.14	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	420	-79.782	3.147	270	-25.35	<.0001	0.05
CO2*WL*H_rate	WD	400	280	WD	400	420	-17.200	2.652	270	-6.49	<.0001	0.05
CO2*WL*H_rate	WD	400	280	FC	700	0	81.596	3.219	270	25.35	<.0001	0.05
CO2*WL*H_rate	WD	400	280	FC	700	280	16.400	2.345	270	6.99	<.0001	0.05
CO2*WL*H_rate	WD	400	280	FC	700	420	-10.512	3.181	270	-3.31	0.0011	0.05
CO2*WL*H_rate	WD	400	280	WD	700	0	70.240	3.200	270	21.95	<.0001	0.05
CO2*WL*H_rate	WD	400	280	WD	700	280	9.348	2.279	270	4.1	<.0001	0.05
CO2*WL*H_rate	WD	400	280	WD	700	420	-16.251	3.112	270	-5.22	<.0001	0.05
CO2*WL*H_rate	WD	400	420	FC	700	0	98.796	3.226	270	30.63	<.0001	0.05

Table B6: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	420	FC	700	280	33.600	3.184	270	10.55	<.0001	0.05
CO2*WL*H_rate	WD	400	420	FC	700	420	6.688	2.365	270	2.83	0.005	0.05
CO2*WL*H_rate	WD	400	420	WD	700	0	87.440	3.207	270	27.26	<.0001	0.05
CO2*WL*H_rate	WD	400	420	WD	700	280	26.548	3.133	270	8.48	<.0001	0.05
CO2*WL*H_rate	WD	400	420	WD	700	420	0.948	2.267	270	0.42	0.6761	0.05
CO2*WL*H_rate	FC	700	0	FC	700	280	-65.196	2.734	270	-23.85	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	420	-92.108	2.739	270	-33.62	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-11.356	1.920	270	-5.91	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	280	-72.248	2.783	270	-25.96	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	420	-97.847	2.767	270	-35.36	<.0001	0.05
CO2*WL*H_rate	FC	700	280	FC	700	420	-26.912	2.684	270	-10.03	<.0001	0.05
CO2*WL*H_rate	FC	700	280	WD	700	0	53.840	2.817	270	19.11	<.0001	0.05
CO2*WL*H_rate	FC	700	280	WD	700	280	-7.052	1.695	270	-4.16	<.0001	0.05
CO2*WL*H_rate	FC	700	280	WD	700	420	-32.651	2.713	270	-12.04	<.0001	0.05
CO2*WL*H_rate	FC	700	420	WD	700	0	80.752	2.823	270	28.61	<.0001	0.05
CO2*WL*H_rate	FC	700	420	WD	700	280	19.860	2.735	270	7.26	<.0001	0.05
CO2*WL*H_rate	FC	700	420	WD	700	420	-5.739	1.683	270	-3.41	0.0007	0.05
CO2*WL*H_rate	WD	700	0	WD	700	280	-60.892	2.762	270	-22.05	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	420	-86.491	2.746	270	-31.5	<.0001	0.05
CO2*WL*H_rate	WD	700	280	WD	700	420	-25.600	2.655	270	-9.64	<.0001	0.05

Table B7. Analysis of variance (ANOVA) for total biomass for the two studied species.

Sources of Variance	Velvetleaf		Palmer amaranth	
	F- value	P- value	F- value	P- value
CO ₂ Concentration ([CO ₂])	6.69	0.1226	2.35	0.2652
Water Level (WL)	9.64	0.0900	8.45	0.1008
[CO ₂] x WL	6.19	0.0137	20.17	<.0001
Herbicide rate (H_rate)	161.21	0.0002	104.62	0.0004
[CO ₂] x H_rate	3.50	0.0322	0.26	0.6091
WL x H_rate	1.46	0.2356	0.58	0.5590
[CO ₂] x WL x H_rate	1.26	0.2852	15.93	0.0001

Table B8. Velvetleaf total biomass obtained 21 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	280	2.281	0.396	183	5.76	<.0001	0.05
CO2*H_rate	-	400	0	-	400	420	4.867	0.396	183	12.28	<.0001	0.05
CO2*H_rate	-	400	0	-	700	0	-2.737	0.896	183	-3.06	0.0026	0.05
CO2*H_rate	-	400	0	-	700	280	-0.054	0.911	183	-0.06	0.9529	0.05
CO2*H_rate	-	400	0	-	700	420	3.396	0.910	183	3.73	0.0003	0.05
CO2*H_rate	-	400	280	-	400	420	2.586	0.382	183	6.78	<.0001	0.05
CO2*H_rate	-	400	280	-	700	0	-5.018	0.908	183	-5.53	<.0001	0.05
CO2*H_rate	-	400	280	-	700	280	-2.334	0.886	183	-2.64	0.0091	0.05
CO2*H_rate	-	400	280	-	700	420	1.115	0.904	183	1.23	0.219	0.05
CO2*H_rate	-	400	420	-	700	0	-7.604	0.908	183	-8.37	<.0001	0.05
CO2*H_rate	-	400	420	-	700	280	-4.921	0.905	183	-5.44	<.0001	0.05
CO2*H_rate	-	400	420	-	700	420	-1.471	0.886	183	-1.66	0.0983	0.05
CO2*H_rate	-	700	0	-	700	280	2.683	0.394	183	6.81	<.0001	0.05
CO2*H_rate	-	700	0	-	700	420	6.133	0.393	183	15.62	<.0001	0.05
CO2*H_rate	-	700	280	-	700	420	3.449	0.384	183	8.98	<.0001	0.05
CO2*WL	FC	400	-	WD	400	-	0.931	0.501	183	1.86	0.065	0.05
CO2*WL	FC	400	-	FC	700	-	-2.677	0.866	183	-3.09	0.0023	0.05
CO2*WL	FC	400	-	WD	700	-	-0.754	0.961	183	-0.78	0.4336	0.05
CO2*WL	WD	400	-	FC	700	-	-3.608	0.960	183	-3.76	0.0002	0.05
CO2*WL	WD	400	-	WD	700	-	-1.685	0.867	183	-1.94	0.0536	0.05
CO2*WL	FC	700	-	WD	700	-	1.923	0.501	183	3.84	0.0002	0.05

Table B9. Palmer amaranth total biomass.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	280	255.26	27.2181	143	9.38	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	105.78	21.3432	143	4.96	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	280	300.55	27.1988	143	11.05	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	53.7908	30.6557	143	1.75	0.0815	0.05
CO2*WL*H_rate	FC	400	0	FC	700	280	216.93	34.5772	143	6.27	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	420	307.28	34.8799	143	8.81	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-13.7582	30.6557	143	-0.45	0.6543	0.05
CO2*WL*H_rate	FC	400	0	WD	700	280	252.04	34.5772	143	7.29	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	420	299.71	35.5226	143	8.44	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	400	0	-149.48	27.2084	143	-5.49	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	400	280	45.2922	21.3558	143	2.12	0.0357	0.05
CO2*WL*H_rate	FC	400	280	FC	700	0	-201.47	35.0256	143	-5.75	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	700	280	-38.3329	30.4606	143	-1.26	0.2103	0.05
CO2*WL*H_rate	FC	400	280	FC	700	420	52.0193	35.0225	143	1.49	0.1397	0.05
CO2*WL*H_rate	FC	400	280	WD	700	0	-269.02	35.0256	143	-7.68	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	700	280	-3.2189	30.4606	143	-0.11	0.916	0.05
CO2*WL*H_rate	FC	400	280	WD	700	420	44.4512	35.6608	143	1.25	0.2146	0.05
CO2*WL*H_rate	WD	400	0	WD	400	280	194.77	27.2087	143	7.16	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	-51.99	30.7084	143	-1.69	0.0926	0.05
CO2*WL*H_rate	WD	400	0	FC	700	280	111.15	34.6283	143	3.21	0.0016	0.05
CO2*WL*H_rate	WD	400	0	FC	700	420	201.5	34.9261	143	5.77	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	-119.54	30.7084	143	-3.89	0.0002	0.05
CO2*WL*H_rate	WD	400	0	WD	700	280	146.26	34.6283	143	4.22	<.0001	0.05

Table B9: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	0	WD	700	420	193.93	35.5674	143	5.45	<.0001	0.05
CO2*WL*H_rate	WD	400	280	FC	700	0	-246.76	35.0332	143	-7.04	<.0001	0.05
CO2*WL*H_rate	WD	400	280	FC	700	280	-83.6251	30.4619	143	-2.75	0.0068	0.05
CO2*WL*H_rate	WD	400	280	FC	700	420	6.7271	35.024	143	0.19	0.848	0.05
CO2*WL*H_rate	WD	400	280	WD	700	0	-314.31	35.0332	143	-8.97	<.0001	0.05
CO2*WL*H_rate	WD	400	280	WD	700	280	-48.511	30.4619	143	-1.59	0.1135	0.05
CO2*WL*H_rate	WD	400	280	WD	700	420	-0.8409	35.6628	143	-0.02	0.9812	0.05
CO2*WL*H_rate	FC	700	0	FC	700	280	163.14	25.6139	143	6.37	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	420	253.49	26.0254	143	9.74	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-67.549	20.0098	143	-3.38	0.0009	0.05
CO2*WL*H_rate	FC	700	0	WD	700	280	198.25	25.6139	143	7.74	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	420	245.92	26.8803	143	9.15	<.0001	0.05
CO2*WL*H_rate	FC	700	280	FC	700	420	90.3522	25.6168	143	3.53	0.0006	0.05
CO2*WL*H_rate	FC	700	280	WD	700	0	-230.69	25.6139	143	-9.01	<.0001	0.05
CO2*WL*H_rate	FC	700	280	WD	700	280	35.114	18.9274	143	1.86	0.0656	0.05
CO2*WL*H_rate	FC	700	280	WD	700	420	82.7841	26.4901	143	3.13	0.0022	0.05
CO2*WL*H_rate	FC	700	420	WD	700	0	-321.04	26.0254	143	-12.34	<.0001	0.05
CO2*WL*H_rate	FC	700	420	WD	700	280	-55.2382	25.6168	143	-2.16	0.0327	0.05
CO2*WL*H_rate	FC	700	420	WD	700	420	-7.5681	21.1067	143	-0.36	0.7205	0.05
CO2*WL*H_rate	WD	700	0	WD	700	280	265.8	25.6139	143	10.38	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	420	313.47	26.8803	143	11.66	<.0001	0.05
CO2*WL*H_rate	WD	700	280	WD	700	420	47.6701	26.4901	143	1.8	0.074	0.05

Table B10. Analysis of variance (ANOVA) for Palmer amaranth shoot biomass.

Sources of Variance	Palmer amaranth	
	F- value	P- value
CO ₂ Concentration ([CO ₂])	5.76	0.1385
Water Level (WL)	13.60	0.0663
[CO ₂] x WL	10.51	0.0013
Herbicide rate (H_rate)	142.95	0.0002
[CO ₂] x H_rate	15.81	<.0001
WL x H_rate	3.40	0.0348
[CO ₂] x WL x H_rate	10.13	<.0001

Table B11. Palmer amaranth shoot biomass.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	280	7.7956	0.654	273	11.92	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	400	420	10.1803	0.6536	273	15.57	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	2.556	0.5313	273	4.81	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	280	8.762	0.6547	273	13.38	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	420	10.4864	0.6515	273	16.1	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	2.3055	0.6342	273	3.64	0.0003	0.05
CO2*WL*H_rate	FC	400	0	FC	700	280	6.0131	0.7536	273	7.98	<.0001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	420	8.4068	0.7688	273	10.93	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	1.2772	0.6483	273	1.97	0.0498	0.05
CO2*WL*H_rate	FC	400	0	WD	700	280	7.4272	0.7562	273	9.82	<.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	700	420	8.2682	0.7536	273	10.97	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	400	420	2.3848	0.5973	273	3.99	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	400	0	-5.2396	0.6232	273	-8.41	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	400	280	0.9664	0.4225	273	2.29	0.023	0.05
CO2*WL*H_rate	FC	400	280	WD	400	420	2.6909	0.5949	273	4.52	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	700	0	-5.4901	0.7135	273	-7.69	<.0001	0.05
CO2*WL*H_rate	FC	400	280	FC	700	280	-1.7825	0.5659	273	-3.15	0.0018	0.05
CO2*WL*H_rate	FC	400	280	FC	700	420	0.6112	0.7216	273	0.85	0.3977	0.05
CO2*WL*H_rate	FC	400	280	WD	700	0	-6.5184	0.7262	273	-8.98	<.0001	0.05
CO2*WL*H_rate	FC	400	280	WD	700	280	-0.3684	0.569	273	-0.65	0.5179	0.05
CO2*WL*H_rate	FC	400	280	WD	700	420	0.4726	0.7058	273	0.67	0.5037	0.05
CO2*WL*H_rate	FC	400	420	WD	400	0	-7.6243	0.6225	273	-12.25	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	400	280	-1.4184	0.5982	273	-2.37	0.0184	0.05

Table B11: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	420	WD	400	420	0.3061	0.419	273	0.73	0.4657	0.05
CO2*WL*H_rate	FC	400	420	FC	700	0	-7.8748	0.7128	273	-11.05	<.0001	0.05
CO2*WL*H_rate	FC	400	420	FC	700	280	-4.1672	0.7051	273	-5.91	<.0001	0.05
CO2*WL*H_rate	FC	400	420	FC	700	420	-1.7735	0.5882	273	-3.02	0.0028	0.05
CO2*WL*H_rate	FC	400	420	WD	700	0	-8.9032	0.7255	273	-12.27	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	700	280	-2.7531	0.708	273	-3.89	0.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	700	420	-1.9121	0.5656	273	-3.38	0.0008	0.05
CO2*WL*H_rate	WD	400	0	WD	400	280	6.206	0.6243	273	9.94	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	400	420	7.9305	0.6207	273	12.78	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	-0.2505	0.6034	273	-0.42	0.6784	0.05
CO2*WL*H_rate	WD	400	0	FC	700	280	3.4571	0.7264	273	4.76	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	420	5.8508	0.7423	273	7.88	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	-1.2788	0.6181	273	-2.07	0.0395	0.05
CO2*WL*H_rate	WD	400	0	WD	700	280	4.8712	0.7291	273	6.68	<.0001	0.05
CO2*WL*H_rate	WD	400	0	WD	700	420	5.7122	0.7264	273	7.86	<.0001	0.05
CO2*WL*H_rate	WD	400	280	WD	400	420	1.7245	0.5956	273	2.9	0.0041	0.05
CO2*WL*H_rate	WD	400	280	FC	700	0	-6.4565	0.7144	273	-9.04	<.0001	0.05
CO2*WL*H_rate	WD	400	280	FC	700	280	-2.7489	0.5665	273	-4.85	<.0001	0.05
CO2*WL*H_rate	WD	400	280	FC	700	420	-0.3552	0.7224	273	-0.49	0.6234	0.05
CO2*WL*H_rate	WD	400	280	WD	700	0	-7.4848	0.7271	273	-10.29	<.0001	0.05
CO2*WL*H_rate	WD	400	280	WD	700	280	-1.3348	0.5695	273	-2.34	0.0198	0.05
CO2*WL*H_rate	WD	400	280	WD	700	420	-0.4938	0.7067	273	-0.7	0.4853	0.05
CO2*WL*H_rate	WD	400	420	FC	700	0	-8.1809	0.7113	273	-11.5	<.0001	0.05
CO2*WL*H_rate	WD	400	420	FC	700	280	-4.4734	0.7034	273	-6.36	<.0001	0.05

Table B11: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	420	FC	700	420	-2.0797	0.5857	273	-3.55	0.0005	0.05
CO2*WL*H_rate	WD	400	420	WD	700	0	-9.2093	0.724	273	-12.72	<.0001	0.05
CO2*WL*H_rate	WD	400	420	WD	700	280	-3.0593	0.7063	273	-4.33	<.0001	0.05
CO2*WL*H_rate	WD	400	420	WD	700	420	-2.2183	0.563	273	-3.94	0.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	280	3.7076	0.6004	273	6.18	<.0001	0.05
CO2*WL*H_rate	FC	700	0	FC	700	420	6.1013	0.6202	273	9.84	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	-1.0283	0.4593	273	-2.24	0.026	0.05
CO2*WL*H_rate	FC	700	0	WD	700	280	5.1217	0.6035	273	8.49	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	420	5.9627	0.6004	273	9.93	<.0001	0.05
CO2*WL*H_rate	FC	700	280	FC	700	420	2.3937	0.6115	273	3.91	0.0001	0.05
CO2*WL*H_rate	FC	700	280	WD	700	0	-4.7359	0.6153	273	-7.7	<.0001	0.05
CO2*WL*H_rate	FC	700	280	WD	700	280	1.4141	0.4186	273	3.38	0.0008	0.05
CO2*WL*H_rate	FC	700	280	WD	700	420	2.2551	0.5909	273	3.82	0.0002	0.05
CO2*WL*H_rate	FC	700	420	WD	700	0	-7.1296	0.6347	273	-11.23	<.0001	0.05
CO2*WL*H_rate	FC	700	420	WD	700	280	-0.9796	0.6148	273	-1.59	0.1122	0.05
CO2*WL*H_rate	FC	700	420	WD	700	420	-0.1386	0.446	273	-0.31	0.7561	0.05
CO2*WL*H_rate	WD	700	0	WD	700	280	6.15	0.6183	273	9.95	<.0001	0.05
CO2*WL*H_rate	WD	700	0	WD	700	420	6.991	0.6153	273	11.36	<.0001	0.05
CO2*WL*H_rate	WD	700	280	WD	700	420	0.841	0.5941	273	1.42	0.158	0.05

Table B12. Analysis of variance (ANOVA) for root/shoot ratio.

Sources of Variance	Velvetleaf		Palmer amaranth	
	F- value	P- value	F- value	P- value
CO ₂ Concentration ([CO ₂])	0.01	0.9459	0.27	0.6566
Water Level (WL)	2.97	0.2272	6.74	0.1218
[CO ₂] x WL	2.15	0.1441	2.20	0.1399
Herbicide rate (H_rate)	22.12	0.0069	7.34	0.0458
[CO ₂] x H_rate	15.82	<.0001	6.00	0.0155
WL x H_rate	3.16	0.0451	0.63	0.5333
[CO ₂] x WL x H_rate	2.16	0.1189	2.54	0.1135

Table B13. Velvetleaf root/shoot ratio.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	280	-0.138	0.036	176	-3.81	0.0002	0.05
CO2*H_rate	-	400	0	-	400	420	-0.173	0.036	176	-4.75	<.0001	0.05
CO2*H_rate	-	400	0	-	700	0	0.017	0.042	176	0.41	0.6823	0.05
CO2*H_rate	-	400	0	-	700	280	-0.069	0.051	176	-1.34	0.1827	0.05
CO2*H_rate	-	400	0	-	700	420	-0.251	0.051	176	-4.89	<.0001	0.05
CO2*H_rate	-	400	280	-	400	420	-0.034	0.035	176	-0.97	0.3324	0.05
CO2*H_rate	-	400	280	-	700	0	0.156	0.051	176	3.06	0.0026	0.05
CO2*H_rate	-	400	280	-	700	280	0.070	0.041	176	1.71	0.0892	0.05
CO2*H_rate	-	400	280	-	700	420	-0.113	0.051	176	-2.22	0.0274	0.05
CO2*H_rate	-	400	420	-	700	0	0.190	0.051	176	3.73	0.0003	0.05
CO2*H_rate	-	400	420	-	700	280	0.104	0.051	176	2.05	0.0417	0.05
CO2*H_rate	-	400	420	-	700	420	-0.078	0.041	176	-1.91	0.0578	0.05
CO2*H_rate	-	700	0	-	700	280	-0.086	0.036	176	-2.4	0.0173	0.05
CO2*H_rate	-	700	0	-	700	420	-0.268	0.036	176	-7.5	<.0001	0.05
CO2*H_rate	-	700	280	-	700	420	-0.182	0.035	176	-5.15	<.0001	0.05
WL*H_rate	FC	-	0	FC	-	280	-0.086	0.036	176	-2.39	0.0178	0.05
WL*H_rate	FC	-	0	FC	-	420	-0.226	0.036	176	-6.27	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	0	-0.011	0.023	176	-0.49	0.625	0.05
WL*H_rate	FC	-	0	WD	-	280	-0.149	0.037	176	-4.01	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	420	-0.226	0.037	176	-6.07	<.0001	0.05
WL*H_rate	FC	-	280	FC	-	420	-0.140	0.035	176	-3.96	0.0001	0.05
WL*H_rate	FC	-	280	WD	-	0	0.075	0.037	176	2.03	0.0444	0.05
WL*H_rate	FC	-	280	WD	-	280	-0.063	0.021	176	-3.04	0.0027	0.05

Table B13: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t 	Alpha
WL*H_rate	FC	-	280	WD	-	420	-0.140	0.037	176	-3.83	0.0002	0.05
WL*H_rate	FC	-	420	WD	-	0	0.214	0.037	176	5.8	<.0001	0.05
WL*H_rate	FC	-	420	WD	-	280	0.076	0.037	176	2.09	0.038	0.05
WL*H_rate	FC	-	420	WD	-	420	-0.001	0.021	176	-0.04	0.9689	0.05
WL*H_rate	WD	-	0	WD	-	280	-0.138	0.036	176	-3.85	0.0002	0.05
WL*H_rate	WD	-	0	WD	-	420	-0.215	0.036	176	-5.98	<.0001	0.05
WL*H_rate	WD	-	280	WD	-	420	-0.077	0.036	176	-2.17	0.0311	0.05

Table B14. Palmer amaranth root/shoot ratio.

Differences of Least Squares Means												
Effect	CO2	H_rate	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha	Lower	Upper
CO2*H_rate	400	0	400	280	-0.124	0.030	142	-4.2	<.0001	0.05	-0.18	-0.07
CO2*H_rate	400	0	700	0	-0.074	0.051	142	-1.45	0.1498	0.05	-0.18	0.03
CO2*H_rate	400	0	700	280	-0.099	0.051	142	-1.95	0.0529	0.05	-0.20	0.00
CO2*H_rate	400	0	700	420	-0.061	0.052	142	-1.17	0.2451	0.05	-0.16	0.04
CO2*H_rate	400	280	700	0	0.050	0.052	142	0.97	0.3313	0.05	-0.05	0.15
CO2*H_rate	400	280	700	280	0.025	0.051	142	0.49	0.6236	0.05	-0.08	0.13
CO2*H_rate	400	280	700	420	0.064	0.053	142	1.21	0.2275	0.05	-0.04	0.17
CO2*H_rate	700	0	700	280	-0.025	0.028	142	-0.91	0.365	0.05	-0.08	0.03
CO2*H_rate	700	0	700	420	0.013	0.030	142	0.45	0.6552	0.05	-0.05	0.07
CO2*H_rate	700	280	700	420	0.038	0.029	142	1.31	0.1921	0.05	-0.02	0.10

Table B15. Analysis of variance (ANOVA) for CO₂ assimilation rate 5 DAT.

Sources of Variance	Palmer amaranth	
	F- value	P- value
CO ₂ Concentration ([CO ₂])	2.03	0.3894
Water Level (WL)	0.58	0.5863
[CO ₂] x WL	1.27	0.2625
Herbicide rate (H_rate)	111.08	0.0089
[CO ₂] x H_rate	5.17	0.0070
WL x H_rate	1.06	0.3502
[CO ₂] x WL x H_rate	2.25	0.1103

Table B16. Palmer amaranth CO₂ assimilation rate at 5 DAT.

Differences of Least Squares Means												
Effect	CO2	H_rate	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha	Lower	Upper
CO2*H_rate	400	0	400	280	0.918	0.133	118	6.89	<.0001	0.05	0.65	1.18
CO2*H_rate	400	0	400	420	1.491	0.140	118	10.63	<.0001	0.05	1.21	1.77
CO2*H_rate	400	0	700	0	-0.113	0.183	118	-0.62	0.5388	0.05	-0.47	0.25
CO2*H_rate	400	0	700	280	1.299	0.196	118	6.63	<.0001	0.05	0.91	1.69
CO2*H_rate	400	0	700	420	1.869	0.196	118	9.54	<.0001	0.05	1.48	2.26
CO2*H_rate	400	280	400	420	0.574	0.140	118	4.09	<.0001	0.05	0.30	0.85
CO2*H_rate	400	280	700	0	-1.030	0.199	118	-5.18	<.0001	0.05	-1.42	-0.64
CO2*H_rate	400	280	700	280	0.382	0.180	118	2.12	0.0357	0.05	0.03	0.74
CO2*H_rate	400	280	700	420	0.951	0.196	118	4.85	<.0001	0.05	0.56	1.34
CO2*H_rate	400	420	700	0	-1.604	0.203	118	-7.89	<.0001	0.05	-2.01	-1.20
CO2*H_rate	400	420	700	280	-0.192	0.201	118	-0.96	0.3403	0.05	-0.59	0.21
CO2*H_rate	400	420	700	420	0.378	0.185	118	2.04	0.0433	0.05	0.01	0.74
CO2*H_rate	700	0	700	280	1.412	0.157	118	8.97	<.0001	0.05	1.10	1.72
CO2*H_rate	700	0	700	420	1.982	0.157	118	12.59	<.0001	0.05	1.67	2.29
CO2*H_rate	700	280	700	420	0.570	0.154	118	3.7	0.0003	0.05	0.26	0.87

Table B17. Analysis of variance (ANOVA) for *g_s* 5 DAT.

Sources of Variance	Palmer amaranth	
	F- value	P- value
CO ₂ Concentration ([CO ₂])	1.65	0.4215
Water Level (WL)	0.36	0.6546
[CO ₂] x WL	0.07	0.7947
Herbicide rate (H _{rate})	10.73	0.0853
[CO ₂] x H _{rate}	3.10	0.0490
WL x H _{rate}	0.39	0.6805
[CO ₂] x WL x H _{rate}	2.20	0.1159

Table B18. Palmer amaranth g_s at 5 DAT.

Differences of Least Squares Means												
Effect	CO2	H_rate	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha	Lower	Upper
CO2*H_rate	400	0	400	280	0.046	0.012	115	3.91	0.0002	0.05	0.02	0.07
CO2*H_rate	400	0	400	420	0.065	0.013	115	5.13	<.0001	0.05	0.04	0.09
CO2*H_rate	400	0	700	0	-0.013	0.026	115	-0.51	0.6118	0.05	-0.06	0.04
CO2*H_rate	400	0	700	280	0.019	0.027	115	0.71	0.4816	0.05	-0.03	0.07
CO2*H_rate	400	0	700	420	0.012	0.027	115	0.45	0.6558	0.05	-0.04	0.07
CO2*H_rate	400	280	400	420	0.019	0.013	115	1.47	0.1443	0.05	-0.01	0.04
CO2*H_rate	400	280	700	0	-0.060	0.027	115	-2.22	0.0285	0.05	-0.11	-0.01
CO2*H_rate	400	280	700	280	-0.028	0.026	115	-1.06	0.2901	0.05	-0.08	0.02
CO2*H_rate	400	280	700	420	-0.035	0.027	115	-1.29	0.1994	0.05	-0.09	0.02
CO2*H_rate	400	420	700	0	-0.078	0.027	115	-2.87	0.0049	0.05	-0.13	-0.02
CO2*H_rate	400	420	700	280	-0.046	0.027	115	-1.7	0.0927	0.05	-0.10	0.01
CO2*H_rate	400	420	700	420	-0.053	0.026	115	-2.03	0.0451	0.05	-0.10	0.00
CO2*H_rate	700	0	700	280	0.032	0.014	115	2.27	0.0249	0.05	0.00	0.06
CO2*H_rate	700	0	700	420	0.025	0.014	115	1.8	0.0751	0.05	0.00	0.05
CO2*H_rate	700	280	700	420	-0.007	0.014	115	-0.51	0.6135	0.05	-0.03	0.02

Table B19. Analysis of variance (ANOVA) for CO₂ assimilation rate at 10 DAT for both studied species.

Sources of Variance	Velvetleaf		Palmer amaranth	
	F- value	P- value	F- value	P- value
CO ₂ Concentration ([CO ₂])	0.83	0.4589	1.06	0.4120
Water Level (WL)	0.02	0.9044	4.47	0.1687
[CO ₂] x WL	5.32	0.0223	0.01	0.9323
Herbicide rate (H_rate)	12.49	0.0190	25.62	0.0052
[CO ₂] x H_rate	2.03	0.1349	4.85	0.0090
WL x H_rate	2.13	0.1219	3.36	0.0372
[CO ₂] x WL x H_rate	5.00	0.0078	1.70	0.1866

Table B20. Velvetleaf CO₂ assimilation rate at 10 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	0	FC	400	280	2.6063	1.2619	177	2.07	0.0403	0.05
CO2*WL*H_rate	FC	400	0	FC	400	420	4.9647	1.2677	177	3.92	0.0001	0.05
CO2*WL*H_rate	FC	400	0	WD	400	0	-0.6657	0.8431	177	-0.79	0.4308	0.05
CO2*WL*H_rate	FC	400	0	WD	400	280	1.5999	1.3876	177	1.15	0.2504	0.05
CO2*WL*H_rate	FC	400	0	WD	400	420	5.0441	1.3795	177	3.66	0.0003	0.05
CO2*WL*H_rate	FC	400	0	FC	700	0	-3.3731	1.3972	177	-2.41	0.0168	0.05
CO2*WL*H_rate	FC	400	0	FC	700	280	1.2096	1.7899	177	0.68	0.5001	0.05
CO2*WL*H_rate	FC	400	0	FC	700	420	4.4092	1.7765	177	2.48	0.014	0.05
CO2*WL*H_rate	FC	400	0	WD	700	0	-1.0202	1.5127	177	-0.67	0.5009	0.05
CO2*WL*H_rate	FC	400	0	WD	700	280	1.8865	1.8673	177	1.01	0.3137	0.05
CO2*WL*H_rate	FC	400	0	WD	700	420	3.4815	1.8758	177	1.86	0.0651	0.05
CO2*WL*H_rate	FC	400	280	FC	400	420	2.3583	1.2717	177	1.85	0.0653	0.05
CO2*WL*H_rate	FC	400	280	WD	400	0	-3.272	1.3922	177	-2.35	0.0199	0.05
CO2*WL*H_rate	FC	400	280	WD	400	280	-1.0064	0.8486	177	-1.19	0.2372	0.05
CO2*WL*H_rate	FC	400	280	WD	400	420	2.4377	1.383	177	1.76	0.0797	0.05
CO2*WL*H_rate	FC	400	280	FC	700	0	-5.9795	1.7824	177	-3.35	0.001	0.05
CO2*WL*H_rate	FC	400	280	FC	700	280	-1.3968	1.4152	177	-0.99	0.325	0.05
CO2*WL*H_rate	FC	400	280	FC	700	420	1.8029	1.7793	177	1.01	0.3123	0.05
CO2*WL*H_rate	FC	400	280	WD	700	0	-3.6265	1.8744	177	-1.93	0.0546	0.05
CO2*WL*H_rate	FC	400	280	WD	700	280	-0.7198	1.5107	177	-0.48	0.6343	0.05
CO2*WL*H_rate	FC	400	280	WD	700	420	0.8752	1.8781	177	0.47	0.6418	0.05
CO2*WL*H_rate	FC	400	420	WD	400	0	-5.6304	1.3981	177	-4.03	<.0001	0.05
CO2*WL*H_rate	FC	400	420	WD	400	280	-3.3648	1.3971	177	-2.41	0.0171	0.05

Table B20: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	FC	400	420	WD	400	420	0.07937	0.8429	177	0.09	0.9251	0.05
CO2*WL*H_rate	FC	400	420	FC	700	0	-8.3378	1.787	177	-4.67	<.0001	0.05
CO2*WL*H_rate	FC	400	420	FC	700	280	-3.7551	1.7972	177	-2.09	0.0381	0.05
CO2*WL*H_rate	FC	400	420	FC	700	420	-0.5555	1.4032	177	-0.4	0.6927	0.05
CO2*WL*H_rate	FC	400	420	WD	700	0	-5.9849	1.879	177	-3.19	0.0017	0.05
CO2*WL*H_rate	FC	400	420	WD	700	280	-3.0782	1.8741	177	-1.64	0.1023	0.05
CO2*WL*H_rate	FC	400	420	WD	700	420	-1.4832	1.526	177	-0.97	0.3324	0.05
CO2*WL*H_rate	WD	400	0	WD	400	280	2.2656	1.2764	177	1.78	0.0776	0.05
CO2*WL*H_rate	WD	400	0	WD	400	420	5.7098	1.2679	177	4.5	<.0001	0.05
CO2*WL*H_rate	WD	400	0	FC	700	0	-2.7074	1.5158	177	-1.79	0.0758	0.05
CO2*WL*H_rate	WD	400	0	FC	700	280	1.8753	1.8828	177	1	0.3206	0.05
CO2*WL*H_rate	WD	400	0	FC	700	420	5.0749	1.8711	177	2.71	0.0073	0.05
CO2*WL*H_rate	WD	400	0	WD	700	0	-0.3545	1.4116	177	-0.25	0.802	0.05
CO2*WL*H_rate	WD	400	0	WD	700	280	2.5522	1.7871	177	1.43	0.155	0.05
CO2*WL*H_rate	WD	400	0	WD	700	420	4.1472	1.7959	177	2.31	0.0221	0.05
CO2*WL*H_rate	WD	400	280	WD	400	420	3.4441	1.2674	177	2.72	0.0072	0.05
CO2*WL*H_rate	WD	400	280	FC	700	0	-4.973	1.8737	177	-2.65	0.0087	0.05
CO2*WL*H_rate	WD	400	280	FC	700	280	-0.3903	1.5276	177	-0.26	0.7986	0.05
CO2*WL*H_rate	WD	400	280	FC	700	420	2.8093	1.8707	177	1.5	0.1349	0.05
CO2*WL*H_rate	WD	400	280	WD	700	0	-2.6201	1.7908	177	-1.46	0.1452	0.05
CO2*WL*H_rate	WD	400	280	WD	700	280	0.2866	1.4057	177	0.2	0.8387	0.05
CO2*WL*H_rate	WD	400	280	WD	700	420	1.8816	1.795	177	1.05	0.296	0.05
CO2*WL*H_rate	WD	400	420	FC	700	0	-8.4172	1.8677	177	-4.51	<.0001	0.05
CO2*WL*H_rate	WD	400	420	FC	700	280	-3.8345	1.8763	177	-2.04	0.0425	0.05

Table B20: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*WL*H_rate	WD	400	420	FC	700	420	-0.6349	1.5042	177	-0.42	0.6735	0.05
CO2*WL*H_rate	WD	400	420	WD	700	0	-6.0642	1.7845	177	-3.4	0.0008	0.05
CO2*WL*H_rate	WD	400	420	WD	700	280	-3.1575	1.7799	177	-1.77	0.0778	0.05
CO2*WL*H_rate	WD	400	420	WD	700	420	-1.5625	1.4088	177	-1.11	0.2689	0.05
CO2*WL*H_rate	FC	700	0	FC	700	280	4.5827	1.2988	177	3.53	0.0005	0.05
CO2*WL*H_rate	FC	700	0	FC	700	420	7.7823	1.2798	177	6.08	<.0001	0.05
CO2*WL*H_rate	FC	700	0	WD	700	0	2.3529	0.8764	177	2.68	0.008	0.05
CO2*WL*H_rate	FC	700	0	WD	700	280	5.2596	1.4028	177	3.75	0.0002	0.05
CO2*WL*H_rate	FC	700	0	WD	700	420	6.8546	1.4138	177	4.85	<.0001	0.05
CO2*WL*H_rate	FC	700	280	FC	700	420	3.1996	1.293	177	2.47	0.0143	0.05
CO2*WL*H_rate	FC	700	280	WD	700	0	-2.2298	1.4216	177	-1.57	0.1185	0.05
CO2*WL*H_rate	FC	700	280	WD	700	280	0.6769	0.8877	177	0.76	0.4467	0.05
CO2*WL*H_rate	FC	700	280	WD	700	420	2.2719	1.4268	177	1.59	0.1131	0.05
CO2*WL*H_rate	FC	700	420	WD	700	0	-5.4294	1.4046	177	-3.87	0.0002	0.05
CO2*WL*H_rate	FC	700	420	WD	700	280	-2.5227	1.3987	177	-1.8	0.073	0.05
CO2*WL*H_rate	FC	700	420	WD	700	420	-0.9277	0.8789	177	-1.06	0.2926	0.05
CO2*WL*H_rate	WD	700	0	WD	700	280	2.9067	1.2905	177	2.25	0.0255	0.05
CO2*WL*H_rate	WD	700	0	WD	700	420	4.5017	1.3022	177	3.46	0.0007	0.05
CO2*WL*H_rate	WD	700	280	WD	700	420	1.595	1.2959	177	1.23	0.2201	0.05
CO2*WL	FC	400	-	WD	400	-	-0.5309	0.6722	177	-0.79	0.4307	0.05
CO2*WL	FC	400	-	FC	700	-	-1.7751	1.3011	177	-1.36	0.1742	0.05
CO2*WL	FC	400	-	WD	700	-	-1.0744	1.4193	177	-0.76	0.4501	0.05
CO2*WL	WD	400	-	FC	700	-	-1.2442	1.4189	177	-0.88	0.3817	0.05
CO2*WL	WD	400	-	WD	700	-	-0.5435	1.3025	177	-0.42	0.677	0.05

Table B20: Continued

Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t 	Alpha
CO2*WL	FC	700	-	WD	700	-	0.7007	0.6867	177	1.02	0.3089	0.05

Table B21. Palmer amaranth CO₂ assimilation rate at 10 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	280	1.1214	0.2644	164	4.24	<.0001	0.05
CO2*H_rate	-	400	0	-	400	420	2.0455	0.265	164	7.72	<.0001	0.05
CO2*H_rate	-	400	0	-	700	0	0.6891	0.3719	164	1.85	0.0657	0.05
CO2*H_rate	-	400	0	-	700	280	1.3823	0.4364	164	3.17	0.0018	0.05
CO2*H_rate	-	400	0	-	700	420	2.1973	0.44	164	4.99	<.0001	0.05
CO2*H_rate	-	400	280	-	400	420	0.9241	0.2644	164	3.49	0.0006	0.05
CO2*H_rate	-	400	280	-	700	0	-0.4323	0.4369	164	-0.99	0.3239	0.05
CO2*H_rate	-	400	280	-	700	280	0.2609	0.3703	164	0.7	0.4821	0.05
CO2*H_rate	-	400	280	-	700	420	1.0759	0.4396	164	2.45	0.0155	0.05
CO2*H_rate	-	400	420	-	700	0	-1.3564	0.4372	164	-3.1	0.0023	0.05
CO2*H_rate	-	400	420	-	700	280	-0.6632	0.4364	164	-1.52	0.1305	0.05
CO2*H_rate	-	400	420	-	700	420	0.1518	0.3749	164	0.4	0.6861	0.05
CO2*H_rate	-	700	0	-	700	280	0.6932	0.2588	164	2.68	0.0082	0.05
CO2*H_rate	-	700	0	-	700	420	1.5082	0.2652	164	5.69	<.0001	0.05
CO2*H_rate	-	700	280	-	700	420	0.815	0.2639	164	3.09	0.0024	0.05
WL*H_rate	FC	-	0	FC	-	280	0.9101	0.2622	164	3.47	0.0007	0.05
WL*H_rate	FC	-	0	FC	-	420	1.9881	0.2649	164	7.5	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	0	0.3723	0.1494	164	2.49	0.0137	0.05
WL*H_rate	FC	-	0	WD	-	280	1.2768	0.2724	164	4.69	<.0001	0.05
WL*H_rate	FC	-	0	WD	-	420	1.9379	0.2763	164	7.01	<.0001	0.05
WL*H_rate	FC	-	280	FC	-	420	1.0779	0.2652	164	4.07	<.0001	0.05
WL*H_rate	FC	-	280	WD	-	0	-0.5378	0.2745	164	-1.96	0.0518	0.05
WL*H_rate	FC	-	280	WD	-	280	0.3666	0.1457	164	2.52	0.0128	0.05

Table B21: Continued

Effect	WL	CO2	H_rate	WL	CO2	_H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha
WL*H_rate	FC	-	280	WD	-	420	1.0278	0.2765	164	3.72	0.0003	0.05
WL*H_rate	FC	-	420	WD	-	0	-1.6158	0.277	164	-5.83	<.0001	0.05
WL*H_rate	FC	-	420	WD	-	280	-0.7113	0.2749	164	-2.59	0.0105	0.05
WL*H_rate	FC	-	420	WD	-	420	-0.05017	0.1572	164	-0.32	0.75	0.05
WL*H_rate	WD	-	0	WD	-	280	0.9045	0.2609	164	3.47	0.0007	0.05
WL*H_rate	WD	-	0	WD	-	420	1.5656	0.2651	164	5.91	<.0001	0.05
WL*H_rate	WD	-	280	WD	-	420	0.6611	0.263	164	2.51	0.0129	0.05

Table B22. Analysis of variance (ANOVA) for gs at 10 DAT for both studied species.

Sources of Variance	Velvetleaf		Palmer	
	F- value	P- value	F- value	P- value
CO ₂ Concentration ([CO ₂])	0.76	0.4747	0.01	0.9323
Water Level (WL)	2.30	0.2684	22.81	0.0412
[CO ₂] x WL	12.11	0.0006	0.21	0.6468
Herbicide rate (H_rate)	3.72	0.1221	8.90	0.0337
[CO ₂] x H_rate	16.15	<.0001	3.02	0.0514
WL x H_rate	1.51	0.2248	0.22	0.8014
[CO ₂] x WL x H_rate	1.15	0.3184	1.35	0.2623

Table B23. Velvetleaf g_s measured at 10 DAT.

Differences of Least Squares Means												
Effect	WL	CO2	H_rate	WL	CO2	_H_rate	Estimate	S. Error	DF	tValue	Pr> t	Alpha
CO2*H_rate	-	400	0	-	400	280	0.042	0.020	175	2.09	0.0377	0.05
CO2*H_rate	-	400	0	-	400	420	0.084	0.020	175	4.22	<.0001	0.05
CO2*H_rate	-	400	0	-	700	0	0.048	0.024	175	2.04	0.0426	0.05
CO2*H_rate	-	400	0	-	700	280	0.070	0.030	175	2.34	0.0202	0.05
CO2*H_rate	-	400	0	-	700	420	0.067	0.030	175	2.27	0.0246	0.05
CO2*H_rate	-	400	280	-	400	420	0.042	0.020	175	2.12	0.0354	0.05
CO2*H_rate	-	400	280	-	700	0	0.006	0.030	175	0.2	0.8379	0.05
CO2*H_rate	-	400	280	-	700	280	0.028	0.024	175	1.18	0.2393	0.05
CO2*H_rate	-	400	280	-	700	420	0.025	0.030	175	0.85	0.3938	0.05
CO2*H_rate	-	400	420	-	700	0	-0.036	0.030	175	-1.22	0.224	0.05
CO2*H_rate	-	400	420	-	700	280	-0.015	0.030	175	-0.49	0.6255	0.05
CO2*H_rate	-	400	420	-	700	420	-0.017	0.023	175	-0.72	0.4702	0.05
CO2*H_rate	-	700	0	-	700	280	0.022	0.020	175	1.08	0.2828	0.05
CO2*H_rate	-	700	0	-	700	420	0.019	0.020	175	0.96	0.3377	0.05
CO2*H_rate	-	700	280	-	700	420	-0.002	0.020	175	-0.12	0.9043	0.05
CO2*WL	FC	400	-	WD	400	-	-0.026	0.007	175	-3.45	0.0007	0.05
CO2*WL	FC	400	-	FC	700	-	0.003	0.023	175	0.12	0.902	0.05
CO2*WL	FC	400	-	WD	700	-	0.011	0.023	175	0.46	0.6431	0.05
CO2*WL	WD	400	-	FC	700	-	0.029	0.023	175	1.23	0.2213	0.05
CO2*WL	WD	400	-	WD	700	-	0.037	0.023	175	1.59	0.1148	0.05
CO2*WL	FC	700	-	WD	700	-	0.008	0.008	175	1.04	0.3017	0.05

Table B24. Palmer amaranth g_s measured at 10 DAT.

Differences of Least Squares Means												
Effect	WL	H_rate	WL	H_rate	Estimate	S.Error	DF	tValue	Pr> t	Alpha	Lower	Upper
H_rate	-	0	-	280	0.03573	0.01214	4	2.94	0.0423	0.05	0.002	0.069
H_rate	-	0	-	420	0.05029	0.01232	4	4.08	0.0151	0.05	0.016	0.085
H_rate	-	280	-	420	0.01457	0.01231	4	1.18	0.3023	0.05	-0.020	0.049
WL	FC	-	WD	-	0.02337	0.004893	2	4.78	0.0412	0.05	0.002	0.044

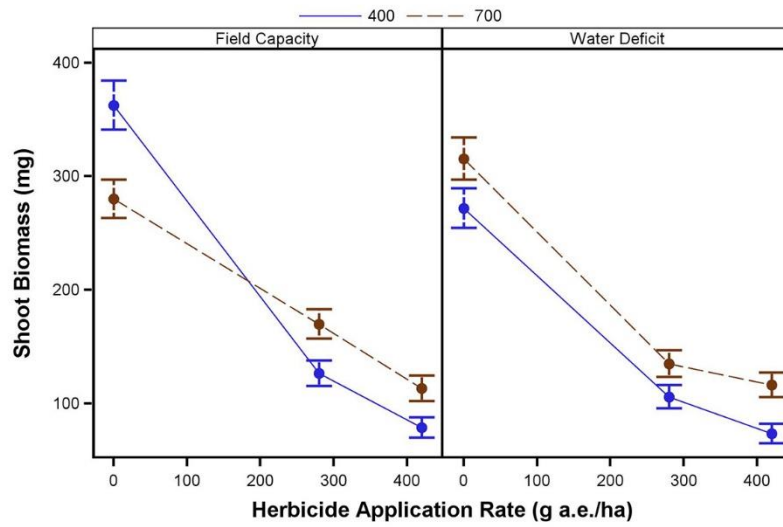


Figure B1. Palmer amaranth shoot biomass collected at 21 DAT. Error bars represent \pm standard errors of means.

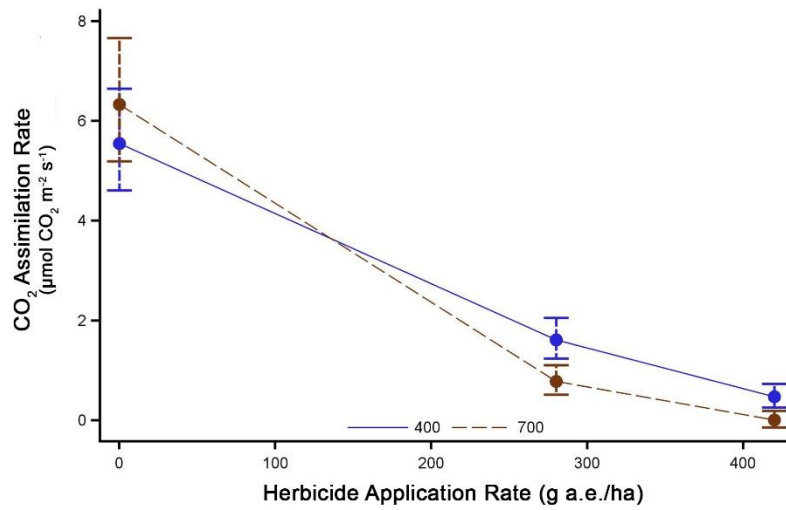


Figure B2. Palmer amaranth CO₂ Assimilation rate at 5 DAT. Error bars represent \pm standard errors of means.

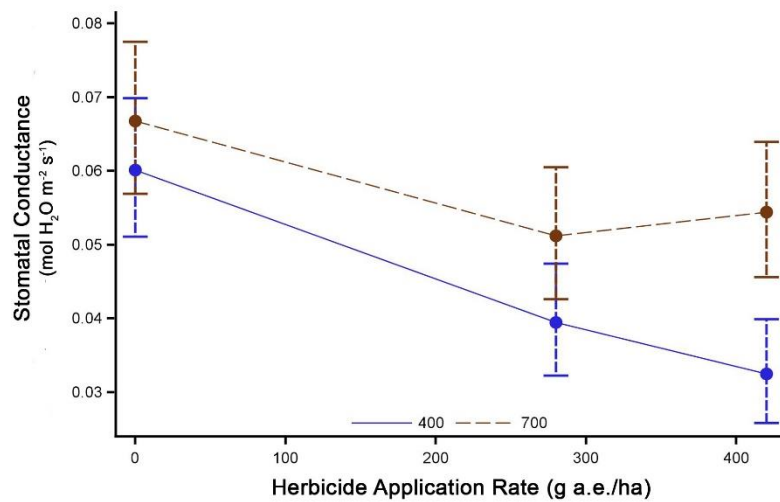


Figure B3. Palmer amaranth g_s at 5 DAT. Error bars represent \pm standard errors of means.