The role of a foliar nutrient product in relieving herbicide-induced defects in crop growth and development in *Zea mays*, *Triticum aestivum*, and *Glycine max*

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August 2017

A thesis submitted to McGill University in partial fulfilment of the requirements of the degree of Master of Science

Summary

As the rate of carbon dioxide assimilation via photosynthesis is closely related to crop growth and development, there are concerns that herbicide-induced decreases in photosynthetic rate could be negatively impacting crop yields. Furthermore, as many mineral elements have direct or indirect roles in maintaining the photosynthetic apparatus, this study aims to investigate the efficacy of foliar nutrient solutions containing N, P, K, Mn, Zn, B and Mo in reversing herbicide-induced photosynthetic suppression. The products Crop Booster (CB), Crop Booster 2.0 (CB2) and RR Soy Booster 2.0 (SB2) were tested in the field setting for their capacity to relieve the phytotoxic effects associated with herbicide usage in wheat (*Triticum aestivum*), corn (*Zea mays)*, and soybean (*Glycine max*). During the first field season, post-emergence treatments consisted of water only (control), herbicide only, and a combination of herbicide and CB; bromoxynil/MCPA, glyphosate plus atrazine, and glyphosate/fomesafen were used in wheat, corn and soybean, respectively. During the second crop year, post-emergence treatments consisted of water only (control), herbicide only, CB2 only and a combination of herbicide and CB2; bromoxynil/MCPA and glyphosate plus atrazine/dicamba were used in wheat and corn, respectively. For soybean, SB2 and the herbicide combination glyphosate plus chlorimuron ethyl were used. Experiments with CB2 usage in spring wheat were repeated in the greenhouse setting. The nutrient formulations were applied in conjunction with the herbicide treatments at the third trifoliate stage for soybean, sixth to eighth leaf stage for corn and sixth to early flag leaf stage in wheat. Photosynthetic variables, growth variables and harvest variables were measured to determine the treatments' effects on plant development. Both CB2 and SB2 resulted in partial recovery of the negative herbicide effects on photosynthetic rate under field conditions. However, the photosynthetic recovery did not result in changes in the growth variables at most stages, or increase harvest variables. Under greenhouse conditions, while CB2 application did not result in photosynthetic recovery, there were statistically significant differences in height and dry weight due to the application of bromoxynil/MCPA.

Keywords: Photosynthesis, Triticum aestevium, Zea maize, Glycine max, herbicides

Résumé

Puisque l'assimilation du gaz carbonique via la photosynthèse par les plantes cultivées est étroitement liée à leur croissance et développement, une baisse du taux de photosynthèse induite par les herbicides peut avoir un effet négatif sur les rendements des cultures. De plus, comme plusieurs éléments minéraux portent un effet direct ou indirect sur la photosynthèse, cette étude vise à étudier l'efficacité des solutions nutritives foliaires contenant N, P, K, Mn, Zn, B, et Mo en réparant la suppression de la photosynthèse causée par l'application des herbicides. Les produits Coup de Fouet (CB), Crop Booster 2.0 (CB2), et RR Soy Booster 2.0 ont été testés dans les champs afin d'identifier leur capacité à soulager les effets phytotoxiques associés avec l'application des herbicides en le blé (*Triticum aestivum*), le maïs (*Zea mays)*, et le soja (*Glycine max*). Pendant la première saison, les applications de post-levée consistées de : contrôle (H_2O), seulement les herbicides, et une combination des herbicides et CB2 ; bromoxynil/MCPA, glyphosate et atrazine, et glyphosate/fomesafen ont été appliqué sur le blé de printemps, le maïs, et le soja respectivement. La deuxième saison, les applications de post-levée sur le maïs et le blé de printemps consistées de contrôle (H_2O), seulement les herbicides, seulement le CB2, et une combination des herbicides et CB2 ; bromoxynil/MCPA et glyphosate plus atrazine/dicamba ont été appliqué sur le blé de printemps et le maïs respectivement. Sur le soja, SB2 et les herbicides glyphosate et chlorimuron ethyl ont été appliqués. Les formulations nutrititives ont été appliquées en liason avec les herbicides lorsque le soja est au stade de la troisième feuille trifoliée, le maïs est au stade de la sixième feuille, et le blé est au stade de la sixième feuille. Les variables photosynthétiques, les variables de croissance (la hauteur, la surface foliaire, et la masse sèche), et les variables de récolte ont été notés pour évaluer les effets des traitements sur le développement des plantes cultivées. L'application de CB2 portait un rétablissement partiel du taux de photosynthèse dans les champs. Cependant, le rétablissement partiel n'avait aucun effet sur ni la plupart des variables de croissance, ni les variables de récolte. Dans la serre, bien que l'application de CB2 n'eût pas un effet sur le taux de photosynthèse, il y avait des différences statistiquement significatives dans la hauteur et la masse sèche associée avec l'application de bromoxynil/MCPA.

Mots clés : Photosynthèse, Triticum aestevium, Zea maize, Glycine max

Acknowledgements

Firstly, I would like to express the utmost appreciation for my thesis advisor, Dr. Donald Smith. His guidance was instrumental in the progression of my thesis and he always encouraged me to be open to new ideas.

I would also like to acknowledge my co-supervisor Dr. Olivia Wilkins for her support throughout my thesis project. She provided me with sound and invaluable advice for my research as well as my future.

I would also like to thank my committee members Dr. Jean-Benoit Charron and Dr. Asim Biswas for providing input towards my project and promoting meaningful progress to my project.

I would also like to thank the technicians Peter Kirby, Marc Samoisette, and Guy Rimmer as well as the post-doctoral fellows Selvakumari Arunchalem, Sowmyalakshmi Subramanian, and Timothy Schwinghamer for their expert advice in the planning, execution, analysis of my projects.

I would also like to thank my laboratory members for their support during my thesis. Their kindness and light-heartedness promoted an atmosphere conducive to high-quality research.

Furthermore, I would like to thank Axter Agrosciences Inc., Natural Sciences and Engineering Research Council of Canada, Tri-Council Networks of Centers of Excellence, and Biofuelnet Canada for providing funding to complete my master's project.

Finally, I would like to thank my parents and close family members for their undying support and encouragement during my master's degree. Without them, I would not be here.

Thank you.
Olivia Haley

Table of Contents

List of Tables

List of Figures

Introduction:

With higher energy costs and increased awareness of environmental issues, there is a profound importance placed on the development and integration of cost-effective agrochemicals into current agronomic practices. Furthermore, as yield performance is the key economic driver for crop producers, the development of agrochemicals which increase crop productivity is a priority, though the development and deployment of these technologies remains a challenge for the agricultural sector. Among these technologies are foliar nutrient formulations which function as crop growth stimulators to increase the efficacy of current products to in turn increase crop yields.

The foliar application of nutrients is rapidly becoming more popular, likely due to their ease of application and largely positive effects on crop productivity (Serecon Management Consulting Inc, October 2011). Investigations into the foliar application of nutrients has revealed that these increases in yield may be a result of increased tolerances to environmental stresses such as salt stress (Hu et al., 2008), and water stress (Gill and Tuteja, 2010). While the focus of foliar nutrition is placed on alleviating environmental stresses, few studies have delved into the alleviation of herbicide stress; a substantial stress considering herbicides are applied by 69% of Canadian farmers per year (Statistics Canada, 2013). Although herbicides were designed to eliminate weed species, crop species often suffer as well due to the inability to rapidly detoxify the herbicides. Furthermore, depending on the duration of the metabolic stress, this could adversely affect the productivity of the crops (Creech et al., 2004). Therefore, the development of technologies to combat herbicide-induced decreases in crop productivity is a step towards improving commercial agricultural practices and performance.

While foliar nutrition is becoming more widely-used, there is a pronounced lack of literature investigating the restorative effects of nutrients on herbicide-affected plant metabolism. Thus, the long-term purpose of this project is to understand the physiological mechanisms by which the products of Axter Agrosciences Inc. stimulate plant growth under herbicide-induced stress conditions. To investigate this purpose, this project will delve into the negative effects of herbicides on various metabolic pathways, and the potential of the products to rescue or partially recover these effects. As many of the herbicides introduced in the experiment have a negative

effect on plant photosynthesis, and most of the micronutrients in the supplied formulations have some direct or indirect involvement in maintaining the photosynthetic machinery, this project will explore the effects of the products from the perspective of photosynthesis.

This project was developed conjointly with Axter Agrosciences Inc., a Québec-based agrochemical company focused on producing foliar nutrient formulations for Canadian crop markets to increase crop yield and quality. Since 1991, the company has tested a wide range of foliar nutrient solutions for their synergistic yield enhancement properties when tank-mixed with post-emergent herbicides. Selected products are either general formulations for a wide range of crops or crop-specific formulations which vary based on the presence and concentration of diverse nutrients. The Smith laboratory formed a collaboration with Axter Agrosciences, Inc. to investigate and evaluate the effects of the products under field and laboratory conditions. Preliminary trials demonstrated that the nutrient formulations enhanced crop growth in the presence of herbicide-induced stress conditions. However, additional characterization to determine the mechanism of the Axter products is necessary to maximize their potential and further increase production of a wider range of crops.

Accordingly, there are multiple hypotheses associated with this project:

(1) The use of herbicides is expected to decrease the rate of photosynthesis unilaterally for all tested crop species. Subsequently,

(2) The growth and harvest variables of crops exposed to herbicides is expected to result in a decrease in these variables.

(3) The use of foliar nutrient supplementation in the presence of herbicides will result in the partial recovery of photosynthesis, thus mediating the negative effects of herbicides on photosynthetic rate.

(4) The growth and harvest variables of crops exposed to a combination of herbicide and foliar

nutrients is expected to exhibit no change in these variables relative to those treated with water only.

Furthermore, the various products utilized in this experiment and their nutrient composition are as follows (Table I):

Table I: The concentration of nutrients in Crop Booster (CB), Crop Booster 2 (CB2), and Soy Booster (SB2) formulations										
	N %	P %	K %	B %	Mn %	Mo %	Zn %	S %	Co %	SA
CB	15	3	6	0.02	0.05	0.05	0.05	0	0	N
CB2	15	3	6	0.02	0.05	0.05	0.05	2	0	Y
SB2	6	18	6	0.02	0.05	0.01	0.05	5	0.01	Y

The concentration of nitrogen (N), phosphorous (P), potassium (K), boron (B), manganese (Mn), molybdenum (Mo), zinc (Zn), sulfur (S), cobalt (Co) and salicylic acid (SA) present in Crop Booster (CB), Crop Booster 2 (CB2), and Soy Booster 2 (SB2) formulations. The formulations were either classified as containing (Y) or not containing (N) salicylic acid (SA).

Literature Review

Section 1: Herbicide-induced alterations in plant metabolism

Due to the fierce competition for space and resources between crop plants and non-crop plants (weeds) the harnessing of chemical herbicides to eliminate a wide range of non-crop species increased crop yields and revolutionized many agricultural industries (Bruinsma, 1962). Under optimal conditions, the correct herbicide will eliminate the target weed species with little to no effect on the yield or quality of the non-target crop species. This is due to the presence of metabolic pathways in crop species (which are absent in non-crop species), that convert lethal herbicide molecules into non-lethal compounds for storage, excretion, or exudation (Devine et al., 1992; Edwards and Dixon, 2005; Oliveira et al., 2001). Herbicide degradation typically proceeds in four phases: conversion, conjugation, secondary conversion (optional), and deposition (Van Eerd et al., 2003). However, the inability to degrade the toxic molecules completely or rapidly results in a host of metabolic symptoms depending on the active site of the herbicide.

There are twenty-seven groups based on herbicide active site, however most herbicides currently used fall into groups: 1 - 10, 13 - 15, 19, 22, and 27. These active sites are exclusively proteins, most involved in metabolic pathways crucial for the biosynthesis of multiple biological molecules. There are nine major metabolic pathways a herbicide can inhibit: amino acid synthesis (groups 2, 9), growth regulation (groups 4, 19), photosynthesis (groups 5, 6, 7), cell membrane function (groups 14, 22), seedling shoot growth (group 15), seedling root growth (group 3), lipid synthesis (group 1), nitrogen metabolism (group 10), and pigment synthesis (groups 13, 27). As the herbicides utilised in this experiment fall within the first four categories, only these categories will be discussed.

Section 1.1 Herbicides as Photosystem II inhibitors

The conversion of light energy and carbon dioxide into water and sugars (photosynthesis) is catalyzed by reactions occurring within the thylakoid membrane of the chloroplast. The thylakoid membrane is composed of appressed and non-appressed regions which contain the

light harvesting pigment-protein complexes photosystems I and II; these systems are responsible for the coupled reduction of carbon and oxidation of water reactions which are mediated with the input of light energy. Each system contains its own set of specific polypeptides, pigments, electron donors/acceptors, and a reaction center at which electrons are shuttled via low energy and high energy carriers. The target of herbicides in groups 5-7 is photosystem II (PSII). PSII is a multi-subunit membrane protein composed of a dimeric oxygen evolving reaction centre surrounded by peripheral antennae composed of light-harvesting protein complexes (Nosek et al., 2017). The herbicides in this experiment bind competitively and reversibly to the plastiquinone binding site of the Q_B portion of the D1 subunit at the "herbicide binding pocket" – a pocket created by the loose binding of the Q_B subunit to the D1 subunit (Draber et al., 1991). This binding prevents the flow of electrons between the subunits, effectively halting the flow of electrons from PSII to PSI (Keren et al., 1997). This blockage of the electron transport system impairs the dissipation of excess energy, resulting in photodamage due to the accumulation of reactive oxygen species (ROS), specifically singlet oxygen (Fufezan et al., 2002).

Phytotoxic damage is primarily confined to the D1 protein of the PSII core reaction centre where inactivation due to ROS is rapidly detected (Krieger-Liszkay, 2004). Ironically, the primary culprit for the generation of ROS is the light-harvesting pigment chlorophyll. While this pigment is optimized for the absorption and conversion of excitation energy harvested from light, if the excess energy is not dissipated and/or quenched, the chlorophyll molecule undergoes a phase change to form the chlorophyll triplet state (Krieger-Liszkay, 2004; Rutherford and Krieger-Liszkay, 2001). This triplet state, despite having a lower energy excited state, has a longer half-life and reacts readily with oxygen to produce singlet oxygen molecules.

Section 1.2 Herbicides as Amino Acid Synthesis Inhibitors: Shikimate Pathway Inhibitiors

Glyphosate (*N*-phosphonomethyl glycine), patented in 1974 by Monsanto, is the most widely-used herbicide in the world and renowned for its low soil residual activity, broad spectrum coverage, systemicity in susceptible plants, and low toxicity to humans. Its role in inhibiting amino acid synthesis was first uncovered by Jaworski (1972) and the site of inhibition, EPSPS synthase, was identified by Steinrücken and Amrhein (1980). This enzyme, produced in the

cytoplasm and stored in the stroma of the chloroplast, is critical in the shikimate pathway; a metabolic pathway responsible for biosynthesis of the aromatic amino acids tryptophan, phenylalanine, and tyrosine, as well as a host of secondary metabolites including vitamins, lignins, alkaloids, and phenolic compounds. Glyphosate, using a phosphate carrier to cross the plasma membranes of the cell and chloroplast, reversibly inhibits the action of this enzyme through competitive and non-competitive methods (Denis and Delrot, 1993).

Under optimal conditions, 5-enolpyruvylshikimate 3-phosphate synthase (EPSPS) converts shikimate 3-phosphate (S3P) to 5-enolpyruvylshikimate 3-phosphate (EPSP) via the transfer of the enolpyruvyl moiety from phosphoenolpyruvate (PEP). However, glyphosate, being a transition-state structural analog of PEP, is a competitive inhibitor relative to the binding of PEP to EPSPS and a non-competitive inhibitor relative to the binding of S3P to EPSPS. Essentially, as S3P forms a complex with EPSP synthase, glyphosate binds to the enzyme, and due to the stability of the glyphosate:S3P:EPSPS complex, the reversal rate is relatively slow thus preventing the binding of PEP and leading to the inhibition of EPSPS function (Dill, 2005).

The inhibition of the shikimate pathway results in the decreased rates of protein synthesis resulting from the depletion of aromatic amino acid pools within the plant. Furthermore, with the reduction in chorisimate, a metabolite produced from the alteration of the shikimate pathway, there is an increased flow of carbon away from the photosynthetic carbon reduction cycle which results in a decrease in photosynthetic rate (Janda et al., 2014). The lethal effect of this herbicide on non-resistant crop species prompted the development of glyphosate-resistant (GR) crops such as corn and soybean. The genetic alterations consist of the inclusion of a transgene encoding a bacterial glyphosate-insensitive EPSPS, resulting in an alternative pathway to producing the necessary proteins and metabolites. However, Zobiole et al. (2010) demonstrated that the production of secondary metabolites, as well as photosynthesis, is still affected.

Section 1.3 Herbicides as Amino Acid Synthesis Inhibitors: Acetolactate Synthase Inhibitors

The acetolactate synthase (ALS) inhibitor herbicide family is popular due to its members' potency, selectivity, broad-spectrum application, and relatively minute amounts required for use (Mazur and Falco, 1989). Since the introduction of the first ALS herbicide, chlorsulfuron in 1982, this family has rapidly expanded to include a multitude of chemicals differing in potency while maintaining the same active site. ALS is a key enzyme in the biosynthesis of the branched amino acids leucine, isoleucine, and valine (Ray, 1984). Like EPSPS, ALS is produced in the cytoplasm then translocated to the chloroplast where it uses pyruvate and threonine as its substrates. Studies of ALS in pea plants have uncovered six ALS subunits whose binding is dependent on the presence of flavin adenine dinucleotide (FAD) (Shimizu et al., 2002). The four larger subunits are responsible for the catalytic action of ALS whereas the two smaller subunits are regulatory subunits responsible for the regulation of ALS via negative feedback inhibition with leucine, valine, and isoleucine (Cobb and Reade, 2011; Dezfulian et al., 2017).

The ALS enzyme often containing FAD, thiamine pyrophosphate, and magnesium (Mg^{2+}), reacts in a biphasic manner (Babczinski and Zelinski, 1991; Cobb and Reade, 2011). During the first phase, a pyruvate molecule binds to thiamine pyrophosphate (TPP) at the active site and is subsequently decarboxylated to yield a molecule of carbon dioxide. In the second phase, a second pyruvate molecule reacts with the remaining enzyme-substrate complex and releases acetolactate; this molecule then undergoes multiple reaction pathways to yield isoleucine, valine, and leucine. ALS inhibitors bind slowly, yet tightly to the enzyme-substrate complex resulting from the first phase of the biphasic ALS reaction to inhibit the binding of the second pyruvate molecule (LaRossa and Schloss, 1984; Lonhienne et al., 2016). This process is can occur via competitive and/or non-competitive inhibition and without a constant supply of leucine, isoleucine and valine, protein reserves eventually deplete, resulting in death.

Section 1.4 Herbicides as Synthetic Auxins

The use of synthetic auxins as herbicides began as early as 1941 with the development and introduction of the first auxin-herbicides MCPA and 2,4-D (Cobb and Reade, 2010a). Due to

their efficiency in eliminating a wide range of broadleaf plants and grasses at low doses and cost-effectiveness, this family of herbicides is the most widely used (Cobb and Reade, 2010a). Their success pushed the chemical industry not only to broadening this family with its structural analogs, but also to develop novel herbicide groups. There exist five chemical groups within the auxin-herbicide group: phenoxyalkanoic acids, benzoic acids, pyridines, aromatic carboxymethyl derivatives, and quinoline carboxylic acids (Cobb and Reade, 2010a). These auxin-herbicides simulate the compound auxin to cause an intensified growth response in affected plants, ultimately leading to their death.

Auxin (indol-3-yl-acetic acid or IAA) is an endogenous plant growth regulator that is crucial to many plant functions including: cell division, differentiation, and elongation. It is for this reason that processes such as leaf, flower and fruit development depend on the synthesis and transport of auxin (Ljung, 2013). The biosynthesis of auxin proceeds via tryptophan-dependent, and tryptophan independent pathways. As the accumulation of auxin results in cell growth disorders and eventually cell death, the plant has a tightly controlled system of concentration-dependent auxin synthesis and degradation. Exogenous (synthetic) auxins, often introduced to tissues at 100 times the endogenous concentration, can simulate, or inhibit plant growth depending on the concentration (Cobb and Reade, 2010a).

Synthetic auxin herbicides are rapidly absorbed by the leaf and transported throughout the plant via a series of well-reported transporters where the relatively high amounts of exogenous auxin results in the deterioration of cell growth processes due to the auxin-induced ethylene response (Thompson and Cobb, 1987). High concentrations of auxin results in an uncontrolled production of ethylene, consequently leading to a negative root and stem growth response (Hansen and Grossmann, 2000; Swarup et al., 2007) hydrogen peroxide which incites tissue damage (Grossmann et al., 2001), and abscisic acid (ABA) which induces stomatal closure and subsequently arrests carbon assimilation by photosynthesis.

Section 1.5 Herbicides as Cell Membrane Disruptors: PPO inhibitors

The inhibition of protoporphyrinogen IX oxidase (PPO) as a method of herbicide activity has been used since the 1970's with the introduction of diphenyl ethers to combat competitive grass species (Cobb and Reade, 2010b). However, there are presently four chemical classes in the PPO inhibitor category: diphenyl ethers, *N*-phenylnitrogen heterocycles, aryl triazinones and pyrimidinediones (Grossmann et al., 2010). These herbicides inhibit PPO, a critical chloroplastic enzyme in the production of tetrapyrroles – a diverse class of molecules containing four pyrrole rings, which assume pivotal roles in the light harvesting, energy transduction, signal transduction, and detoxification pathways (Beale, 1990; Schlicke et al., 2015).
Higher plants utilize four classes of tetrapyrroles: chlorophyll, heme, siroheme, and phytochromobilin (Tanaka and Tanaka, 2007). Chlorophyll and heme are produced using the same protoporphyrin IX precursor, differing notably in the mineral element which they chelate; chlorophylls utilize magnesium whereas hemes utilize iron. Chlorophylls are major light-harvesting molecules which are crucial in the transfer of light energy to power photosynthesis. Hemes, notably cytochromes and phytochromes, function in the transport of electrons or protons to form high energy molecules such as ATP. The protoporphyrin IX precursor required to form these diverse molecules is produced from the conversion of protoporphyrinogen IX by PPO (Böger and Wakabayashi, 2012). The mechanism for PPO inhibition by herbicides however, is unknown.

While the mechanism of enzyme inhibition is unclear for all herbicides in the PPO inhibitor category, the resulting symptoms are indicative of the light-dependent production of oxygen radicals. With the inhibition of PPO, there is a rapid accumulation of protoporphyrinogen IX which is readily oxidized by oxygen produced in the chloroplast (Grossmann et al., 2010; Wakabayashi and Böger, 2004). When protophyrinogen IX is exposed to light, these molecules react with oxygen to form singlet oxygen and oxygen radicals which proceed to rapidly degrade various lipid membranes, proteins, and DNA (Lermontova and Grimm, 2000). This leads to a multitude of problems including: ion leakage and water loss from the cell, the inhibition of photosynthesis, formation of ethylene, bleaching of chloroplast pigments, tissue necrosis, and ultimately growth inhibition and plant death (Grossmann et al., 2010).

Section 2 Micronutrients and Foliar applications

Higher plants require 17 nutrients to complete their life cycle: carbon (C), hydrogen (H), oxygen (O), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sulfur (S), zinc (Zn), copper (Cu), iron (Fe), manganese (Mn), boron (B), molybdenum (Mo), chlorine (Cl), and nickel (Ni) (Fageria, 2016). Nitrogen, phosphorous, potassium, calcium, magnesium, and sulphur are known as macronutrients (Maathuis, 2009). The last seven elements, are known as micronutrients, mineral elements essential for plant growth in much smaller quantities than those of the ten preceding macronutrients. Many cellular and metabolic aspects of plant development involve the utilization of micronutrients as catalytically active cofactors which activate or stabilize protein structures (Hänsch and Mendel, 2009). Micronutrients with multiple oxidation states participate in redox reactions whereas those with single oxidation states bear a more structural role. However, as the levels defining a deficiency or toxicity of a mineral nutrient varies amongst different species, tissues, and stages of the life cycle, the establishment of proper nutrient regimes for field applications is relatively new (Barker and Pilbeam, 2015).

Fields with inadequate nutritional regimens experience severely limited crop production resulting from the inaccessibility of mineral nutrients during one or more stages of the crop's life cycle. While soil amendments are the most popular method of correcting nutritional deficiencies in the field, foliar application systems are rapidly becoming more practised due to their simplicity and cost-effectiveness (Girma et al., 2007). Their efficacy relies on the permeability of the cuticular membrane to organic ions, inorganic ions and dissociated molecules (Fageria et al., 2009), occasionally taking advantage of open stomata (Eichert and Burkhardt, 2001). Although higher plants can absorb macronutrients foliarly, they are rarely applied as foliar sprays to correct a nutrient deficiency due to the relatively high concentration required and potential for burning (Fageria et al., 2009). On the contrary, micronutrient deficiencies are readily mediated with foliar supplementation.

Due to their rapid incorporation into plant systems, foliar treatments are most efficiently used as supplements to a soil nutrient management program to correct short-term deficiencies (Fageria et al., 2009; Kannan and Charnel, 1986). These nutrient deficiencies are often the result of stress-

induced physiologic imbalances brought by environmental conditions such as salinity, water stress, and low levels of light (Bohnert and Sheveleva, 1998; Gill and Tuteja, 2010; Hu et al., 2008). To this effect, various studies have indicated that combinations of micronutrients in foliar treatments can increase the plants' tolerance to abiotic and biotic stresses. For instance, Rahimizadeh et al. (2007) demonstrated the beneficial effects of micronutrients on antioxidant enzyme metabolism in drought stressed sunflower plants. Garcia-Mina (2006) found a relationship between mineral nutrition and pathogen defence (biotic stress) mechanisms in citrus plants. Furthermore, as many anti-oxidative enzymes contain these elements, the availability of micronutrients is strongly linked to the plant's anti-oxidative ability (Gill and Tuteja, 2010). The concept of utilizing micronutrients to heighten the stress response has led to the generation of soil-micronutrient fortification products and investments in soil nutrient management strategies. As the formulations used in this experiment contain differential amounts of boron (B), manganese (Mn), molybdenum (Mo), zinc (Zn), sulfur (S), and cobalt (Co) the emphasis will be placed on these nutrients.

Section 2.1 The role of manganese in plant metabolism

Manganese (Mn) is absorbed from the soil in the form of Mn^{2+}, its most dominant and stable form. Due to its many oxidation states, Mn readily serves as a cofactor in an estimated 35 plant enzymes as either a catalytically active metal, or an enzyme activator (Burnell, 1988). Furthermore, these enzymes play a role in a wide range of metabolic pathways including: photosynthesis, respiration, and protein synthesis (Burnell, 1988).

The Role of Manganese in Maintaining the Photosynthetic Machinery

Having multiple oxidation states, Mn facilitates the oxidation-reduction hydrolytic reactions of the oxygen evolving reaction centre of photosystem II (PSII) (Barker and Pilbeam, 2015). Within the reaction centre, four manganese atoms form a mixed-valent metalloid Mn-cluster linked to a calcium atom and a halide (Umena et al., 2011; Yachandra, 2005). Through a series of oxidation state changes (S_0 to S_5) the manganese cluster binds two water molecules and withdraws four electrons, releasing four protons and one molecule of oxygen (Kok et al., 1970; Yachandra, 2005). Several of the PSII components, including the manganese atoms, are then recycled and

the reaction centre reforms.

The mechanism of manganese-induced photosynthetic expression has been well studied in wheat (*Triticum aestivum*) and corn (*Zea mays* L.) (Gong et al., 2010a; Kriedemann et al., 1985). Due to its catalytic role in the oxygen-evolving complex of PSII, alterations in manganese availability affects the photosynthetic capability and eventually structural/functional integrity of the chloroplast (Gong et al., 2010b). The inability to replenish manganese leads to the accumulation of singlet oxygen radicals which inactivate the reaction centre (Husted et al., 2009), degrades the structure of the lamellar network and results in the uneven distribution of the stroma (Weiland et al., 1975). However, resupplying Mn restores the number of PSII protein pigment units in the thylakoid membrane (Gong et al., 2010a; Simpson and Robinson, 1984).

The Role of Manganese in the Stress Response

In addition to its role in the PSII reaction centre, manganese serves as a catalytic cofactor for a range of anti-oxidative enzymes which serve as reactive oxygen species (ROS) scavengers. ROS are highly reactive oxygen containing species which cause the degradation of a wide variety of biological molecules such as membrane lipids, DNA, and proteins in high quantities (Bowler et al., 1991). Superoxide dismutase (SOD) is one of the principle anti-oxidative enzymes found in the mitochondria, chloroplast, and peroxisome which is responsible for the dismutation of the superoxide radical to form hydrogen peroxide (which is subsequently degraded into water by peroxidases/catalases) (Bowler et al., 1989). Mn^{3+} complexes with SOD to form the site-specific metalloenzyme Mn-SOD which functions as a crucial regulator of reactive oxygen species in the mitochondria and peroxisome (Bowler et al., 1991; Zelko et al., 2002). The mechanism of this enzyme relies on the positively charged active cite which attracts the negatively charged superoxide radicals, and a manganese cofactor which donates an electron to reduce the oxygen molecule and form hydrogen peroxide, with the recruitment of protons (Asada, 1994). This key enzyme in the antioxidative repertoire is shown to be active in a variety of stress induced conditions (Beyer and Fridovich, 1987; Bowler et al., 1991; Zhang and Kirkham, 1994). To this effect, there is also rigorous investigation into the exploitation of Mn to combat these stresses.

Slooten et al. (1995) showed that the overexpression of Mn-SOD can lead to increased tolerances to oxidative damage. In terms of abiotic stress, an increase in Mn-SOD has been reported to contribute to drought tolerance (Wu et al., 1999), tolerance to freezing (McKersie et al., 1993), herbicide damage (Bowler et al., 1991) and salinity (Wang et al., 2004). Subsequently, foliarly applied manganese has been shown to increase abiotic and biotic resistance as well as improve nitrogen fixation. Morab et al. (2003) found that supplementing soybean with a 0.3% $MnSO_4$ foliar treatment decreased the "percent disease index of soybean" of a susceptible soybean variety.

Section 2.2 The role of zinc in plant metabolism

Zinc is absorbed from the soil in the form of its only oxidation state: Zn^{2+}. The essentiality of zinc as a mineral nutrient was first reported by Mazé in 1915 and subsequently demonstrated in barley and sunflower by Sommer and Lipman in 1926 (Broadley et al., 2007). In plants, zinc assumes a crucial role in enzymes of protein synthesis and maintaining the structural integrity of biomembranes (Bettger and O'Dell, 1981; Cakmak, 2000). Although zinc cannot participate in oxidation-reduction reactions due to having only one oxidation state, over 300 enzymes use the element as a metallic cofactor (Ibs et al., 2002). Furthermore, an estimated 1200 proteins are predicted to contain, bind, or transport Zn^{++} including a large number of zinc-finger containing proteins and transcription factors, oxidoreductases, and hydrolytic enzymes such as metalloproteases (Cakmak, 2000; Krämer and Clemens, 2005).

The Role of Zinc in Membrane Integrity

The structural integrity of biomembranes relies heavily on zinc availability. Zinc, complexed with polypeptides and cysteine, binds to phospholipid and sulfhydryl groups of biological membranes to protect the membrane lipids from oxidative damage (Barker and Pilbeam, 2015). Zinc deficiency results in a decreased concentration of key constituents of biological membranes, such as unsaturated fatty acids, phospholipids and reactive sulphydryl (-SH) groups (Cakmak and Marschner, 1988; Rengel, 1995). Membranes lacking these constituents become "leaky", allowing for an increased exudation of molecules as salts exuded in the solutes from the leaf and/or root epidermis. Furthermore, the leaking of the nutrients phosphorous and chlorine from

the roots of zinc-deficient wheat plants is also observed (Welch et al., 1982; Welch and Norvell, 1993). (Cakmak and Marschner, 1988) noted the leakage of solutes, principally potassium K^+, amino acids, sugars, and phenolics, from the roots of various plant species; this was also reversible with zinc supplementation. Furthermore, the instability of the cell membranes allows deficiencies in zinc to manifest in various ways including: reduction in leaf size and shoot girth, incrustations on the leaf due to leakage of solutes (Welch et al., 1982) and inhibition of root growth (Lin et al., 2005).

The Role of Zinc in the Stress Response

As a part of the anti-oxidative response, zinc has multiple benefits as a free molecule as well as when complexed with other micronutrients in enzymes. As a free molecule, Zn protects cellular membranes from oxidative damage through the inhibition of NADPH oxidase, a membrane-bound enzyme which reduces molecular oxygen to produce superoxide radicals that would otherwise cause the peroxidation of biomembranes (Bettger and O'Dell, 1981; Cakmak, 2000). When complexed with copper, these elements are incorporated into Cu/Zn-SOD, a chloroplastic membrane-bound form of SOD that rapidly degrades the superoxide radicals produced by the oversaturation of PSI with electrons that bind to oxygen and produce O^-_2 (Alscher et al., 2002; Ogawa et al., 1995). This prevents the generation and accumulation of hydrogen peroxide and hydroxyl (OH^-) radicals within the chloroplast, protecting the photosynthetic machinery in the membrane of the thylakoid (Asada, 2006). In Zn-deficient plants, activities of H_2O_2 scavenging enzymes were restricted (Broadley et al., 2007). However, like Mn-SOD, the enhanced expression of Cu/Zn-SOD confers resistance against multiple types of stress conditions.

Despite the evolutionary and genetic deviation of Cu/Zn-SOD from Mn-SOD (Zelko et al., 2002), the overexpression of Cu/Zn-SOD also increases tolerances to various stresses. For example, Iturbe-Ormaetxe et al. (1998) found that increasing levels of cytosolic and chloroplastic Cu/Zn-SOD conferred resistance to stress induced by the herbicide paraquat and water deficit. Furthermore, Cu/Zn-SOD is associated with increased tolerance to salt stress (Hernandez et al., 2000) and is hypothesized to be more effective in increasing tolerance to salt stress than Mn-SOD (Barker and Pilbeam, 2015). In fact, Hernandez et al. (2000) found that salt tolerant

varieties of pea showed a higher expression of Cu/Zn-SOD than salt-intolerant species and Prashanth et al. (2008) demonstrated how mangrove-derived Cu/Zn-SOD can increase salt stress tolerance in rice.

Section 2.3 The role of boron in plant metabolism

While the presence of boron in plant tissues was confirmed by Agulhon (1910) and its beneficial effects on corn investigated by Mazé (1919), it wasn't deemed an essential nutrient until critical investigations in *Vicia faba* L. made by Warrington (1923) (Barker and Pilbeam, 2015). Inadequate B supply plagues many agricultural industries worldwide, ultimately leading to severely decreased yields on many soils (Gupta, 1980; Koshiba et al., 2009). The quantity of boron required for growth is variable with graminaceous monocot species requiring the lowest levels of B and higher plant species requiring high levels (Hu et al., 1996). Furthermore, due to the inefficiency of most plant species in translocating B, plants require a constant supply of B throughout their lifecycle (Koshiba et al., 2009). Boron has three valence states +3, +2, and +1 and is most readily absorbed by plants in the form of undissasociated boric acid $B(OH)_3$. The function of B in the crosslinking of rhamnogalacturonan II in the cell wall has been well studied, however, boron has been implicated in a wide variety of biological processes, including protein synthesis, transport of sugars, respiration, RNA and carbohydrate metabolism, the metabolism of plant hormones (indole acetic acid), cell wall synthesis and lignification (Barker and Pilbeam, 2015). Thus, plants respond to B deficiencies with a range of physiological and metabolic alterations unconfirmed to be linked to boron's role in cell wall integrity (Blevins and Lukaszewski, 1998; Camacho-Cristóbal et al., 2008).

The Role of Boron in Cell Wall Cross-linking and Cell Membrane Functionality

Like Zinc, Boron atoms play a pivotal role in the maintainance of composition, structure, and function of membranes (Brown and Bassil, 2011; Goldbach and Wimmer, 2007) particularly in the leaves and the roots. In the form of boric acid, boron atoms form reversible diester bonds with the hydroxyl radicals of cis-diols (a configuration common to sugars) to form strong boron-diol complexes linked by diester bonds (Goldbach and Wimmer, 2007). Within the cell wall,

boron complexes with the rhamnogalacturonan II component of cell wall pectic polysaccharides, forming boron-rhamnogalacturonan II (B-RG II). Through covalent linkage, boron facilitates the formation of RG II dimers that form the constituents of cell walls (Matoh and Kobayashi, 1998). This structure allows for proper control and maintenance of turgor pressure within the plants cells (O'neill et al., 2001).

Similar to zinc-deficiency, a boron-deficient environment results in the deformation of the plasma membrane, leading to electrolyte leakage from the roots and the shoots. The development of deformed, brittle leaves and roots of plants submitted to boron-deficiency (Pfeffer et al., 1998) was attributed to the inhibition of dimeric B-RGII pectin recycling (Wimmer et al., 2015). Consequent to the decrease B-RGII crosslinking, the stability and pore size of the cell wall is severely reduced (Fleischer et al., 1999), leading to a cell wall that is unstructured, thick, and less flexible (Findeklee and Goldbach, 1996) and deteriorated root structure (Gupta, 1983; Martín-Rejano et al., 2011). These rapid physical alterations result in the depolarisation of the cell membranes and consequently the leakage of K^+, phenolics, amino acids, sucrose (Cakmak et al., 1995; Schon et al., 1990) and can affect the uptake of other mineral nutrients (Pollard et al., 1977).

The Role of Boron in the Uptake and Transport of Water

A deficiency in B limits the uptake and transport of water through alterations in root growth, stomatal structure and activity, and phloem and xylem structure. Cakmak et al. (1995) found that boron deficiency caused a decreased shoot and root growth which could adversely affect the absorption of water and nutrients from the soil. Plants subjected to low B supply have altered structures in xylem and phloem vessel elements (Dell and Huang, 1997; Wimmer and Eichert, 2013) which decreases the rate of water transport throughout the plant. Zhao and Oosterhuis (2003) noted impairments in stomatal responsiveness, likely a result of reduced stomatal density and deformation of guard cells (Wimmer and Eichert, 2013). These changes have been demonstrated to affect transpiration rates as well as photosynthesis (Sharma and Ramchandra, 1990).

Malformations of the vascular tissues are a common occurrence under B deficient conditions and are thought to be a consequence of perturbations in auxin metabolism (Martín-Rejano et al., 2011). A continuous supply of water to the leaves via xylem vessels is crucial in the maintenance of photosynthetic activity (Wimmer and Eichert, 2013). With B deficiency, there is poor development and disintegration of xylem elements with decreases in xylem diameter, and malformation of the tracheids (Hajiboland et al., 2012; Liu et al., 2013). Furthermore, there is a poor differentiation of the phloem and the cell wall becomes thick (de Oliveira et al., 2006; Hirsch and Torrey, 1980; Spurr, 1957). The lack of water movement through the vascular tissues inhibits the plants ability to photosynthesize; resulting in cell death.

Disturbances of the stomata limit transpiration and the conductance of water. In B-deficient plants, the stomatal apertures were found to be smaller than in B-sufficient plants which resulted in the hindrance of stomatal conductance (Sharma and Ramchandra, 1990). In cotton plants, B-deficiency caused a 30-80% reduction in stomatal conductance dependent on the duration of the deficient conditions. Furthermore, the guard cells were found to be reduced in number and malformed, preventing the normal opening of the stomata (Wimmer et al., 2015). This malfunction of the guard cells is hypothesized to result from impaired potassium uptake and transport into the guard cells as observed in turnips subjected to B-deficiency (Hajiboland et al., 2012; Roth-Bejerano and Itai, 1981).

Section 2.4 The role of molybdenum in plant metabolism

Molybdenum exists in four oxidation states, Mo^{3+}, Mo^{4+}, Mo^{5+} and Mo^{6+}, however, the most stable form, and the form absorbed from the soil by plants is the hexavalent Mo^{6+}. Although only approximately 40 plant enzymes contain molybdenum, these proteins can be extremely important, being involved in nitrogen assimilation, sulfur metabolism, phytohormone biosynthesis, and stress reactions (Mendel and Hänsch, 2002; Schwarz and Mendel, 2006). In the majority of molybdenum-containing enzymes the molybdenum cofactor (MoCo) contains a pterin group that aids in the transferring of electrons to and from the molybdenum atom (Schwarz et al., 2009). This cofactor, along with molybdenum's many oxidation states, allow this atom to be a prime candidate for oxidation-reduction reactions.

The Role of Molybdenum in Root Nodulation

In leguminous plants, such as soybean, beneficial microorganisms (rhizobia) living in the root nodules use the enzyme nitrogenase to fix atmospheric nitrogen (N_2). Nitrogenase is a complex of two enzymes: dinitrogenase (a Mo-Fe protein) and dinitrogenase reductase (an Fe-protein) (Seefeldt et al., 2009). The molybdenum-iron cofactor FeMoCo of nitrogenase contains one 4Fe-3S cluster and one 1M-3Fe-3S cluster joined by three inorganic sulphur atoms; this configuration allows for a high reducing power of nitrogen (Kim et al., 1993). On the other hand, this renders the enzyme highly sensitive to inactivation by molecular oxygen; this is mediated by superoxide dismutase and the iron protein leghaemoglobin which bind to excess molecular oxygen (Monk et al., 1989; Zhao et al., 2007). Although this results in a convenient source of nitrogen, due to the dual-importance of molybdenum for nodulation and activation of nitrogenase, leguminous plants are sensitive to molybdenum deficiencies.

Section 2.6 The role of cobalt in leguminous plants

Like molybdenum, cobalt aids in the growth and development of nodulated plants such as soybean (Hewitt and Bond, 1966). However, its status as an essential plant micronutrient is still debated as its physiological function remains poorly understood (Bakkaus et al., 2005). Cobalt is absorbed in the form Co^{2+} and stored primarily in the roots and the leaves where is associates with various enzymes and co-enzymes (Barker and Pilbeam, 2015; Chatterjee and Chatterjee, 2000). The beneficial effects of cobalt application on leguminous plants have been largely attributed to symbiotic microorganisms (Ahmed and Evans, 1960; De Hertogh et al., 1964; Nicholas et al., 1962a; Nicholas et al., 1962b; Palit et al., 1994). Cobalt, in the form of vitamin B_{12}, is essential for the growth of various organisms which fix inorganic nitrogen. Ahmed and Evans (1960) first demonstrated that applied cobalt is used primarily by the rhizobacteria in the soybean nodules and the plant itself did not necessarily directly benefit from an increased cobalt supply. This was later attributed to a potential need for cobalt in the formation of nitrate reductase as the activity of this enzyme decreased in cobalt-deficient cells (Nicholas et al., 1962a).

Section 3 The role of sulfur in plant metabolism:

Sulfur has been recognized as an essential element for plant nutrition for over 200 years, with its first recorded use in agriculture dating back to the 1700's (Duke and Reisenauer, 1986). It is absorbed as sulfate (SO_4^{-2}) and stored in the vacuole where its release is heavily regulated by a series of pathways (Hawkesford and De Kok, 2006). Sulfur requirements can depend on species and developmental stage, with higher plants needing the element for producing amino acids (cysteine, methionine), oligopeptides, vitamins, coenzymes, and iron-sulfur clusters of enzymes (Hawkesford and De Kok, 2006; Saito, 2004; Vauclare et al., 2002). Together, these compounds play important roles in primary metabolism, protein synthesis, regulating stress responses, and photosynthesis (Lunde et al., 2008; Rausch and Wachter, 2005).

The role of sulfur in the stress response:

A highly complex network of sulfur-containing defence compounds (SDCs) plays a key role in mitigating the plant response to abiotic and biotic stress conditions (Rausch and Wachter, 2005). Of these compounds, which range from elemental sulfur to sulfur-rich proteins, glutathione is commonly implicated in protecting the plant against herbicide-induced stress (Madamanchi et al., 1994; Rausch and Wachter, 2005). Glutathione (GSH) is located in the leaves and roots in its reduced form. Under oxidative conditions, GSH is oxidized to form GSSG, and then recycled by glutathione reductase which converts GSSG back to GSH. The ascorbate-glutathione cycle, active in the chloroplast and cytosol, plays a major role in scavenging reactive oxygen species (ROS) produced by herbicides; a decrease in sulfur availability can adversely affect glutathione concentrations (Nikiforova et al., 2005) and thus ROS scavenging capacity (Alla et al., 2008; Mittler et al., 2004). Madamanchi et al. (1994) demonstrated the protective characteristics of GSH, finding that applying SO_2 significantly increased mean GSSG content and reduced mean leaf injury due to herbicide application in a range of pea cultivars. Furthermore, experiments by Alla et al. (2008) concluded that tolerance of a cultivar to a herbicide depended heavily on the concentration of GSH and GSH-associated enzymes. Herbicides which induced GSH production decreased the accumulation of ROS in the leaves and were less phytotoxic.

The role of sulfur in photosynthesis:

The decline in photosynthesis which accompanies a sulfur deficiency is primarily caused by a decrease in chlorophyll content (Nikiforova et al., 2005) and rate of photosynthesis per unit chlorophyll (Burke et al., 1986; Terry, 1976), although decreases in leaf development and maximum leaf area are also observed (Burke et al., 1986). Consequently, both photosystems I and II are adversely affected, with the efficiency decreasing by 61% and 31% respectively (Lunde et al., 2008). Furthermore, the content of RUBISCO is significantly decreased (Gilbert et al., 1997; Sexton et al., 1997). This photosynthetic response potentially accounts for subsequent decreases in total plant biomass under sulfur-deficient conditions (Madamanchi et al., 1994; Nikiforova et al., 2005; Terry, 1976).

Section 4 The Role of Salicylic Acid in the Stress Response:

Salicylic acid (SA) is a signaling molecule which mediates defence responses to abiotic and biotic stresses. Many experiments have reported the beneficial effects of SA application on photosynthesis and plant growth under salt-induced (Khodary, 2004), water-deficit induced (Rajasekaran and Blake, 1999), water-excess induced (Singh and Usha, 2003), and herbicide-induced stress conditions (Ananieva et al., 2002; Ananieva et al., 2004). Although the exact mechanism of the SA-regulated stress response is unknown, at least a major portion of it is most likely due to its role in promoting ROS scavenging. While prolonged exposure to SA results in phytotoxic symptoms, single applications increase the activities of antioxidative enzymes such as Cu,Zn-superoxide dismutase (Ananieva et al., 2002; Rao et al., 1997). As such, the effects of herbicides such as paraquat (Ananieva et al., 2002) and clethodim (Radwan, 2012) were mitigated with the application of SA. Due to its ability to enhance plant recovery from photosynthetic stress, SA is predicted to be most effective when combined with herbicides of the photosynthetic inhibitor groups (Gunes et al., 2007; Klepper, 1991).

Section 5 The Effect of Herbicides on Micronutrient Uptake and Translocation

Investigations into herbicide-nutrient interactions have indicated that the main mechanism of herbicide-induced impairment of nutrient uptake occurs in the soil (Kremer et al., 2009). Herbicides accomplish this by complexing with mineral nutrient cations and/or by reducing the

species of micro-organisms capable of converting non-available forms of nutrients into more available forms. Within the soil environment, many herbicides are known to reduce the bioavailability of nutrients the formation of stable, yet often poorly soluble complexes with mineral cations (Bernard et al., 2005; Kobyłecka and Skiba, 2008; Locke and Bryson, 1997). The minerals that are most susceptible to this process are bivalent cations: Cu^{2+}, Zn^{2+}, Cd^{2+}, Ni^{2+}, Co^{2+}, Mn^{2+}, and Pb^{2+} (Kobyłecka and Skiba, 2008). Soil conditions also facilitate this process as a pH of 6-7 is more likely to generate metal-herbicide complexes (Kobyleck and Skiba, 2008). Furthermore, these complexes between mineral nutrients and herbicides are often antagonistic where the mineral cations reduce herbicide efficacy, while there is a notable decrease in availability of the nutrient to be transformed, absorbed, or transported. These effects have been seen in glyphosate (Eker et al., 2006) as well as atrazine.

Recent evidence points to the detrimental effects of herbicides on the soil microbiota with astounding reductions in the concentration of micro-organisms which modify mineral cations into more available forms (Kremer and Means, 2009). Even in herbicide-resistant cultivars, the herbicide is often exuded in small amounts from the roots into the rhizosphere where it has the potential to influence numerous aspects of the soil microbial environment (Kremer and Means, 2009). The effect of herbicides such as glyphosate have been examined by measuring the population distribution of pseudomonads – a genus of bacteria known for their involvement in the degradation of environmental pollutants, plant growth promoting capabilities, and biocontrol of fungi and other microorganisms (Gyamfi et al., 2002; Kremer and Means, 2009). Gyamfi et al. (2006) found that the extent of herbicide-induced declination of pseudomonad population was dependent on at least two factors: stage of application and herbicide combination. While the interactions of herbicides in the uptake and transport of manganese and zinc are well known, there is relatively little evidence for the chemicals effect on boron and molybdenum availability.

Interactions of Herbicides in the Uptake and Transport of Manganese

The effects of herbicides on manganese uptake and translocation are popularly studied with regard to glyphosate. Within the rhizosphere, glyphosate interacts indirectly with manganese to reduce availability for plant uptake, reducing the microbial populations required for manganese

transformation before absorption (Thompson and Huber, 2007; Thompson and Cobb, 1987). Kremer et al. (2009) found that the concentration of fluorescent pseudomonads, contributing to the suppression of fungal pathogens (Schroth and Hancock, 1982) and Mn reduction (Eker et al., 2006; Rengel, 1997) decreased with the application of glyphosate. The disproportionate ratio of Mn-reducers to Mn-oxidizers resulted in an immobility of manganese in the soil, despite its relative abundance. Even with recognizing the detriment of herbicides to the availability of manganese in the soil, the link between the immobilisation of manganese within the plant remains a mystery.

Despite the known methods of glyphosate-mineral nutrient interaction, the results of investigations into the effects of glyphosate on Mn absorption and translocation are highly conflicting. Although some studies have found no significant effect of glyphosate on root or shoot Mn concentration (Nava et al., 2015) many others have reported highly significant effects (Bott et al., 2008; Cakmak et al., 2009). In fact, Cakmak (2009) concluded that glyphosate potentially decreases the availability of Mn by binding to and immobilizing the micronutrient. The work of King et al. (2001) partially supports this hypothesis in finding reduced nodulation and nitrogen fixation following glyphosate usage in some early-season GR soybean cultivars. Furthermore, findings by Skiba et al. (2017) suggest that soils influenced by previous farming practices may be a contributing factor in the effects of synthetic auxin herbicides on mineral nutrient absorption and translocation. Treated plants grown in soil sampled from a farming region showed decreased levels of manganese in the roots and the shoots whereas those grown in soil sampled from a non-farmed rural region indicated decreased levels of manganese only in the shoots (Skiba et al., 2017).

Interactions of Herbicides in the Uptake and Transport of Zinc

The effects of herbicides on reducing zinc availability is highly variable, depending largely upon the herbicide used. Investigations into the effect of the ALS inhibitor chlorsulfuron, used primarily in wheat, have revealed a significant decrease in zinc uptake and root weight (Dong et al., 1995; McLay and Robson, 1992; Osborne and Robson, 1992; Robson and Snowball, 1989). However, these defects in root development and zinc uptake were partially recovered six and

eight weeks after treatment with chlorsulfuron, respectively (Osborne and Robson, 1992). In addition, McLay and Robson (1992) found that both the cell membrane disruptor diclofop-methyl decreased both shoot and root weight, as well as Zn uptake. This investigation also saw that with increasing herbicide concentrations, the roots grew shorter and thicker; a possible consequence of Zn deficiency. On the contrary, not all herbicides yield this response as Robson and Snowball (1989) found that 2,4-D did not affect the zinc status of the plant, or the roots weight or length. However, like with manganese, the uptake of zinc is primarily inhibited through the formation of mineral nutrient-herbicide complexes. (Scroggs et al., 2009; Wang et al., 2006).

Materials and Methods

General field experimentation in summers 2015 and 2016

Field trials were conducted over a period of two years (2015 and 2016) at the Emile A. Lods Agronomy Research Centre at the Macdonald Campus of McGill University in Sainte-Anne-de-Bellevue, QC. All treatments were organized on the field site following a complete randomized complete block design with four blocks. The wheat trials consisted of 14 rows of wheat planted 18 cm apart at a depth of 2 cm and a density of 4,000,000 seeds ha^{-1}. The individual plots were 2.6 m wide and 5 m long. Each corn plot consisted of 8 rows of corn planted 75 cm apart at a depth of 2 cm and a density of 70,000 seeds ha^{-1}. The plots were 6 m wide and 5 m long. The soybean trials consisted of 14 rows of soybeans planted 18 cm apart at a depth of 2 to 2.5 cm and a density of 40,00 seeds ha^{-1}. The plots were 2.6 m wide and 5 m long.

Cultivation of the field sites occurred in the fall and directly before seeding. Soil samples, using a soil core at a depth of 15 cm, were collected prior to seeding and post-harvest for the determination of physio-chemical characteristics. Nitrogen fertilization for corn and wheat was based upon standard agronomic practices for the area, following the results of the soil analyses. The weather data was collected from a site maintained at the Lods Centre, in part to know when conditions following treatments were also environmentally stressful. The plots were not irrigated throughout the growing season.

During the season, each plot was sampled for plant height, leaf area and dry weight at four phenological stages: mid-vegetative, mid-flowering, mid-grain filling, and harvest stage. For wheat, 10 plants were randomly sampled for height and leaf area, and 1 m of plants were sampled for dry weight. Plant height was measured in the vegetative stage by elongating the largest leaf; in the following stages height was recorded as the distance from the ground to the tip of the spike. In corn, plant height was measured at the vegetative stage by elongating the leaves; at the following stages the height was recorded as the distance from the ground to the tip of the flower. In soybean, plant height was measured at the vegetative stage by elongating the leaves; height was recorded as the distance from the ground to the apex. Leaf area was recorded using

the Li-3100 Area Meter; the stems were not included in the measurement. For the dry weight, the samples were dried at 65 degrees Celsius until constant weight.

A Li-Cor 6400 portable photosynthesis meter was used to assess the average photosynthetic rate of each plot. In addition to photosynthetic rate, data on transpiration rate, stomatal aperture, leaf temperature and CO_2 concentration inside the leaf were also collected. The plants were randomly sampled from each plot. For wheat, the measurements were taken from the flag leaf. For corn, the measurements were taken from the second youngest fully unfolded leaf. Plants were randomly selected and measurements were taken from the middle leaf of the second oldest trifoliate. Visual crop injury was observed and noted.

For each crop, the following harvest variables were recorded: fresh seed weight, dry seed weight, moisture content, and harvest index. For corn the number of cobs per plant and seeds per cob were recorded with five replications per plot. For soybean, the number of pods and seeds per plant were recorded with five replications per plot. In wheat, two 1 m samples were taken to calculate the harvest index. In corn and soybean, 5 plant samples were collected to calculate the harvest index.

Conditions of the field season of summer 2015

In the summer of 2015, neither pre-emergence herbicides, nor glyphosate burndowns were applied. All post-emergence treatments were applied with a bicycle sprayer (attempts to obtain manufacturing information of this equipment were not sucessful). In spring wheat, post emergence treatments consisted of Buctril M (1 L ha^{-1}) and Crop Booster (CB) (2.0 L ha^{-1}) applied 44 days after planting (DAP). In corn, post emergence treatments consisted of Roundup Weathermax (1.6 L ha^{-1}) and Aatrex 480 (1.56 L ha^{-1}) and Crop Booster (2.0 L ha^{-1}) applied 37 DAP. In soybean, post-emergence treatments with Flexstar GT (3.5 L ha^{-1}) and Crop Booster (2.0 L ha^{-1}) were made 43 DAP. The photosynthetic variables were recorded one day prior to and one day after the post-emergence treatments.

Conditions of the field season of summer 2016

Due to weed pressure experienced in the summer of 2015, the herbicide application treatment was altered to support a weed-free environment throughout the critical vegetative stages. All pre-emergence herbicides were applied using an aerial sprayer. In corn and soybean, Roundup Weathermax (1.6 L ha^{-1}) was applied to all plots with the HARDI NK-400 spray machine (HARDI, Ohio, USA) equipped with TeeJet Al-11003 spray nozzles (TeeJet Technologies, Illinois, USA) two weeks prior to post-emergence treatment for further weed control. All post-emergence treatments were applied with a hand-pumped pressurized back-pack sprayer. In spring wheat, Focus (177 g ha^{-1}) and Eragon (30 mL ha^{-1}) were applied for pre-emergence weed control. Post emergence treatments with herbicides and foliar nutrient treatments were made 35 days after planting (DAP). In corn, Integrity (1.1 L ha^{-1}) was applied to all plots for pre-emergence weed control. Post emergence treatments with herbicide and foliar nutrient formulations were made 30 DAP. For soybean, Frontier (2.5 L ha^{-1}) and Sencor (1.75 L ha^{-1}) were applied for pre-emergence weed control. Post emergence treatments with herbicide and micronutrient formulations were made 23 DAP. Photosynthetic variables were recorded one and three days prior to and one and three days after the post-emergence treatments. The timeline of

Conditions of the greenhouse experiment of winter 2017

The greenhouse experiment consisted of a randomized complete block design with four blocks and six plants per treatment per block. The plants were sown in Agromix G10 media (Fafard, Massachusetts, USA) at a depth of 1.5 cm. From the seedling to the four-leaf stage the plants were watered with 100 microliters of water per two days. From the four-leaf stage to harvest the plants were irrigated with 140 µL of water per two days. The treatments consisted of Buctril M (1.0 L ha^{-1}) and Crop Booster 2 (2.0 L ha^{-1}) applied 45 days after planting at the sixth leaf to early flag leaf stage. The treatments were applied to individual plants using a handheld sprayer at a distance of 20 cm. One day before and one day after treatment, photosynthetic measurements were recorded using the LI-6400 portable photosynthesis machine. The dry weight and height were recorded at the time of harvest 14 days after treatment.

General Statistical Methodology

The following analyses employ mixed modeling (SAS PROC MIXED with RANDOM block) and generalized linear mixed modeling (SAS PROC GLIMMIX with RANDOM block) that fit error structures with distributions that were not normal. The mixed model formulae have in common fixed effects on the observed variables due to the treatments and random effects due to the blocks. In the cases of non-normal (error) distribution, the distributions were selected for generalized linear mixed models based on the goodness of model fit (Bayesian information criterion). The mean and standard error of data modeled with a Gamma, Gaussian, or inverse Gaussian distributions were inverse-linked to the scale of observation; for those modeled with a logarithmic distribution, the mean was exponentiated to the scale of observation. The statistical significance of differences, and Bonferroni-adjusted limits with 95% confidence intervals, were computed. The Bonferroni adjustment was used as a multiple comparison post-hoc correction. When the data was modeled with the normal, logarithmic, or Gamma distribution, the upper and lower limits of the difference between the means as indicated by the 95% confidence interval were included (whenever the *p*-value of the difference was significant) to indicate the extent of variability expected for the treatment effect; the limits were not calculated for estimates on the inverse Gaussian scale. The differences between natural logged values are unitless ratios that were expressed as percentages. When the photosynthetic rate between the treatment groups did not differ ($\alpha = 0.05$), it was assumed that the experimental units were equally stressed or unstressed, and the data was not included in subsequent analyses.

Results

Corn photosynthetic, growth and harvest variables in the field

Summer 2015 corn photosynthetic measurements in response to herbicide and/or foliar nutrient treatments

The data indicates no difference in photosynthetic rate between the experimental groups one day before post-emergence treatment (Figure 1.1). Furthermore, the experimental treatments did not have a significant effect on the photosynthetic rate (Figure 1.2).

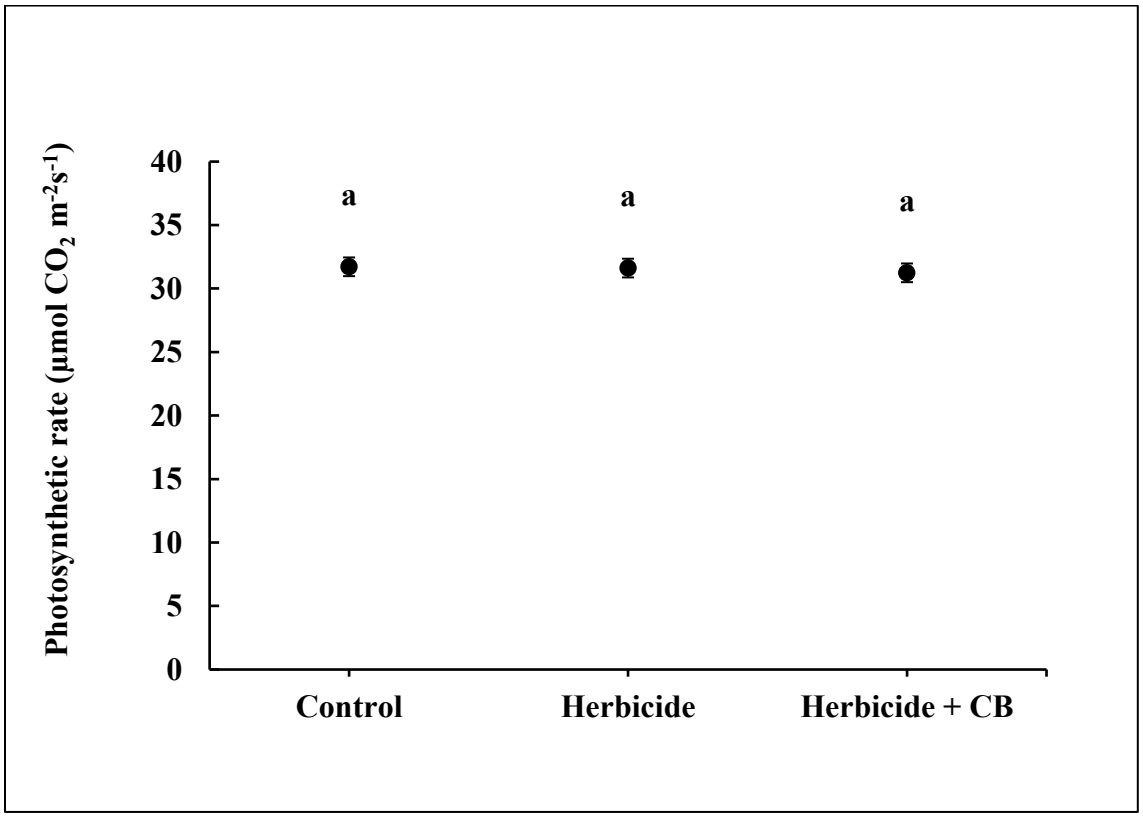

Figure 1.1 LS-Means plot of corn photosynthetic rate one day before post-emergence treatments in summer 2015 (n=12, p=0.891). Whiskers indicate standard error and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

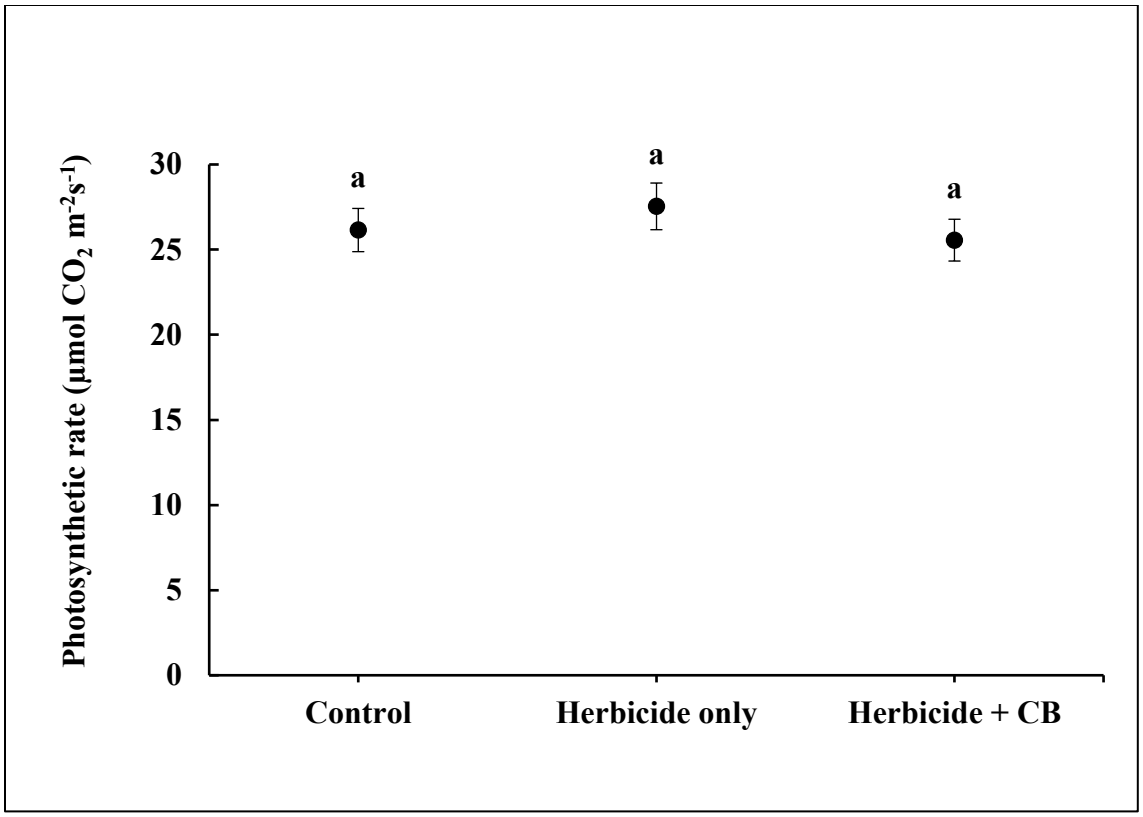

Figure 1.2 Inverse Linked LS-Means plot of corn photosynthetic rate one day after post-emergence treatment in summer 2015 (n=12, p=0.543). Whiskers indicate standard error and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

Summer 2015 corn growth and harvest variables in response to herbicide and/or foliar nutrient treatments

The experimental treatments did not affect the height, leaf area, or dry weight of the corn at most developmental stages (Tables 1.1, 1.2, 1.3). At the mid-flowering stage, the data indicates a difference in leaf area due to treatment (Table 1.2). The water-treated (control) plants would be 429.82 cm^2 to 1653.76 cm^2 larger than the plants treated with a combination of herbicides and Crop Booster. Similarly, the plants treated only with herbicides would be 664.54 cm^2 to 1888.48 cm^2 larger than those subjected to a combination of herbicides and Crop Booster. No significant difference was detected between the water-treated (control) plants and the plants treated only with herbicide. Similarly, there was no effect of the treatments on the harvest index or yield (Table 1.4).

Table 1.1 Summer 2015 corn height in response to herbicide and foliar nutrient applications

Stages	Control	Herbicide only	Herbicides + CB	p-value
Mid-vegetative	93.34 ± 1.92 [a]	96.94 ± 1.92 [a]	92.58 ± 1.92 [a]	0.2539
Mid-flowering	284.55 ± 6.49 [a]	281.65 ± 6.49 [a]	258.50 ± 6.49 [a]	0.0562
Mid-Grain filling	291.45 ± 4.44 [a]	290.85 ± 4.44 [a]	281.50 ± 4.44 [a]	0.2809
Harvest	276.99 ± 4.51 [a]	272.54 ± 4.51 [a]	263.75 ± 4.51 [a]	0.1889

LS-Means with standard error of corn height at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 1.2 Summer 2015 corn leaf area in response to herbicide and foliar nutrient applications

Stages	Control	Herbicide only	Herbicide + CB	p-value
Mid-vegetative	1279.75 ± 99.48 [a]	1251.75 ± 99.48 [a]	920.25 ± 99.48 [a]	0.0341
Mid-flowering	4451.71 ± 131.63 [a]	4686.43 ± 131.63 [a]	3409.92 ± 131.63 [b]	0.0010
Mid-Grain filling	4707.15 ± 212.92 [a]	4247.20 ± 212.92 [a]	4064.05 ± 212.92 [a]	0.1694

LS-Means with standard error of corn leaf area at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 1.3 Summer 2015 corn 5-plant dry weight in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	Herbicide + CB	p-value
Mid-vegetative	62.30 ± 9.72 [a]	73.90 ± 9.72 [a]	39.30 ± 9.72 [a]	0.0741
Mid-flowering	169.21 ± 28.22 [a]	177.99 ± 28.22 [a]	175.30 ± 28.22 [a]	0.9750
Mid-Grain filling	909.91 ± 73.58 [a]	826.54 ± 73.58 [a]	730.23 ± 73.58 [a]	0.2976
Harvest	1290.28 ± 66.36 [a]	1515.78 ± 66.36 [a]	1555.67 ± 66.36 [a]	0.0603

LS-Means with standard error of corn 5-plant dry weight at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 1.8 Summer 2015 corn harvest variables in response to herbicide and foliar nutrient application

	Control	Herbicide only	Herbicide + CB
Harvest index	0.572 ± 0.017 [a]	0.588 ± 0.017 [a]	0.597 ± 0.017 [a]
Harvest yield (kg ha^{-1})	6951.67 ± 196.75 [a]	7259.68 ± 196.75 [a]	6827.32 ± 196.75 [a]

LS-Means with standard error of harvest index (n=20, p=0.412) and yield (kg ha^{-1}) (n=4, p=0.1667) in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Summer 2016 corn photosynthetic measurements in response to herbicide and/or foliar nutrient treatments

The data indicates no difference in photosynthetic rate between the experimental groups one day before post-emergence treatment (Figure 1.3). However, the experimental treatments affected the photosynthetic rate of corn to a highly significant degree (Figure 1.4). Based on the Bonferroni-adjusted limits at 95% confidence, the mean photosynthetic rate of corn plots treated with water only (control) would/should be 21 to 70% higher than mean of plots treated with herbicides only; 4 to 39% higher than the mean of plots treated with CB2 only corn and 3 to 40% higher than the mean of plots treated with a combination of herbicides and CB2. The photosynthetic rate of plots treated with herbicide only would be 29 to 1% lower/slower than the mean of plots treated with CB2 only, and 29 to 1% lower than the mean of plots treated with a combination of herbicides and CB2. The means of photosynthetic rate for plots treated with CB2 only and those treated with a combination of herbicides and CB2 were statistically equivalent.

Furthermore, the effects of the experimental treatments on corn photosynthetic rate were still detectable three days after treatment (Figure 1.4). Based on the Bonferroni-adjusted limits at 95% confidence, the means photosynthetic rate of plots treated with CB2 only would be 0.38 to 4.96 µmol CO_2 m^{-2} s^{-1} lower/slower than those treated with herbicides only, and 4.88 to 0.28 µmol CO_2 m^{-2} s^{-1} lower than the plots treated with a combination of herbicides and CB2. The mean photosynthetic rate for plots treated with water only was statistically equivalent to plots

treated with herbicides only, and plots treated with a combination of herbicides and CB2. There were no statistically significant differences detected between the plots treated with water only and those treated with CB2 only. Moreover, the mean photosynthetic rate of plots treated with herbicides only and those treated with a combination of herbicides and CB2 were statistically equivalent.

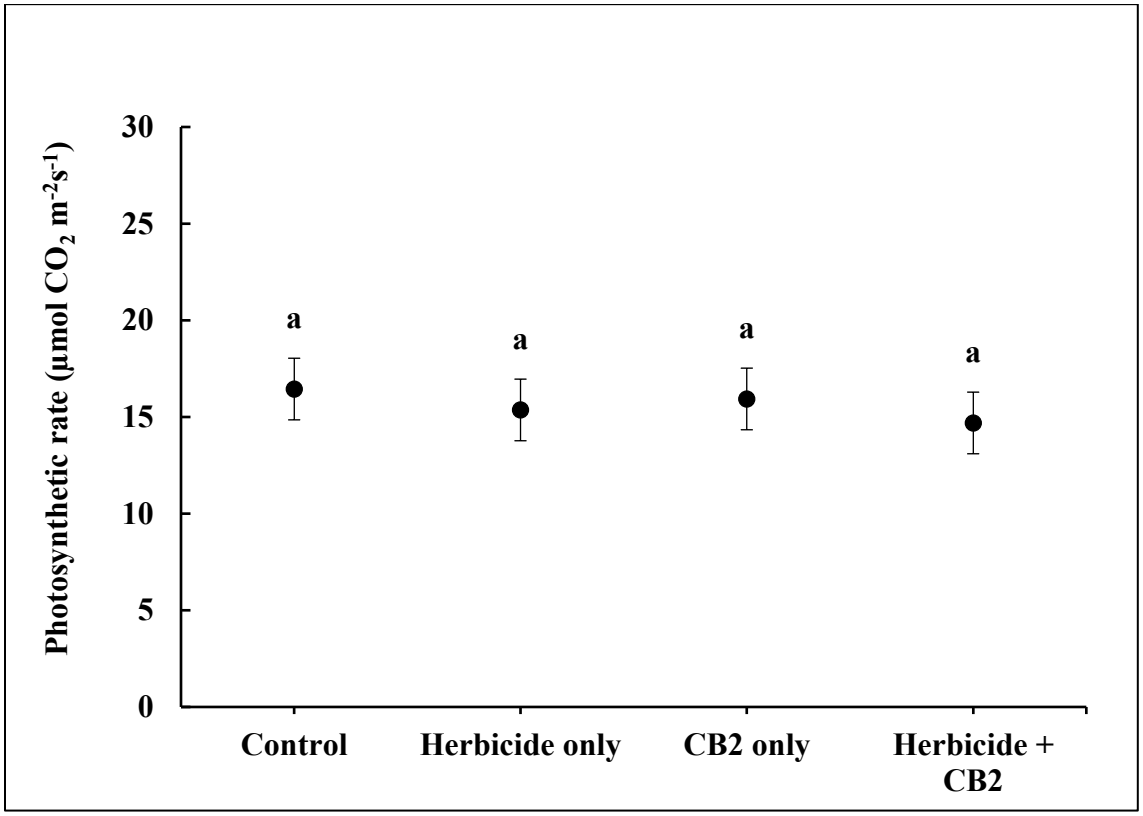

Figure 1.3 LS-Means plot of corn photosynthetic rate one day before post-emergence treatment in summer 2016 (n=20, p=0.5447). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

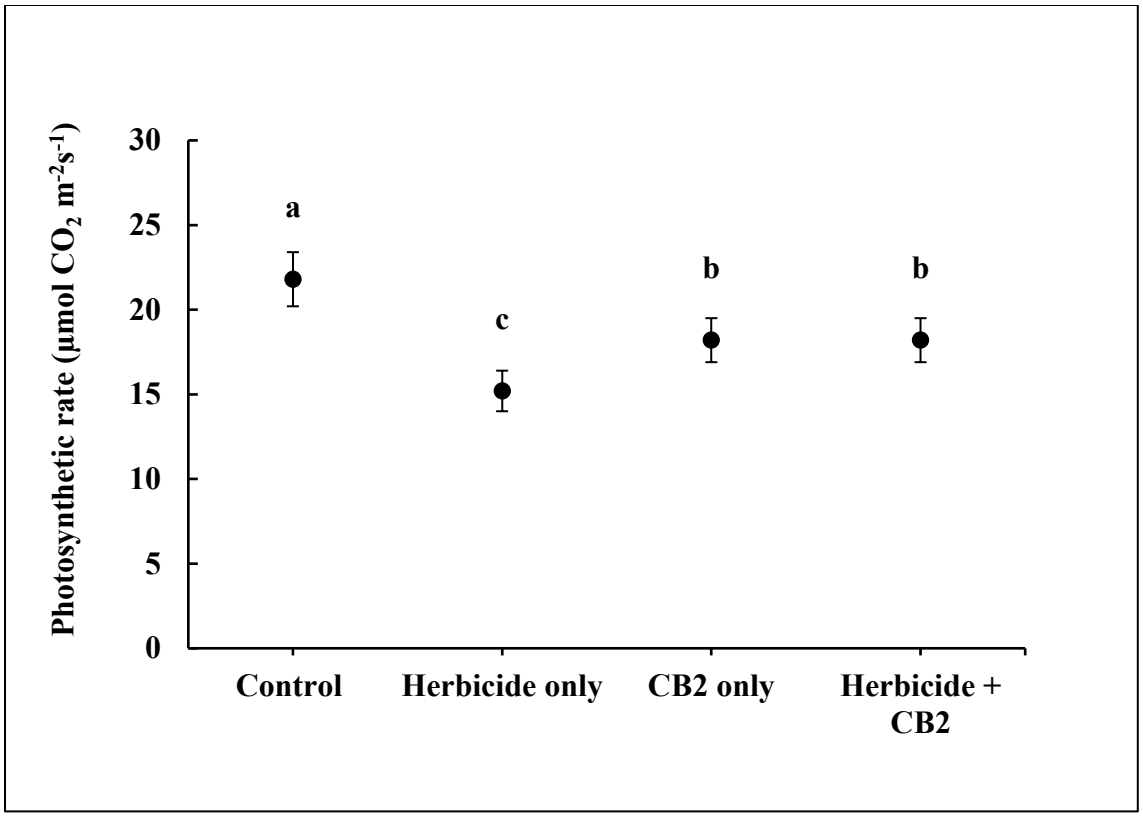

Figure 1.4 LS-Means plot of corn photosynthetic rate one day after post-emergence treatment in summer 2016 (n=20, $p < 0.0001$). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

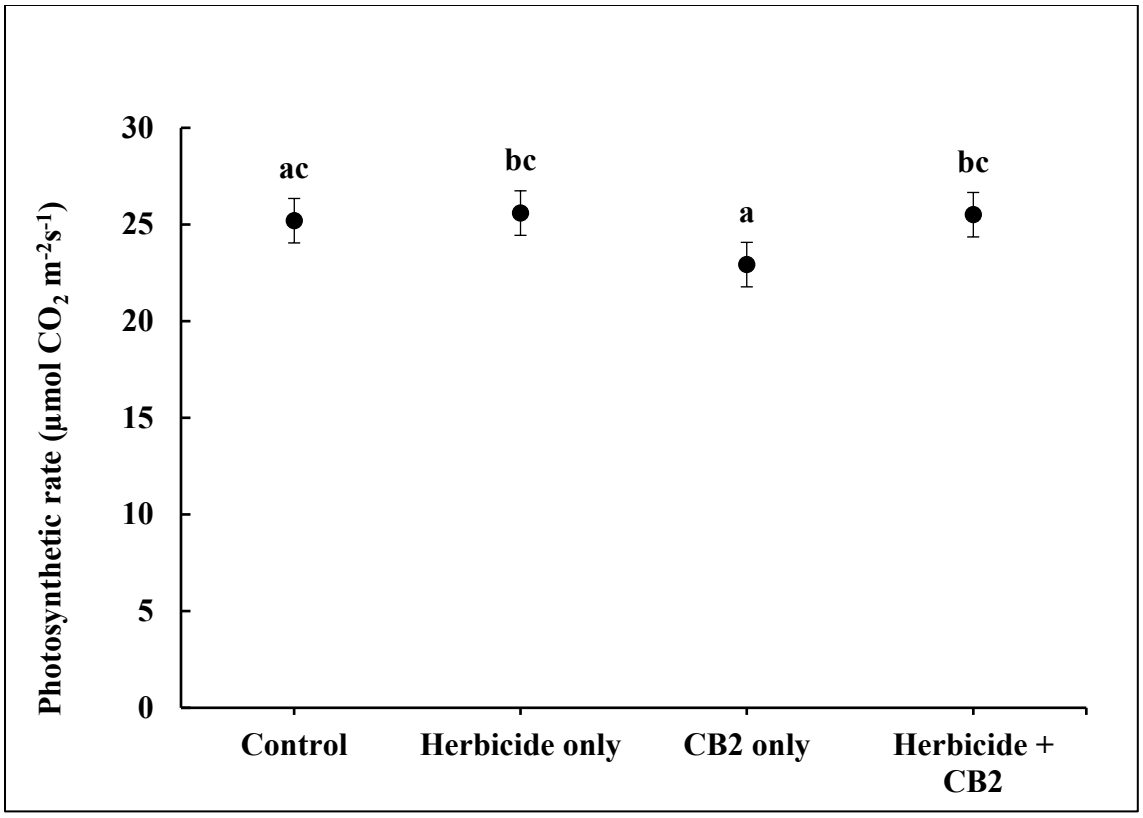

Figure 1.5 LS-Means plot of corn photosynthetic rate three days after post-emergence treatment in summer 2016 (n=20, p=0.0063). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences (α = 0.05) between treatments tested with Bonferroni-adjusted limits.

Summer 2016 growth and harvest variables in response to herbicide and/or foliar nutrient treatments

The experimental treatments did not affect the height, leaf area, or dry weight of the wheat plants at any developmental stage where samples were collected (Table 1.5, 1.6, 1.7). Similarly, there was no effect of the treatments on the yield or harvest index (Table 1.8).

Table 1.5 Summer 2016 corn height in response to herbicide and foliar nutrient applications

Stages	Control	Herbicide only	CB2 only	Herbicide + CB2	p-value
Mid-vegetative	73.30 ± 3.01 [a]	75.81 ± 3.01 [a]	69.45 ± 3.01 [a]	78.48 ± 3.01 [a]	0.2494
Mid-flowering	186.09 ± 6.01 [a]	193.87 ± 6.01 [a]	177.51 ± 6.01 [a]	191.71 ± 6.01 [a]	0.2852
Mid-Grain filling	194.63 ± 17.30 [a]	197.46 ± 17.30 [a]	227.05 ± 17.30 [a]	199.36 ± 17.30 [a]	0.5390
Harvest	153.26 ± 14.77 [a]	173.82 ± 14.77 [a]	142.29 ± 14.77 [a]	164.19 ± 14.77 [a]	0.3145

LS-Means with standard error of summer 2016 corn height at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 1.6 Summer 2016 corn leaf area in response to herbicide and foliar nutrient applications

Stages	Control	Herbicide only	CB2 only	Herbicide + CB2	p-value
Mid-vegetative	918.37 ± 0.32 [a]	1206.04 ± 0.32 [a]	915.98 ± 0.32 [a]	1027.54 ± 0.32 [a]	0.3159
Mid-flowering	2949.62 ± 157.64 [a]	3166.06 ± 157.64 [a]	2684.73 ± 157.64 [a]	3045.34 ± 157.64 [a]	0.2390
Mid-Grain filling	2369.78 ± 115.48 [a]	2678.26 ± 115.48 [a]	2457.40 ± 115.48 [a]	2637.54 ± 115.48 [a]	0.2549

LS-Means with standard error of corn leaf area at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 1.7 Summer 2016 corn 5-plant dry weight in response to herbicide and foliar nutrient applications

Stages	Control	Herbicide only	CB2 only	Herbicide + CB2	p-value
Mid-vegetative	39.85 ± 7.09 [a]	52.36 ± 7.09 [a]	45.24 ± 7.09 [a]	46.80 ± 7.09 [a]	0.4887
Mid-flowering	326.73 ± 4.87 [a]	356.25 ± 24.87 [a]	288.67 ± 24.87 [a]	343.42 ± 24.87 [a]	0.3073
Harvest	189.94 ± 3.04 [a]	187.93 ± 13.04 [a]	197.59 ± 13.04 [a]	205.65 ± 13.04 [a]	0.7181

LS-Means with standard error of corn 5-plant dry weight at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 1.8 Summer 2016 corn harvest variables in response to herbicide and foliar nutrient application

Variable	Control	Herbicide only	CB2 only	Herbicide + CB2	p-value
Harvest index	0.613 ± 0.018^{a}	0.6129 ± 0.018^{a}	0.6427 ± 0.018^{a}	0.6341 ± 0.018^{a}	0.5036
Yield (kg ha^{-1})	5267.97 ± 292.55^{a}	6437.18 ± 292.55^{a}	5425.49 ± 292.55^{a}	6368.67 ± 292.55^{a}	0.0293

LS-Means with standard error of harvest index (n=20) and yield (kg ha^{-1}) (n=4) in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Soybean photosynthetic, growth and harvest variables in the field

The effect of herbicide and nutrient applications on soybean photosynthetic, growth, and harvest variables in field seasons of summer 2015

The data indicates no difference in photosynthetic rate between the experimental groups one day before post-emergence treatment (Figure 2.1). Furthermore, the experimental treatments did not have a significant effect on the photosynthetic rate one day after treatment (Figure 2.2).

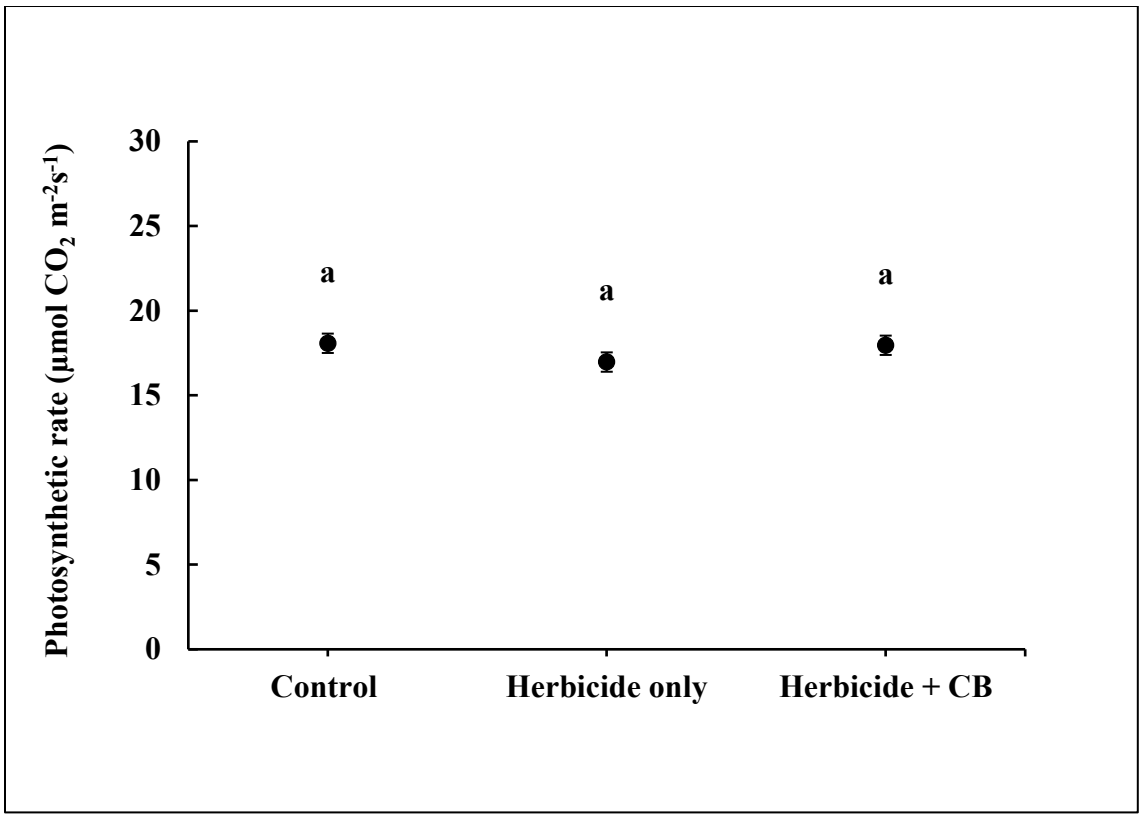

Figure 2.1 LS-Means plot of soybean photosynthetic rate one day before post-emergence treatment in summer 2015 (n=12, p=0.5714). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

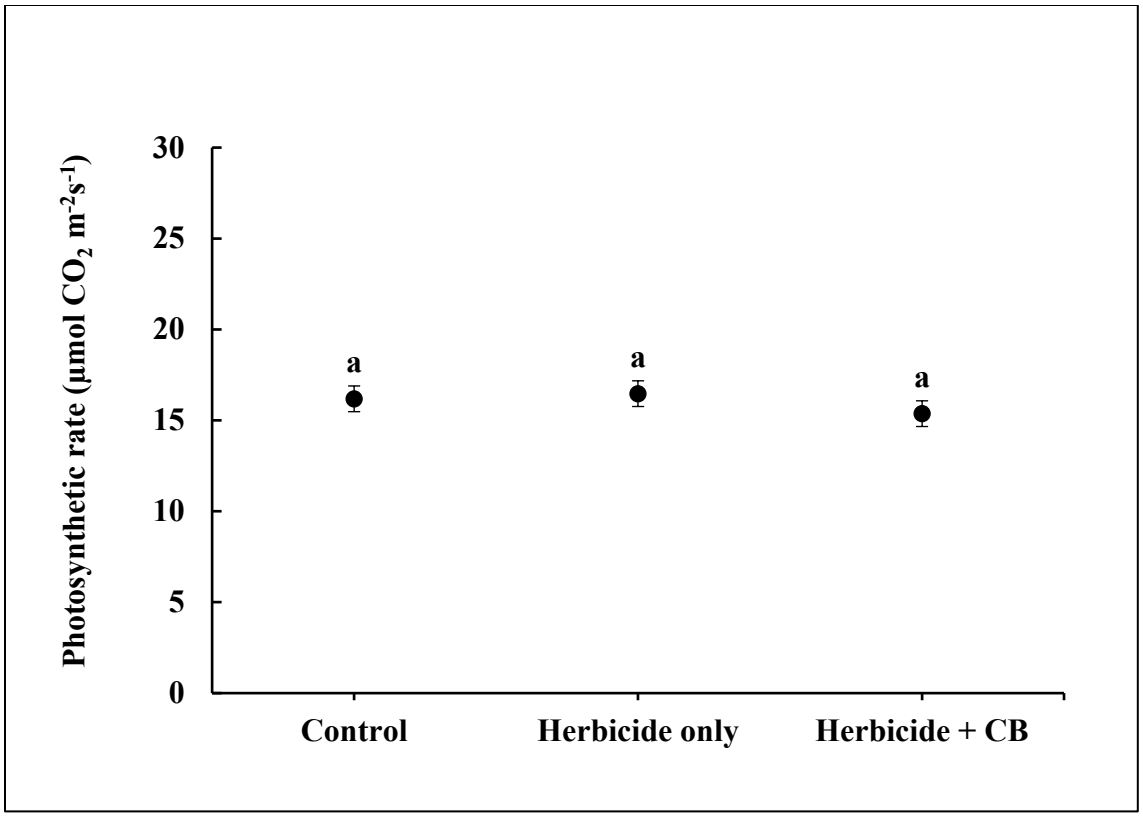

Figure 2.2 LS-Means plot of soybean photosynthetic rate one day after treatment in summer 2015 (n=12, p=0.3483). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$, n=12) between treatments tested with Bonferroni-adjusted limits.

Growth and harvest variables in response to herbicide and nutrient application for soybean in summer 2015

There were no statistically significant differences in height (Table 2.1), leaf area (Table 2.2), or dry weight (Table 2.3) of the soybean due to the experimental treatments at most developmental stages. At the mid-flowering stage, the mean height of plots treated with water only (control) should/would be 0.1596 to 15.1804 cm higher than those treated with a combination of herbicide and Crop Booster. Although there was no effect on the harvest index due to treatment, harvest yield was affected to a highly significant degree (Table 2.4). The Bonferroni-adjusted limits at 95 % confidence indicated that the mean harvest yield of plots treated with water only (control) would/should be 4726.61 to 2648.36 kg ha^{-1} lower than the means of those treated with herbicide only, and 4042.72 to 2585.90 kg ha^{-1} lower than the means of those treated with a combination of herbicide and Crop Booster.

Table 2.1 Summer 2015 soybean height in response to herbicide and foliar nutrient applications

Stages	Control	Herbicide only	Herbicide + CB	p-value
Mid-vegetative	47.09 ± 1.23 [a]	43.65 ± 1.23 [a]	42.13 ± 1.23 [a]	0.0470
Mid-flowering	57.56 ± 2.39 [a]	50.605 ± 2.39 [a]	49.89 ± 2.39 [a]	0.0280
Mid-Grain filling	90.02 ± 3.11 [a]	83.94 ± 3.11 [a]	80.69 ± 3.11 [a]	0.0743
Harvest	82.95 ± 2.50 [a]	79.7 ± 2.50 [a]	74.85 ± 2.50 [a]	0.1173

LS-Means with standard error of soybean height at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 2.2 Summer 2015 soybean leaf area in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	Herbicide + CB	p-value
Mid-vegetative	400.35 ± 22.87 [a]	398.70 ± 22.87 [a]	360.85 ± 22.87 [a]	0.3823
Mid-flowering	405.18 ± 21.29 [a]	393.09 ± 21.29 [a]	359.80 ± 21.29 [a]	0.1137
Mid-Grain filling	542.22 ± 78.42 [a]	899.60 ± 78.42 [a]	664.46 ± 78.42 [a]	0.0461

LS-Means with standard error of soybean leaf area at different developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 2.3 Summer 2015 soybean dry weight in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	Herbicide + CB	p-value
Mid-vegetative	12.28 ± 0.74 [a]	13.325 ± 0.74 [a]	11.85 ± 0.74 [a]	0.3904
Mid-flowering	16.05 ± 0.85 [a]	16.70 ± 0.85 [a]	15.75 ± 0.85 [a]	0.6627
Mid-Grain filling	28.38 ± 4.11 [a]	44.80 ± 4.11 [a]	35.13 ± 4.11 [a]	0.0777
Harvest	8.35 ± 1.71 [a]	10.80 ± 1.71 [a]	8.98 ± 1.71 [a]	0.6008

LS-Means with standard error of soybean dry weight at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 2.4 Summer 2015 soybean harvest variables in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	Herbicide + CB	p-value
Harvest index	0.568 ± 0.018 [a]	0.607 ± 0.020 [a]	0.580 ± 0.020 [a]	0.3956
Yield	2995.91 ± 312.43 [a]	6683.40 ± 336.14 [b]	6310.22 ± 336.14 [b]	0.0003

LS-Means with standard error of the soybean harvest index (n=20) and yield (kg ha^{-1}) (n=4) in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Soybean photosynthetic rates resulting from herbicide and micronutrient usage in 2016

The data indicates no difference in photosynthetic rate one day before post-emergence treatment (Figure 2.3). Furthermore, the experimental treatments have no effect on the photosynthetic rate one day after post-emergence treatment (Figure 2.4).

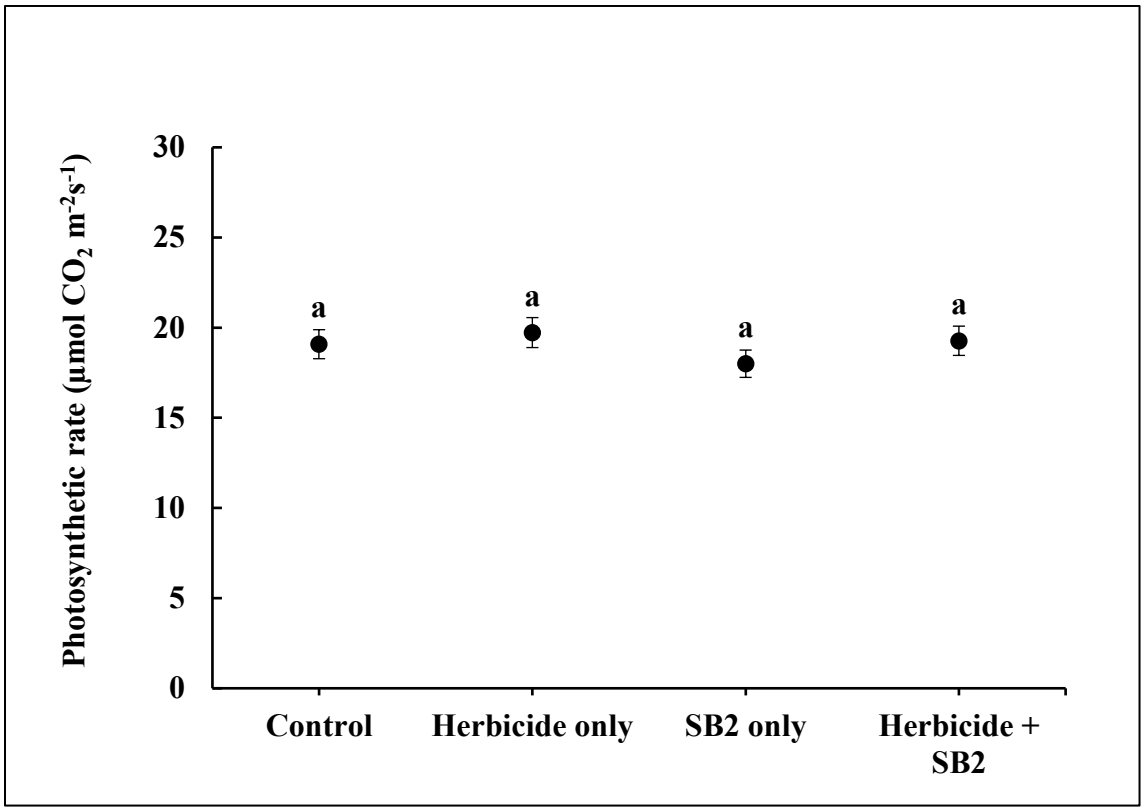

Figure 2.3 Inverse linked LS-Means plot of soybean photosynthetic rate one day before treatment in summer 2016 (n=20, p=0.2828). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

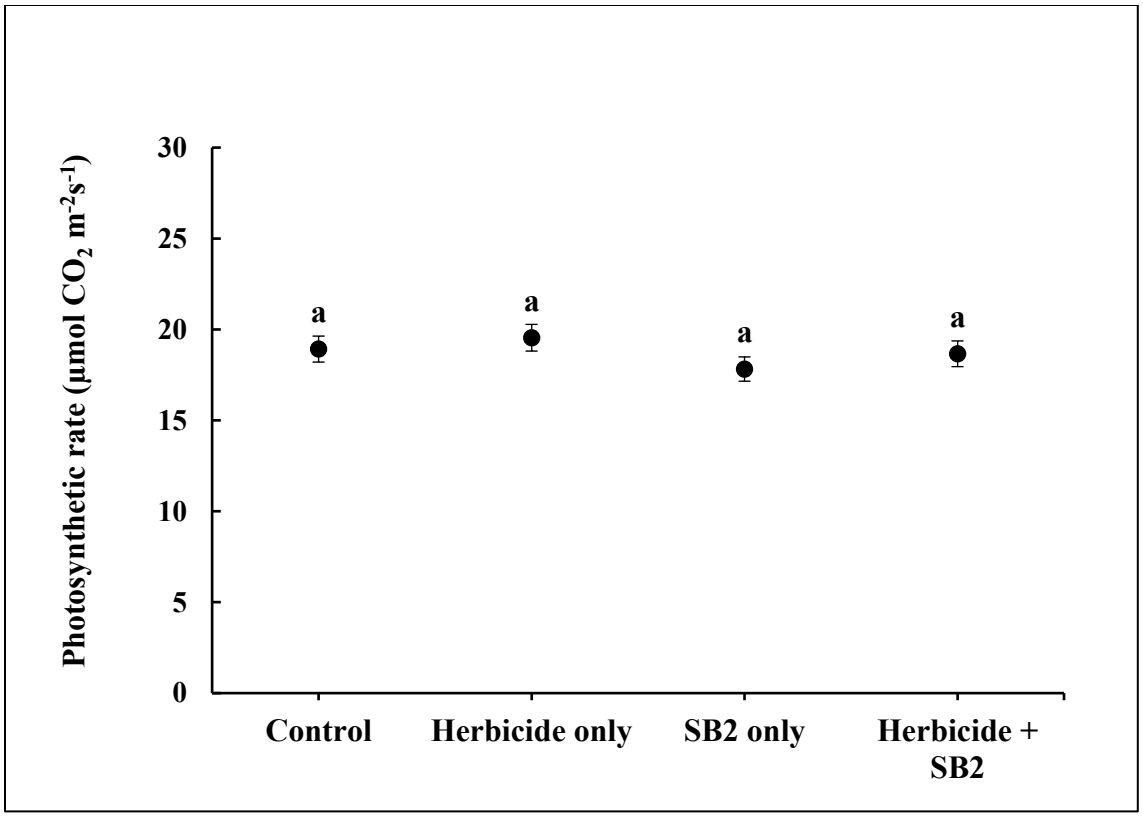

Figure 2.4 Inverse-linked LS-Means plot of soybean photosynthetic rate one day after treatment in summer 2016 (n=20, p=0.6552). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

Growth and harvest variables in response to herbicide and nutrient application for soybean in summer 2016

There were no statistically significant differences in height (Table 2.5), leaf area (Table 2.6), or dry weight (Table 2.7) of the soybean due to the experimental treatments at most developmental stages. Similarly, there were no statistically significant differences in harvest index or yield (Table 2.8).

Table 2.5 Summer 2016 soybean height in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	SB2 only	Herbicide + SB2	p-value
Mid-vegetative	23.55 ± 0.70 [a]	24.05 ± 0.70 [a]	21.78 ± 0.70 [a]	22.25 ± 0.70 [a]	0.1461
Mid-flowering	34.40 ± 1.53 [a]	33.87 ± 1.53 [a]	31.34 ± 1.53 [a]	33.28 ± 1.53 [a]	0.4676
Mid-Grain filling	73.56 ± 2.00 [a]	75.37 ± 2.00 [a]	71.60 ± 2.00 [a]	76.89 ± 2.00 [a]	0.3007
Harvest	69.46 ± 3.67 [a]	72.04 ± 3.67 [a]	73.49 ± 3.67 [a]	72.97 ± 3.67 [a]	0.8448

LS-Means with standard error of summer 2016 soybean height at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 2.6 Summer 2016 soybean leaf area in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	SB2 only	Herbicide + SB2	p-value
Mid-vegetative	126.23 ± 13.30 [a]	116.07 ± 13.30 [a]	122.06 ± 13.30 [a]	116.21 ± 13.30 [a]	0.9352
Mid-flowering	290.22 ± 37.02 [a]	262.26 ± 37.02 [a]	292.00 ± 37.02 [a]	258.52 ± 37.02 [a]	0.8409
Mid-Grain filling	1132.68 ± 155.96 [a]	1504.94 ± 155.96 [a]	1074.52 ± 155.96 [a]	1201.80 ± 155.96 [a]	0.2778

LS-Means with standard error of summer 2016 soybean leaf area at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 2.7 Summer 2016 soybean dry weight in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	SB2 only	Herbicide + SB2	p-value
Mid-vegetative	4.85 ± 0.44 [a]	4.65 ± 0.44 [a]	4.70 ± 0.44 [a]	5.30 ± 0.44 [a]	0.7231
Mid-flowering	13.28 ± 1.40 [a]	12.65 ± 1.40 [a]	12.65 ± 1.40 [a]	11.08 ± 1.40 [a]	0.6209
Harvest	32.38 ± 1.85 [a]	33.51 ± 1.85 [a]	24.96 ± 1.85 [a]	28.39 ± 1.85 [a]	0.0336

LS-Means with standard error of summer 2016 soybean dry weight at four developmental growth stages in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05, n=20) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Table 2.8 Summer 2016 soybean harvest variables in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	SB2 only	Herbicide + SB2	p-value
Harvest index	0.615 ± 0.006 [a]	0.619 ± 0.006 [a]	0.611 ± 0.006 [a]	0.611 ±0.006 [a]	0.2976
Yield (kg/ha)	7468.58 ± 416.69 [a]	7544.27 ± 416.69 [a]	7616.71 ± 416.69 [a]	7234.24 ± 416.69 [a]	0.6705

LS-Means with standard error of the soybean harvest index (n=20) and yield (kg ha^{-1}) (n=4) in response to herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences (α=0.05) between treatments tested with Bonferroni-adjusted limits with 95% confidence.

Wheat photosynthetic, growth and harvest variables in the field

Summer 2015 wheat photosynthetic measurements in response to herbicide and/or foliar nutrient treatment

The data indicates no difference in photosynthetic rate between the experimental groups one day before post-emergence treatment (Figure 3.1). However, the experimental treatments did have a significant effect on the photosynthetic rate one day after treatment (Figure 3.2). According to the Bonferoni-adjusted limits at 95% confidence, the photosynthetic rate of plots treated with water only is expected to be 9.7192 to 15.4641 μmol CO_2 m^{-2} s^{-1} higher/faster than that of the herbicide treated plots, and 12.0963 μmol CO_2 m^{-2} s^{-1} to 17.814 μmol CO_2 m^{-2} s^{-1} higher/faster than that of herbicide and nutrient treated plots. The herbicide treated plots were expected to have between a 0.4954 μmol CO_2 m^{-2} s^{-1} lower/slower rate to 5.2495 μmol CO_2 m^{-2} s^{-1} higher/faster than the herbicide and nutrient treated plots.

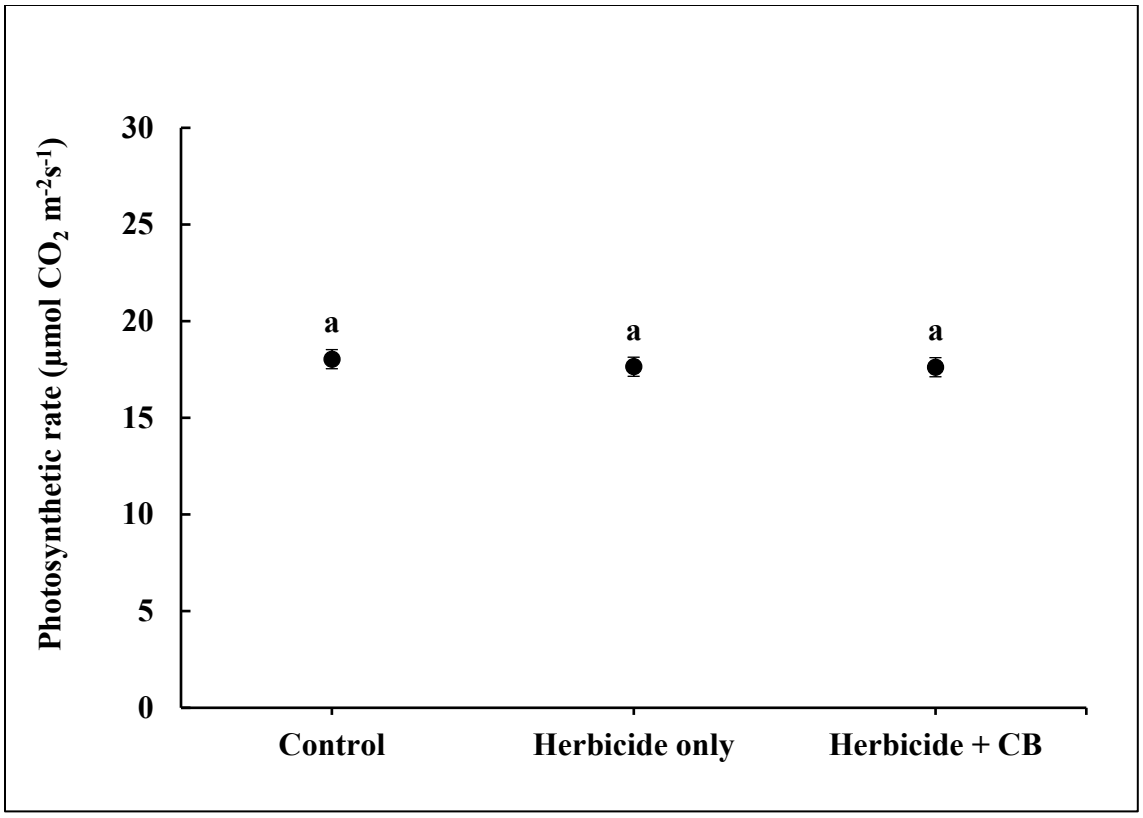

Figure 3.1 LS-Means plot of wheat photosynthetic rate one day before post-emergence treatment in summer 2015 (n=12, p=0.8012). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences (α = 0.05) between treatments tested with Bonferroni-adjusted limits.

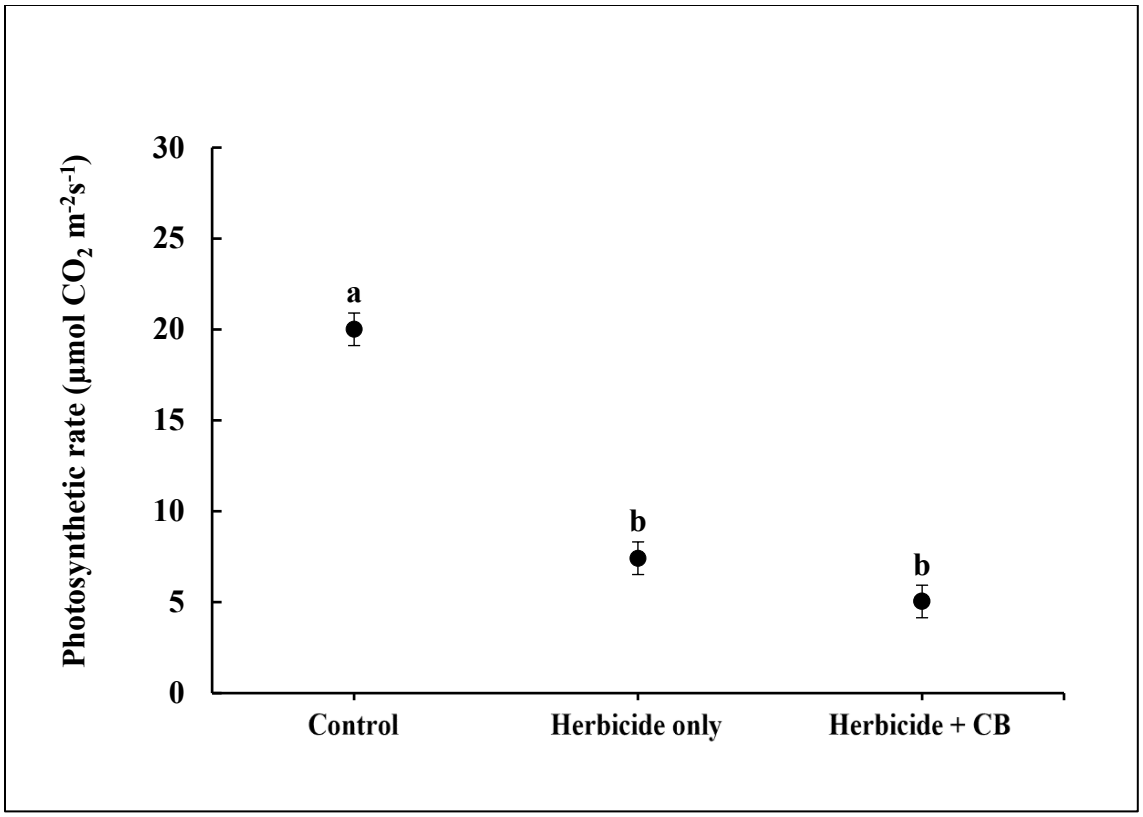

Figure 3.2 LS-Means plot of wheat photosynthetic rate one day after post-emergence treatment in summer 2015 (n=12, $p<0.0001$). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

Summer 2016 wheat growth and harvest variables in response to herbicide and/or foliar nutrient treatment

The experimental treatments did not affect the height, leaf area, or dry weight of the wheat plants at any developmental stage where samples were collected (Table 3.1, 3.2, 3.3). Similarly, there was no effect of the treatments on the yield (Table 3.4).

Table 3.1 Summer 2015 wheat height in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	Herbicide + CB	p-value
Mid-vegetative	78.58 ± 2.66 [a]	77.12 ± 2.66 [a]	78.76 ± 2.66 [a]	p = 0.7486
Mid-flowering	95.31 ± 3.19 [a]	93.47 ± 3.19 [a]	97.27 ± 3.19 [a]	p = 0.4358
Mid-Grain filling	102.69 ± 4.15 [a]	106.97 ± 4.15 [a]	108.92 ± 4.15 [a]	p = 0.3978
Harvest	104.10 ± 2.36 [a]	105.70 ± 2.36 [a]	105.68 ± 2.36 [a]	p = 0.8630

LS-Means of summer 2015 wheat height in response to different herbicide and foliar nutrient treatments at four phenological growth stages. Different letters indicate statistically significant differences (α=0.05, n=40) between treatments tested with Bonferroni-adjusted limits.

Table 3.2 Summer 2015 wheat leaf area in response to herbicide and foliar nutrient application

Stage	Control	Herbicide only	Herbicide + CB	p-value
Mid-vegetative	55.00 ± 3.82[a]	54.90 ± 3.82 [a]	60.85 ± 3.82 [a]	p =0.4939
Mid-flowering	76.60 ± 5.47 [a]	88.05 ± 5.47 [a]	90.30 ± 5.47 [a]	p = 0.1761
Mid-Grain filling	52.35 ± 5.70 [a]	50.00 ± 5.70 [a]	55.65 ± 5.70 [a]	p = 0.6767

LS-Means of summer 2015 wheat leaf area in response to different herbicide and foliar nutrient treatments at four phenological growth stages. Different letters indicate statistically significant differences (α=0.05, n=40) between treatments tested with Bonferroni-adjusted limits.

Table 3.3 Summer 2015 wheat dry weight in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	Herbicide + CB	p-value
Mid-vegetative	83.34 ± 7.22 [a]	67.92 ± 7.22 [a]	75.75 ± 7.22 [a]	p = 0.3616
Mid-flowering	102.80 ± 11.17 [a]	96.23 ± 11.17 [a]	110.38 ± 11.17 [a]	p = 0.6807
Mid-Grain filling	185.65 ± 9.71 [a]	151.55 ± 9.71 [a]	175.52 ± 9.71 [a]	p = 0.0865
Harvest	172.68 ± 10.31[a]	155.41 ± 10.31[a]	166.72 ± 10.31[a]	p = 0.5086

LS-Means of summer 2015 wheat dry weight in response to different herbicide and foliar nutrient treatments at four phenological growth stages. Different letters indicate statistically significant differences (α=0.05, n=4) between treatments tested with Bonferroni-adjusted limits.

Table 3.4 Summer 2015 wheat harvest variables in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	Herbicide + CB	p-value
Harvest index	0.403 ± 0.015 [a]	0.409 ± 0.015 [a]	0.410 ± 0.015 [a]	p = 0.9359
Yield	4921.44 ± 223.11 [a]	4586.12 ± 223.11 [a]	5007.30 ± 223.11 [a]	p = 0.4070

LS-Means of summer 2015 wheat harvest index (n=8) and yield (kg ha^{-1}) (n=4) in response to different herbicide and foliar nutrient treatments at four phenological growth stages. Different letters indicate statistically significant differences (α=0.05) between treatments tested with Bonferroni-adjusted limits.

Summer 2016 wheat photosynthetic measurements in response to herbicide and/or foliar nutrient treatment

The data indicates no difference in photosynthetic rate between the experimental groups before post-emergence treatment (Figure 3.3). However, there were highly statistically significant differences in the photosynthetic rate amongst the treatment groups one day after post-emergence treatment (Figure 3.4). The effects of the experimental treatments on wheat photosynthetic rate were not detectable three days after treatment (Figure 3.5).

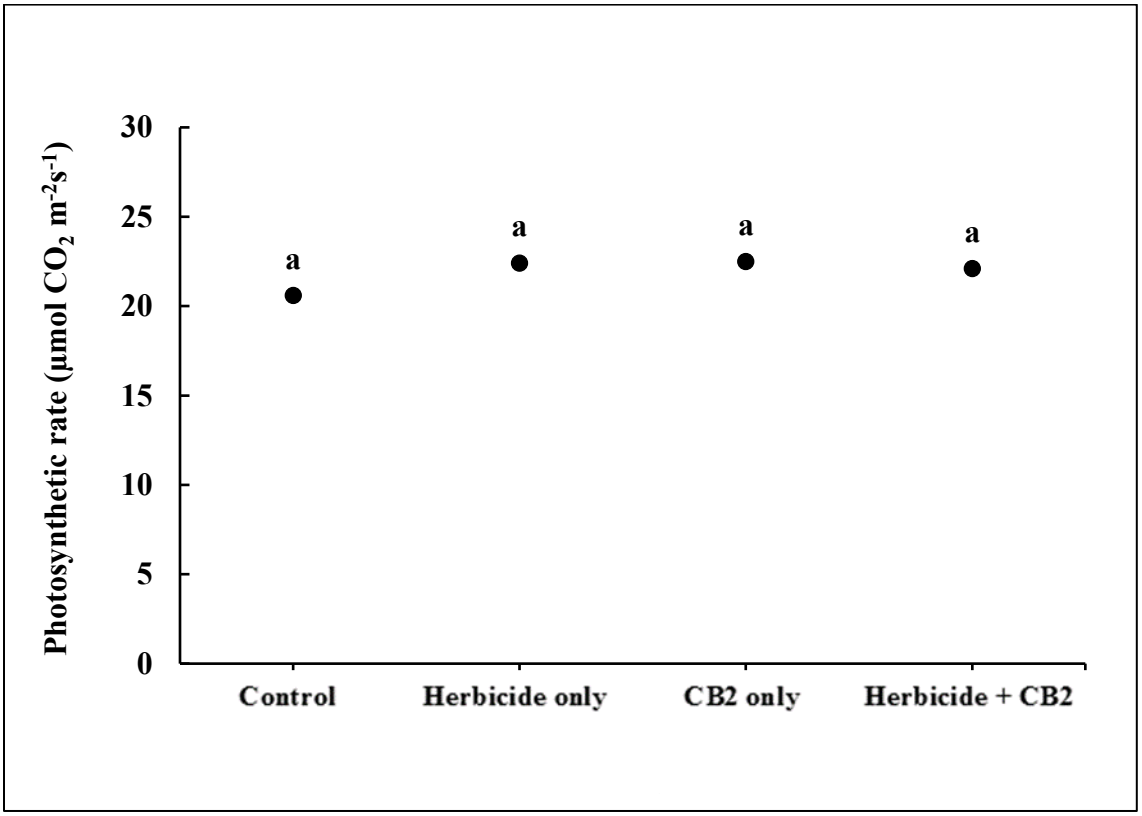

Figure 3.3 LS-Means plot of wheat photosynthetic rate one day prior to post-emergence treatment in summer 2016 (n=12, p=0.8416). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

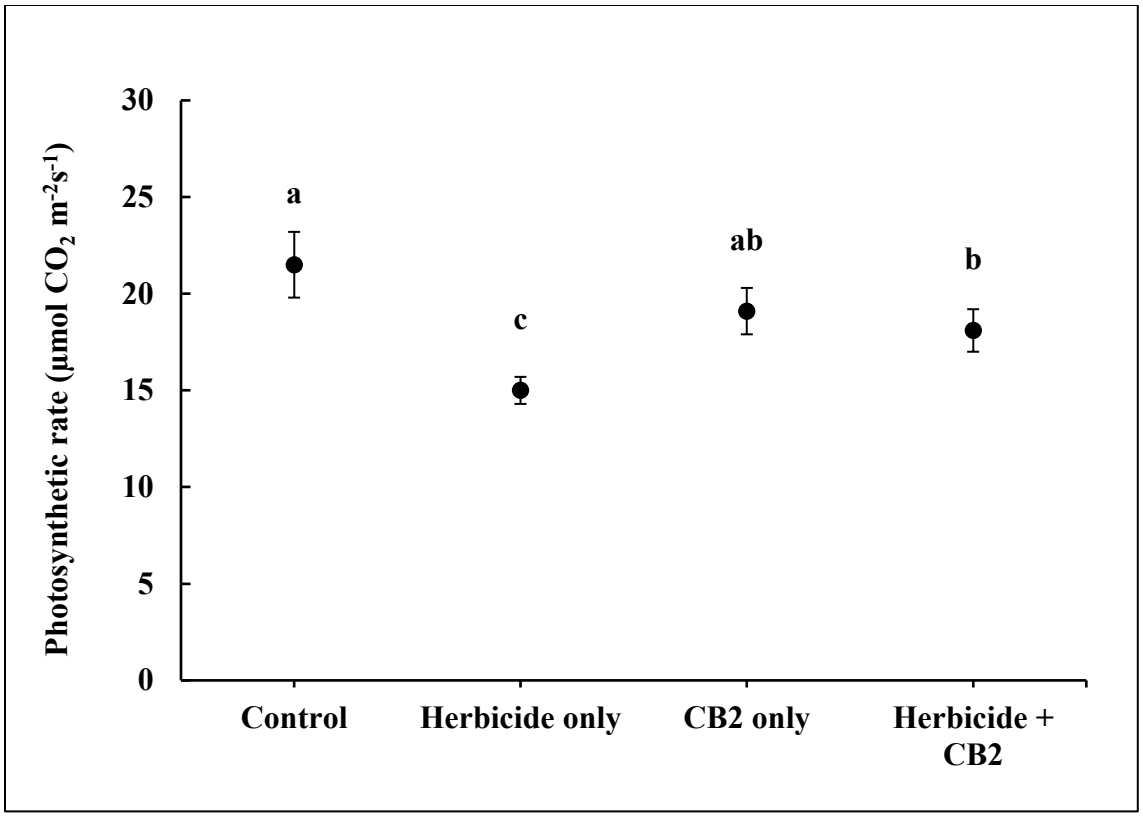

Figure 3.4 LS-Means plot of wheat photosynthetic rate one day after post emergence treatment in summer 2016 (n=20, $p<0.0001$). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

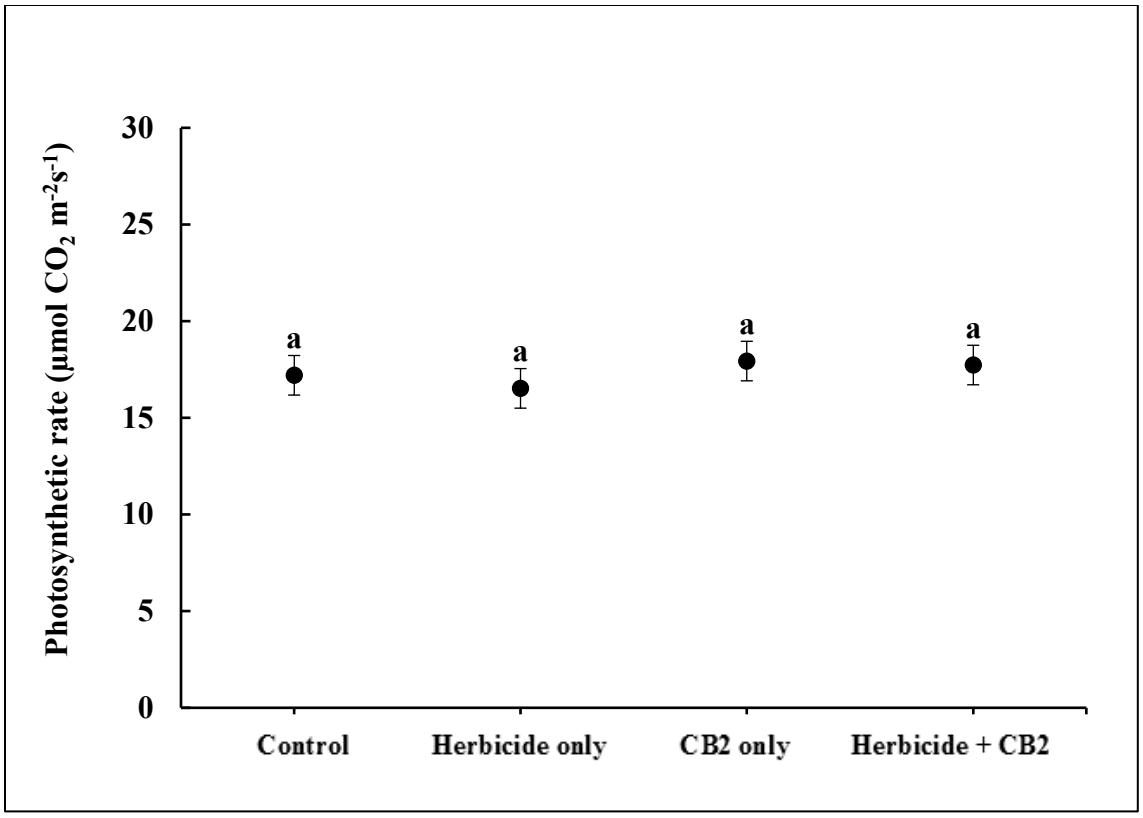

Figure 3.5 LS-Means plot of wheat photosynthetic rate three days after post-emergence treatment in summer 2016 (n=20, p=0.5719). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences (α = 0.05) between treatments tested with Bonferroni-adjusted limits.

Summer 2016 wheat growth and harvest variables in response to herbicide and/or foliar nutrient treatment

The experimental treatments did not affect the height, leaf area, or dry weight of the wheat plants at most developmental stage where samples were collected (Table 3.5, 3.6, 3.7). At the mid-grainfilling stage, the experimental treatments did have a significant effect on the leaf area (Figure 3.6). According to the Bonferoni-adjusted limits at 95% confidence, the leaf area of plots treated with water only is expected to be 5.5879 to 0.04465 cm^2 larger than that of the herbicide treated plots. Similarly, there was no effect of the treatments on the yield (Table 3.8). However, the harvest index of the herbicide only treated plots is expected to be from 0.1358 to 0.01002 units lower than the harvest index of nutrient only treated plots. The results do suggest that further experimentation (possibly with larger sample sizes) is warranted as various p-values are in proximity with an alpha of α=0.05.

Table 3.5 Summer 2016 wheat height in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	CB2 only	Herbicide + CB2	p-value
Mid-vegetative	49.69 ± 1.69 [a]	48.14 ± 1.69 [a]	50.975 ± 1.69 [a]	49.54 ± 1.69 [a]	0.4526
Mid-flowering	79.15	64.59	70.88	65.89	0.2971
Mid-Grain filling	57.97 ± 1.33 [a]	55.39 ± 1.33 [a]	61.12 ± 1.3276 [a]	57.39 ± 1.33 [a]	0.0542
Harvest	57.20 ± 1.7126 [a]	54.39 ± 1.7126 [a]	58.05 ± 1.7126 [a]	55.16 ± 1.71 [a]	0.3252

LS-Means of summer 2016 wheat height in response to different herbicide and foliar nutrient treatments at four phenological growth stages. Different letters indicate statistically significant differences (α=0.05, n=40) between treatments tested with Bonferroni-adjusted limits. Due to non-normal distribution, the graph displays the inverse-linked means at the mid-flowering stage.

Table 3.6 Summer 2016 wheat leaf area in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	CB2 only	Herbicide + CB2	p-value
Mid-vegetative	8.88 ± 1.11 [a]	11.49 ± 1.11 [a]	7.59 ± 1.11 [a]	8.17 ± 1.11[a]	0.1268
Mid-flowering	5.25 ± 0.98 [a]	5.77 ± 0.98 [a]	4.46 ± 0.98 [a]	5.10 ± 0.98 [a]	0.7744
Mid-Grain filling	3.89 ± 0.90 [a]	6.71 ± 0.90 [b]	5.95 ± 0.90 [ab]	5.37 ± 0.90 [ab]	0.0411

LS-Means of summer 2016 wheat leaf area in response to different herbicide and foliar nutrient treatments at four phenological growth stages. Different letters indicate statistically significant differences (α=0.05, n=40) between treatments tested with Bonferroni-adjusted limits.

Table 3.7 Summer 2016 wheat dry weight in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	CB2 only	Herbicide + CB2	p-value
Mid-vegetative	35.28 ± 4.04 [a]	31.78 ± 4.034 [a]	34.83 ± 4.04 [a]	26.11 ± 4.034 [a]	0.3898
Mid-flowering	49.79 ± 6.36 [a]	49.54 ± 6.36 [a]	49.56 ± 6.36 [a]	50.71 ± 6.36 [a]	0.9988
Mid-Grain filling	8.70 ± 1.00 [a]	10.70 ± 1.00 [a]	9.65 ± 1.00 [a]	9.50 ± 1.00 [a]	0.2448
Harvest	59.28 ± 3.4692 [a]	58.65 ± 3.4692 [a]	66.93 ± 3.4692[a]	60.13 ± 3.4692 [a]	0.3352

LS-Means of summer 2016 wheat dry weight in response to different herbicide and foliar nutrient treatments at four phenological growth stages. Different letters indicate statistically significant differences (α=0.05, n=4) between treatments tested with Bonferroni-adjusted limits.

Table 3.8 Summer 2016 wheat dry weight in response to herbicide and foliar nutrient application

Stages	Control	Herbicide only	CB2 only	Herbicide + CB2	p-value
Harvest index	0.391 ± 0.018^{ab}	0.353 ± 0.018^{b}	0.426 ± 0.018^{a}	0.378 ± 0.018^{ab}	0.0225
Yield	2150.31 ± 256.74^{a}	2003.03 ± 256.74^{a}	$2836.76 \ 256.74^{a}$	2356.26 ± 256.74^{a}	0.0520

LS-Means of summer 2016 wheat harvest index (n=8) and yield (kg ha^{-1}) (n=4) in response to different herbicide and foliar nutrient treatments at four phenological growth stages. Different letters indicate statistically significant differences (α=0.05) between treatments tested with Bonferroni-adjusted limits.

Wheat photosynthetic, growth and harvest variables in the greenhouse

Greenhouse 2017 wheat photosynthetic measurements in response to herbicide and/or foliar nutrient treatment

The data indicates no difference in photosynthetic rate between the experimental groups before post-emergence treatment (Figure 3.6). However, the experimental treatments did have a significant effect on the photosynthetic rate one day after treatment (Figure 3.7). According to the Bonferroni-adjusted limits at 95% confidence, the photosynthetic rate of plots treated with water was statistically significantly different from those treated with herbicide only, or a combination of herbicides and Crop Booster 2. Similarly, photosynthetic rate of plots treated with Crop Booster 2 only is statistically significantly different from plots treated with herbicide only, and a combination of herbicide and Crop Booster 2 treated plots. The photosynthetic rates of the herbicide treated plots and those treated with a combination of herbicides and Crop Booster 2 were statistically equivalent. Furthermore, the photosynthetic rates of the water only (control) plots and the plots treated with Crop Booster 2 only were statistically equivalent.

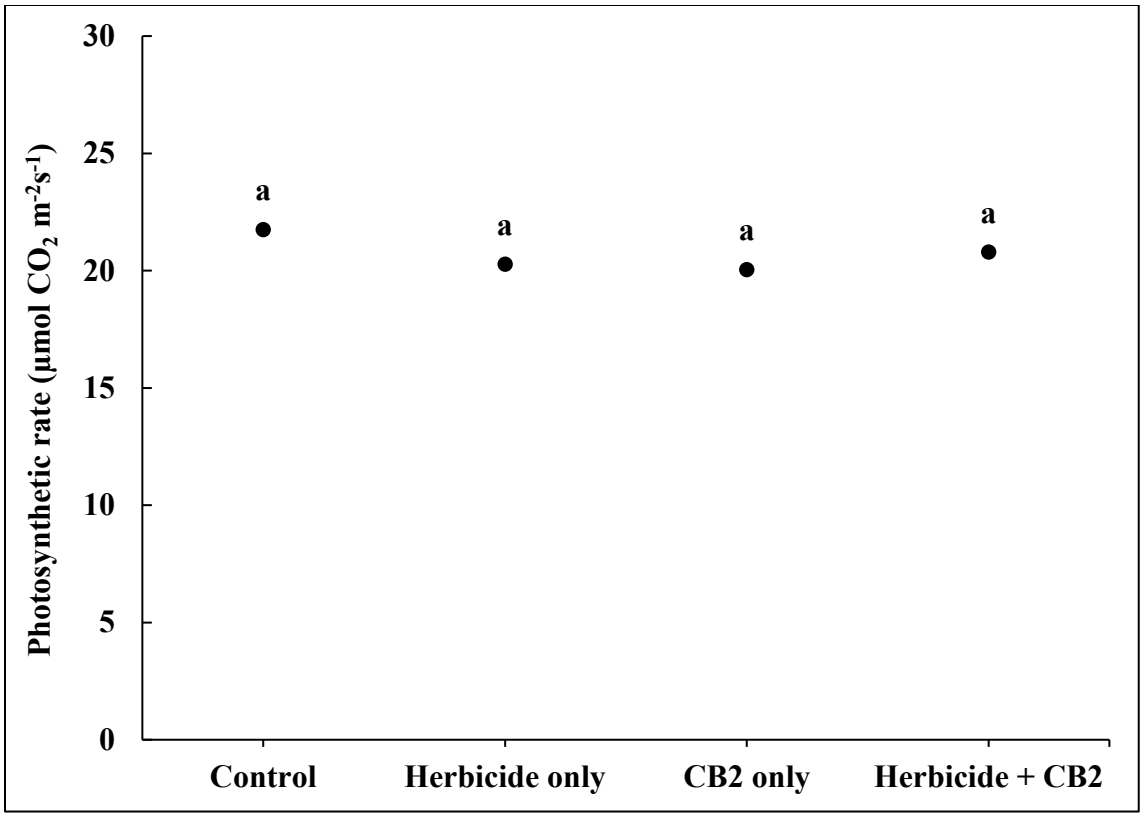

Figure 3.6 LS-Means plot of greenhouse wheat photosynthetic rate one day before post-emergence treatment (n=48, p=0.2365). Different letters indicate statistically significant differences (α = 0.05) between treatments tested with Bonferroni-adjusted limits.

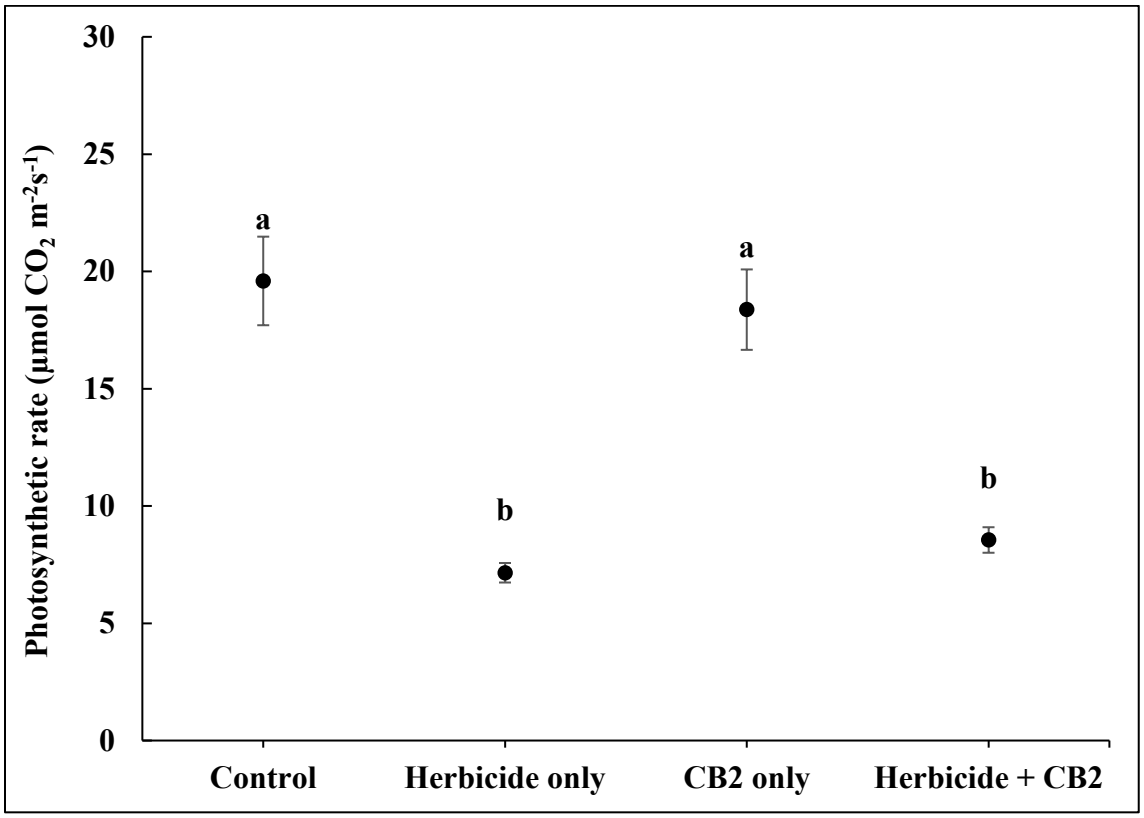

Figure 3.7 LS-Means plot of greenhouse wheat photosynthetic rate one day after post-emergence treatment (n=48, $p < 0.0001$). Whiskers indicate 95% confidence limits and different letters indicate statistically significant differences ($\alpha = 0.05$) between treatments tested with Bonferroni-adjusted limits.

Greenhouse 2017 wheat growth measurements in response to herbicide and/or foliar nutrient treatment

While there were no statistically significant differences detected in regards to leaf area, the experimental treatments affected the plant height and dry weight to a highly significant degree (Figure 3.9). The Bonferroni-adjusted limits at 95 % confidence indicated that the mean plant height of plots treated with water only (control) would/should be 6 to 16% higher than the means of those treated with herbicide only and 5 to 15% higher than the means of those treated with a combination of the herbicide and CB2. The mean of the plots treated with water only was statistically equivalent to those treated with CB2 only. The mean plant height of plots treated with herbicide only would/should be 4 to 13% shorter than that of plots treated with CB2 only. The mean of the plots treated with herbicide only was statistically equivalent to those treated with a combination of herbicide and CB2. Furthermore, the mean plant height of plots treated with CB2 only would be 3 to 14% taller than those treated with a combination of herbicide and CB2.

Moreover, the data indicates a highly significant effect of the treatments on dry weight (Table 3.9). According to the Bonferroni-adjusted limits at 95% confidence, the dry weight of plots treated with water only (control) was statistically significantly different from those treated with herbicide only, or a combination of herbicides and CB2. Similarly, the dry weight of plots treated with CB2 only was statistically significantly different from plots treated with herbicide only, and a combination of herbicide and CB2 treated plots. The dry weight of the herbicide treated plots and those treated with a combination of herbicides and CB2 were statistically equivalent. Furthermore, the dry weight of the water only (control) plots and the plots treated with CB2 only were statistically equivalent.

Table 3.9 Greenhouse 2017 wheat height, leaf area, and dry weight in response to herbicide and foliar nutrient application

Parameter	Control	Herbicide only	CB2 only	Herbicide + CB2
Height	63.42 [a]	57.13 [a]	62.67 [a]	57.86 [a]
Leaf Area	44.60 ± 17.89 [a]	41.84 ± 16.78 [a]	44.17 ± 17.72 [a]	41.52 ± 16.65 [a]
Dry Weight	4.49 ± 1.08 [a]	3.77 ± 0.64 [a]	4.35 ± 0.98 [a]	3.78 ± 0.65 [a]

Inverse-linked LS-Means of greenhouse wheat plant height ($n = 48$, $p < 0.0001$), leaf area ($n = 48$, $p = 0.4605$), and dry weight ($n = 48$, $p < 0.0001$) in response to different herbicide and foliar nutrient treatments. Different letters indicate statistically significant differences ($\alpha=0.05$) between treatments tested with Bonferroni-adjusted limits at 95% confidence.

Discussion

The effects of herbicide application on photosynthetic rate in field conditions

While the negative effects of Buctril M on wheat photosynthetic rate was consistent amongst both years, the photosynthetic rate of the corn was more affected by the herbicide combinations used in 2016 and the photosynthetic rate of the soybean remained unaffected. Since the use of glyphosate alone at the appropriate concentrations does not typically affect the photosynthetic rate of glyphosate-resistant cultivars (Zobiole et al., 2009) the observed effects were likely due to the accompanying herbicides. In the case of corn, the lower concentration of atrazine combined with dicamba in Marksman had a more significant effect on photosynthetic activity. This is highly unexpected as the results obtained by Creech et al. (2004) indicate that higher rates of atrazine typically result in higher photosynthetic suppression as it is a PSII inhibitor. Interestingly, soybean was not sensitive to either herbicide combination used in 2016 containing Classic 25 (an ALS inhibitor), or the 2015 herbicide combination containing fomesafen (a PPO inhibitor). To this effect, the herbicide combinations from 2016 for wheat and corn support the hypothesis that the herbicides either directly or indirectly affected some aspect of photosynthesis (Piñol and Simón, 2009).

Furthermore, corn and wheat experienced the greatest photosynthetic suppression. This is likely due to the presence of at least one direct PSII inhibiting component in the herbicide combination (atrazine and bromoxynil in corn and wheat, respectively). The active ingredient in Classic 25, chlorimuron has an indirect effect on photosynthesis, which would result in a more modest decrease in soybean. These results are consistent with the studies of Creech et al. (2004) which concluded that herbicides directly implicated in photoinhibition principally determined the severity of herbicide-induced photosynthetic stress; for this reason, the soybean being affected to a lesser degree is a reasonable result.

These results also support the temporary effects of herbicides on photosynthetic metabolism in species capable of efficiently degrading the toxic molecules. As seen in Creech et al. (2004) the herbicides exhibited the greatest effect on photosynthetic metabolism one day after treatment; after three days, the effects of the herbicide treatments diminished for all three crops. This is

reasonable as non-target crop species possess the necessary metabolic machinery for degrading herbicides into non-toxic metabolites to be stored, excreted, or exuded (Monaco and Creech, 2004).

The efficacy of foliarly applied nutrients in mediating herbicide-induced photodamage in field conditions

The foliar nutrient products Crop Booster and Crop Booster 2 also had different effects on photosynthetic recovery. In the summer 2015 field trials with corn and soybean, there was no effect of the herbicide combination on photosynthesis, thus there was no effect of the addition of Crop Booster. However, even in wheat subject to herbicide stress, Crop Booster was not able to recover the photosynthetic defects caused by Buctril M. On the contrary, the data from 2016 suggest a modest recovery in photosynthetic rate with the co-application of Crop Booster 2 and Soy Booster 2. Corn and wheat were more responsive than soybean, demonstrating a greater recovery in photosynthetic rate one day after treatment. Moreover, as the wheat was more responsive to Crop Booster 2 than Crop Booster, this suggests the importance of the differing elements in restoring photosynthesis. Unsurprisingly, the effects of foliar treatments are temporary, and benefits experienced due to foliar nutrient co-application disappeared within three days after treatment.

The effect of herbicide application and foliar nutrient co-application on growth and harvest variables in field conditions

Despite the cases of herbicide-induced suppression of photosynthetic rate, there were rarely significant effects of herbicide application on the growth variables (height, leaf area, and dry weight) at most phenological stages (mid-vegetative, mid-flowering, mid-grain filling, harvest) for any of the crops during any year. This supports the hypothesis that the application of herbicides to crop species capable of rapid detoxification results in temporary damage to plant metabolism, and thus does not affect long-term growth or harvest variables (Creech et al., 2004). Studies which have reported significant decreases in growth and/or harvest variables are most likely observing prolonged herbicide stress due to significantly slower detoxification processes. To this effect, if the photosynthetic stress were to last longer in the crops, rescuing the effects

with the use of Crop Booster 2 might be more effective in increasing yields. Consequently, the co-application of foliar nutrients did not affect the growth and/or harvest variables of either crop. Interestingly, the sole application of foliar nutrients did not affect the growth or harvest variables despite the conclusions of the previous literature. The discrepancy is likely due to the experimental solution containing considerably lower concentrations of nutrients than in previous literature which lead to a lesser response to the nutrients.

The effects of herbicide application on photosynthetic rate in greenhouse conditions

Like in field conditions, the application of herbicides in the greenhouse setting resulted in a highly significant reduction in photosynthetic rate for wheat. This is expected as many studies have reported the negative impact of the herbicides in Buctril M on the photosynthetic apparatus under controlled conditions. As suggested by Creech et al. (2004), the interaction between environmental stresses and herbicide applications is a possible culprit for the increased suppression of photosynthetic rate in the field in comparison to in greenhouse conditions.

The efficacy of foliar nutrients in mediating herbicide-induced photodamage and growth variables in greenhouse conditions

The inconsistency of the beneficial effects of foliar nutrient application remains an obstacle for many formulations (David et al., 2005). Although there was a highly significant decrease in photosynthetic rate attributed to herbicide application, there was no significant photosynthetic recovery with the co-application of foliar nutrients the day following treatment in the greenhouse. In reference to the hypothesis proposed by Creech et al. (2004), the foliar nutrient solutions were most likely more effective in field conditions due to the recovery of the environmental stress and herbicide stress interactions which are not present in controlled systems. However, there was a larger effect of the herbicide application on the height and dry weight, likely due to the more intense phytotoxic effects experienced in the greenhouse than in the field (detectable in the photosynthetic rate).

The exacerbating effect of low rainfall and higher temperatures on herbicide treatments

The discrepancy between the photosynthetic results obtained in the field during summer 2016 and the greenhouse during winter 2017 can be potentially explained by examining the

environmental conditions. Plants in field conditions are subjected to environmental stresses due to the wind, sun, plant-plant competition, water, etc. Furthermore, many herbicides have indicated an exacerbating effect of environmental stresses such as heat stress and water-deficit stress on the application of herbicides. The threshold for proper herbicide activity is from 24 to 29 degrees Celsius. In addition to supra-optimal temperatures for herbicide applications at various times one week before and after herbicide application, there was a notable decrease in the overall monthly rainfall at the Emile Lods field site during the critical vegetative stages. In May, when wheat and corn were undergoing the critical vegetative stage, the overall amount of rainfall dwindled to 45.8 cm below the twenty-year average (Figure 4.1). During June, when soybean was undergoing the critical vegetative stages, the total rainfall fell 44 cm below the twenty-year average (Figure 4.2). Knowing this, it is possible that the water-deficit stress and the heat stress which occurred during the summer 2016 field trials interacted with the herbicide stress, resulting in a greater decrease in photosynthetic rate under field conditions than in greenhouse conditions. To this effect, the data suggests that the application of nutrient solutions is not effective in recovering herbicide stress, rather the interaction of herbicide stress and environmental stress.

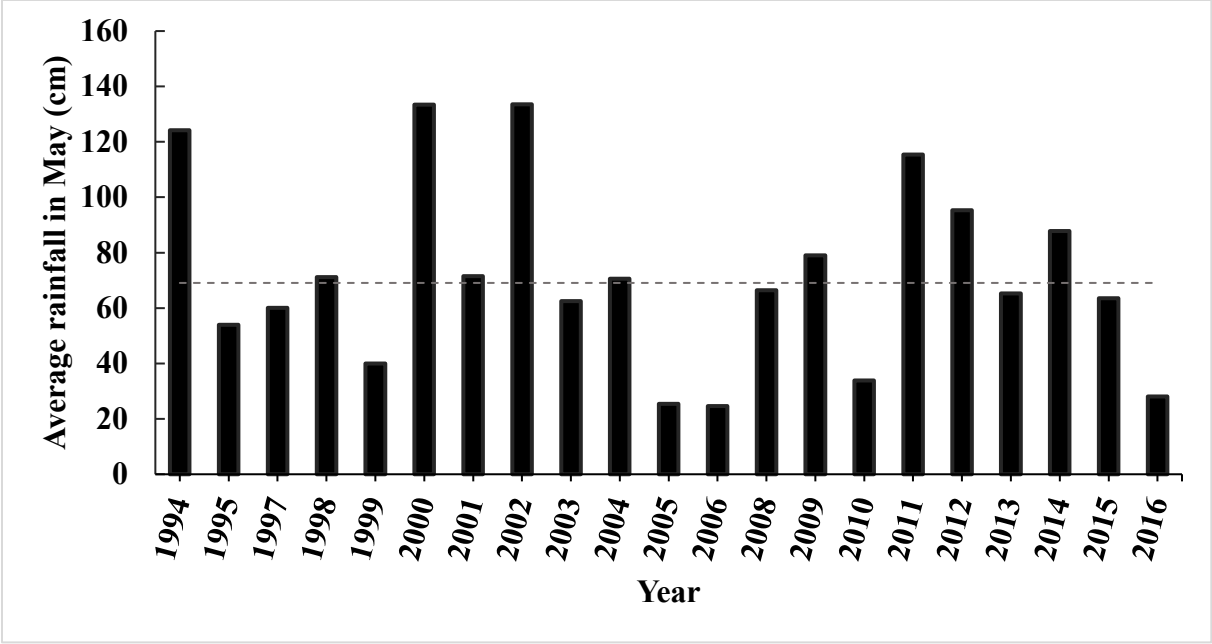

Figure 4.1 Total rainfall (cm) in May as recorded by the Sainte-Anne-de-Bellevue station from 1994 to 2016. Years where rainfall data is missing are absent from the figure.

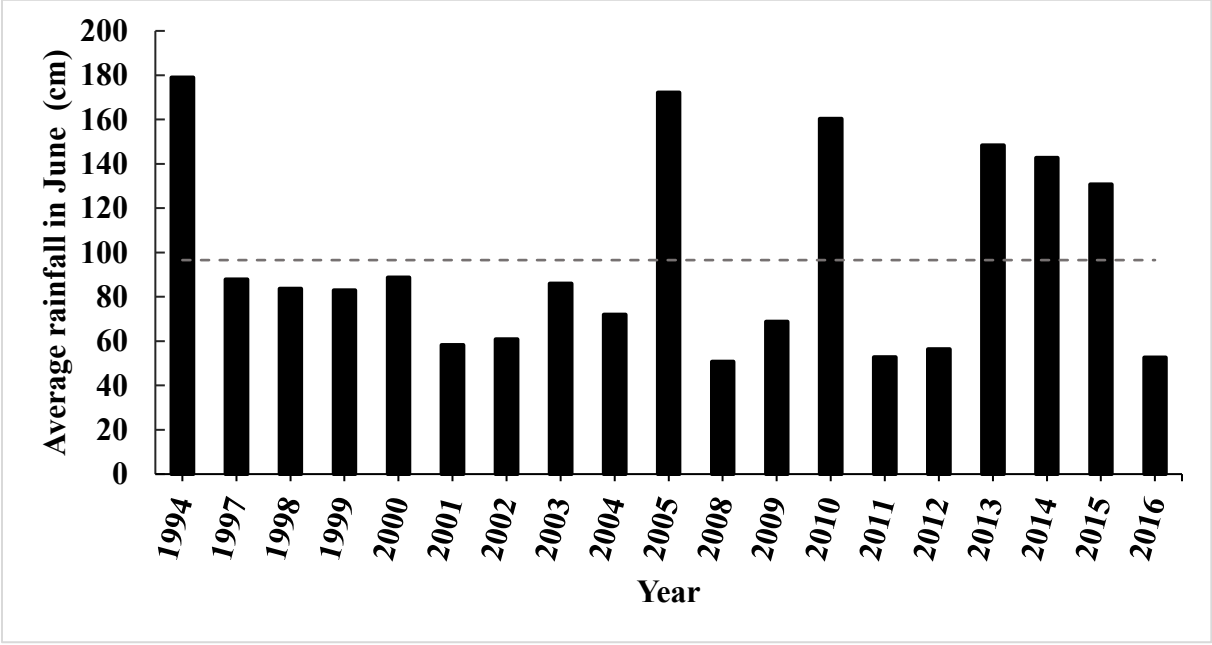

Figure 4.2 Total rainfall (cm) in June as recorded by the Sainte-Anne-de-Bellevue station from 1994 to 2016. Years where rainfall data is missing are absent from the figure.

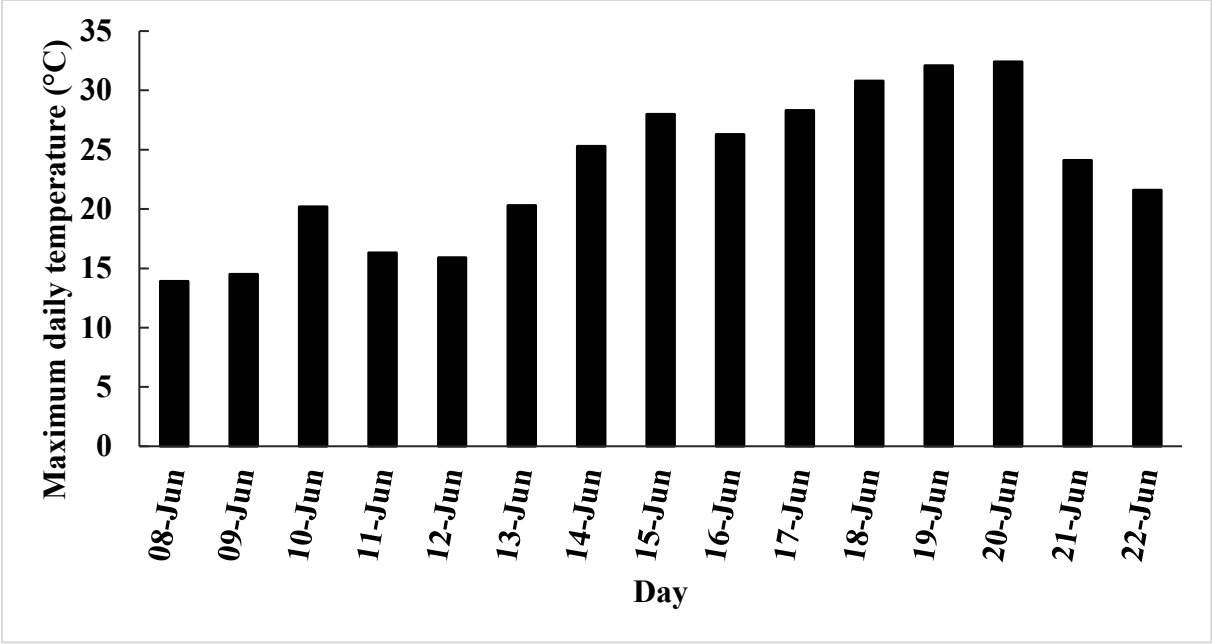

Figure 4.3 Maximum daily temperature (degrees Celcius) one week prior and one week following post-emergence treatment in summer 2016 for wheat and corn.

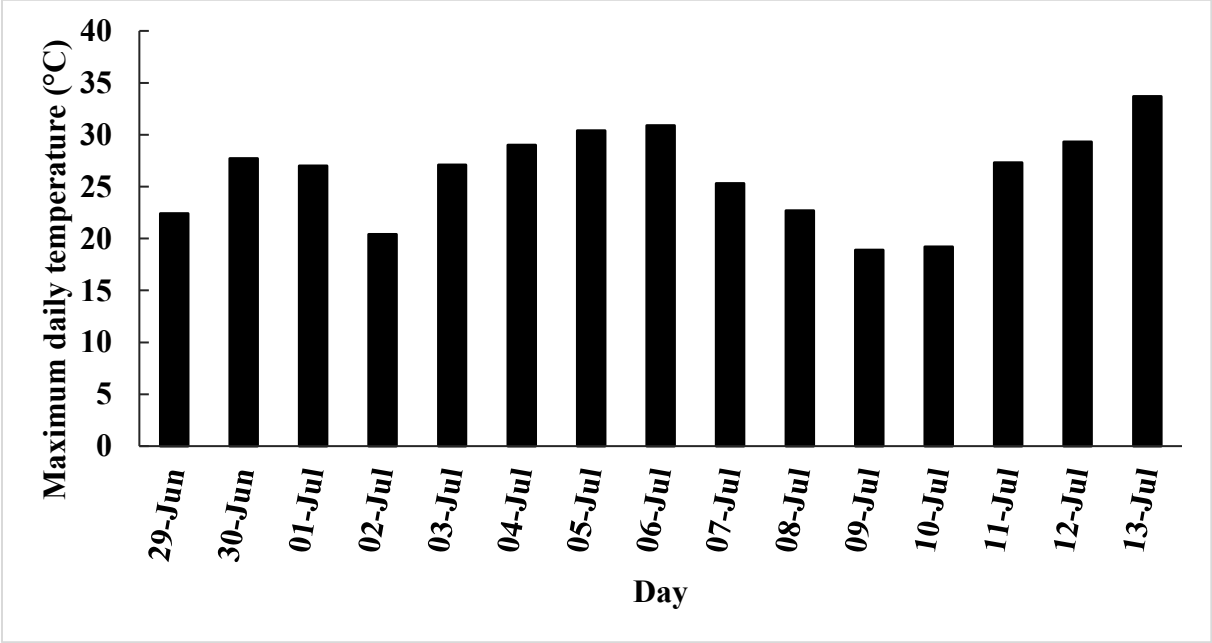

Figure 4.4 Maximum daily temperature (degrees Celsius) one week prior and one week following post-emergence treatment in summer 2016 for soybean

Future directions

The results of this study indicate the photosystem damage consequent to herbicide application regardless of the asymptomatic appearance of the crops. Furthermore, my studies indicate that these effects are partially recovered with the foliar co-application of nutrients under field conditions. Therefore, I have identified that at least on the photosynthetic level, the impairment of function attributed to herbicide-induced stress which can be partially reversed with the co-application of foliar nutrients. However, this study does not address alterations in the metabolic profile under herbicide-foliar nutrient co-application conditions which are potentially responsible for the photosynthetic rate recovery observed under field conditions.

A common metabolite group produced in response to various stress conditions, reactive oxygen species (ROS), are highly reactive chemical species which react with a variety of proteins and often causes the degradation of biological membranes. Many studies have observed the accumulation of reactive oxygen species (ROS) in the chloroplast under PSII herbicide inhibition which damages the thylakoid membrane and the photosystems core proteins. However, there is a lack of literature investigating the effect of foliar nutrient co-applications on ROS production in the chloroplast. In the field, the recovery of photosynthetic rate is likely due to a reduction in ROS as many of the nutrients in the solution are involved in the stress response. Using a variety of ROS-responsive dyes, the generation of ROS can be monitored in the chloroplast of herbicide treated plants and potentially verify the capacity of nutrients to activate pathways with quench ROS.

References

Ahmed, S., and Evans, H. J. (1960). Cobalt: A micronutrient element for the growth of soybean plants under symbiotic conditions. *Soil Science* **90**, 205-210.

Alla, M. M. N., Badawi, A.-H. M., Hassan, N. M., El-Bastawisy, Z. M., and Badran, E. G. (2008). Herbicide tolerance in maize is related to increased levels of glutathione and glutathione-associated enzymes. *Acta Physiologiae Plantarum* **30**, 371-379.

Alscher, R. G., Erturk, N., and Heath, L. S. (2002). Role of superoxide dismutases (SODs) in controlling oxidative stress in plants. *Journal of experimental botany* **53**, 1331-1341.

Ananieva, E. A., Alexieva, V. S., and Popova, L. P. (2002). Treatment with salicylic acid decreases the effects of paraquat on photosynthesis. *Journal of Plant Physiology* **159**, 685-693.

Ananieva, E. A., Christov, K. N., and Popova, L. P. (2004). Exogenous treatment with Salicylic acid leads to increased antioxidant capacity in leaves of barley plants exposed to Paraquat. *Journal of Plant Physiology* **161**, 319-328.

Asada, K. (2006). Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant physiology* **141**, 391-396.

Babczinski, P., and Zelinski, T. (1991). Mode of action of herbicidal ALS-inhibitors on acetolactate synthase from green plant cell cultures, yeast, and Escherichia coli. *Pesticide Science* **31**, 305-323.

Bakkaus, E., Gouget, B., Gallien, J. P., Khodja, H., Carrot, F., Morel, J. L., and Collins, R. (2005). Concentration and distribution of cobalt in higher plants: The use of micro-PIXE spectroscopy. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms* **231**, 350-356.

Barker, A. V., and Pilbeam, D. J. (2015). "Handbook of plant nutrition," CRC press.

Beale, S. I. (1990). Tetrapyrrole metabolism in photosynthetic organisms. *Biosynthesis of Heme and Chlorophylls.*, 278-391.

Bernard, H., Chabalier, P.-F., Chopart, J.-L., Legube, B., and Vauclin, M. (2005). Assessment of herbicide leaching risk in two tropical soils of Reunion Island (France). *Journal of environmental quality* **34**, 534-543.

Bettger, W. J., and O'Dell, B. L. (1981). A critical physiological role of zinc in the structure and function of biomembranes. *Life Sciences* **28**, 1425-1438.

Beyer, W. F., and Fridovich, I. (1987). Assaying for superoxide dismutase activity: some large consequences of minor changes in conditions. *Analytical biochemistry* **161**, 559-566.

Blevins, D. G., and Lukaszewski, K. M. (1998). BORON IN PLANT STRUCTURE AND FUNCTION. *Annual Review of Plant Physiology and Plant Molecular Biology* **49**, 481-500.

Böger, P., and Wakabayashi, K. (2012). "Peroxidizing herbicides," Springer Science & Business Media.

Bohnert, H. J., and Sheveleva, E. (1998). Plant stress adaptations—making metabolism move. *Current opinion in plant biology* **1**, 267-274.

Bott, S., Tesfamariam, T., Candan, H., Cakmak, I., Römheld, V., and Neumann, G. (2008). Glyphosate-induced impairment of plant growth and micronutrient status in glyphosate-resistant soybean (Glycine max L.). *Plant and soil* **312**, 185.

Bowler, C., Alliotte, T., De Loose, M., Van Montagu, M., and Inzé, D. (1989). The induction of manganese superoxide dismutase in response to stress in Nicotiana plumbaginifolia. *The EMBO journal* **8**, 31.

Bowler, C., Slooten, L., Vandenbranden, S., De Rycke, R., Botterman, J., Sybesma, C., Van Montagu, M., and Inzé, D. (1991). Manganese superoxide dismutase can reduce cellular damage mediated by oxygen radicals in transgenic plants. *The EMBO journal* **10**, 1723.

Broadley, M. R., White, P. J., Hammond, J. P., Zelko, I., and Lux, A. (2007). Zinc in plants. *New Phytologist* **173**, 677-702.

Brown, P. H., and Bassil, E. (2011). Overview of the acquisition and utilization of boron, chlorine, copper, manganese, molybdenum, and nickel by plants and prospects for improvement of micronutrient use efficiency. *The Molecular and physiological basis of nutrient use efficiency in crops*, 377-428.

Bruinsma, J. (1962). The effect of 4,6-dinitro-o-cresol (DNOC) on growth, development, and yield of winter rye (Secale cereal L.). *Weed Res*, 2:73-89.

Burke, J. J., Holloway, P., and Dalling, M. J. (1986). The Effect of Sulfur Deficiency on the Organisation and Photosynthetic Capability of Wheat Leaves. *Journal of Plant Physiology* **125**, 371-375.

Burnell, J. N. (1988). The biochemistry of manganese in plants. *In* "Manganese in soils and plants", pp. 125-137. Springer.

Cakmak, I. (2000). Tansley Review No. 111 Possible roles of zinc in protecting plant cells from damage by reactive oxygen species. *The New Phytologist* **146**, 185-205.

Cakmak, I., Kurz, H., and Marschner, H. (1995). Short-term effects of boron, germanium and high light intensity on membrane permeability in boron deficient leaves of sunflower. *Physiologia Plantarum* **95**, 11-18.

Cakmak, I., and Marschner, H. (1988). Increase in membrane permeability and exudation in roots of zinc deficient plants. *Journal of Plant Physiology* **132**, 356-361.

Cakmak, I., Yazici, A., Tutus, Y., and Ozturk, L. (2009). Glyphosate reduced seed and leaf concentrations of calcium, manganese, magnesium, and iron in non-glyphosate resistant soybean. *European Journal of Agronomy* **31**, 114-119.

Camacho-Cristóbal, J. J., Rexach, J., and González-Fontes, A. (2008). Boron in plants: deficiency and toxicity. *Journal of Integrative Plant Biology* **50**, 1247-1255.

Chatterjee, J., and Chatterjee, C. (2000). Phytotoxicity of cobalt, chromium and copper in cauliflower. *Environmental Pollution* **109**, 69-74.

Cobb, A. H., and Reade, J. P. (2011). "Herbicides and plant physiology," John Wiley & Sons.

Cobb, A. H., and Reade, J. P. H. (2010a). Auxin-Type Herbicides. *In* "Herbicides and Plant Physiology", pp. 133-156. Wiley-Blackwell.

Cobb, A. H., and Reade, J. P. H. (2010b). Inhibitors of Pigment Biosynthesis. *In* "Herbicides and Plant Physiology", pp. 116-132. Wiley-Blackwell.

Creech, J. E., Monaco, T. A., and Evans, J. O. (2004). Photosynthetic and growth responses of Zea mays L and four weed species following post-emergence treatments with mesotrione and atrazine. *Pest management science* **60**, 1079-1084.

David, D., Gene, S., and Andy, K. (2005). Boron fertilization of rice with soil and foliar applications. *Crop management* **4**, 0-0.

De Hertogh, A., Mayeux, P. A., and Evans, H. J. (1964). The relationship of cobalt requirement to propionate metabolism in Rhizobium. *Journal of Biological Chemistry* **239**, 2446-2453.

de Oliveira, R. H., Dias Milanez, C. R., Moraes-Dallaqua, M. A., and Rosolem, C. A. (2006). Boron Deficiency Inhibits Petiole and Peduncle Cell Development and Reduces Growth of Cotton. *Journal of Plant Nutrition* **29**, 2035-2048.

Dell, B., and Huang, L. (1997). Physiological response of plants to low boron. *Plant and soil* **193**, 103-120.

Denis, M. H., and Delrot, S. (1993). Carrier-mediated uptake of glyphosate in broad bean (Vicia faba) via a phosphate transporter. *Physiologia Plantarum* **87**, 569-575.

Devine, M., Duke, S. O., and Fedtke, C. (1992). "Physiology of herbicide action," PTR Prentice Hall.

Dezfulian, M. H., Foreman, C., Jalili, E., Pal, M., Dhaliwal, R. K., Roberto, D. K. A., Imre, K. M., Kohalmi, S. E., and Crosby, W. L. (2017). Acetolactate synthase regulatory subunits play divergent and overlapping roles in branched-chain amino acid synthesis and Arabidopsis development. *BMC plant biology* **17**, 71.

Dill, G. M. (2005). Glyphosate-resistant crops: history, status and future. *Pest management science* **61**, 219-224.

Dong, B., Rengel, Z., and Graham, R. D. (1995). Effects of herbicide chlorsulfuron on growth and nutrient uptake parameters of wheat genotypes differing in Zn-efficiency. *Plant and Soil* **173**, 275-282.

Draber, W., Tietjen, K., Kluth, J. F., and Trebst, A. (1991). Herbicides in photosynthesis research. *Angewandte Chemie International Edition* **30**, 1621-1633.

Duke, S. H., and Reisenauer, H. (1986). Roles and requirements of sulfur in plant nutrition. *Sulfur in agriculture*, 123-168.

Edwards, R., and Dixon, D. P. (2005). Plant glutathione transferases. *Methods in enzymology* **401**, 169-186.

Eichert, T., and Burkhardt, J. (2001). Quantification of stomatal uptake of ionic solutes using a new model system. *Journal of Experimental Botany* **52**, 771-781.

Eker, S., Ozturk, L., Yazici, A., Erenoglu, B., Romheld, V., and Cakmak, I. (2006). Foliar-applied glyphosate substantially reduced uptake and transport of iron and manganese in sunflower (Helianthus annuus L.) plants. *Journal of agricultural and food chemistry* **54**, 10019-10025.

Fageria, N. K. (2016). "The use of nutrients in crop plants," CRC press.

Fageria, N. K., Filho, M. P. B., Moreira, A., and Guimarães, C. M. (2009). Foliar Fertilization of Crop Plants. *Journal of Plant Nutrition* **32**, 1044-1064.

Findeklee, P., and Goldbach, H. (1996). Rapid effects of boron deficiency on cell wall elasticity modulus in Cucurbita pepo roots. *Plant Biology* **109**, 463-465.

Fleischer, A., O'Neill, M. A., and Ehwald, R. (1999). The pore size of non-graminaceous plant cell walls is rapidly decreased by borate ester cross-linking of the pectic polysaccharide rhamnogalacturonan II. *Plant Physiology* **121**, 829-838.

Fufezan, C., Rutherford, A. W., and Krieger-Liszkay, A. (2002). Singlet oxygen production in herbicide-treated photosystem II. *FEBS letters* **532**, 407-410.

Garcia-Mina, J. M. (2006). The relationships among mineral nutrition, biostimulation and plant defense mechanisms: an example in citrus plants. *Fertilitas Agrorum* **1**, 83-88.

Gilbert, S. M., Clarkson, D. T., Cambridge, M., Lambers, H., and Hawkesford, M. J. (1997). SO42-deprivation has an early effect on the content of ribulose-1, 5-bisphosphate carboxylase/oxygenase and photosynthesis in young leaves of wheat. *Plant Physiology* **115**, 1231-1239.

Gill, S. S., and Tuteja, N. (2010). Reactive oxygen species and antioxidant machinery in abiotic stress tolerance in crop plants. *Plant physiology and biochemistry* **48**, 909-930.

Girma, K., Martin, K. L., Freeman, K. W., Mosali, J., Teal, R. K., Raun, W. R., Moges, S. M., and Arnall, D. B. (2007). Determination of Optimum Rate and Growth Stage for Foliar-Applied Phosphorus in Corn. *Communications in Soil Science and Plant Analysis* **38**, 1137-1154.

Goldbach, H. E., and Wimmer, M. A. (2007). Boron in plants and animals: Is there a role beyond cell-wall structure? *Journal of Plant Nutrition and Soil Science* **170**, 39-48.

Gong, X., Liu, C., Wang, Y., Zhao, X., Zhou, M., Hong, M., Wang, S., Li, N., and Hong, F. (2010a). Inhibition of the photosynthesis in maize caused by manganese deficiency. *Cereal Research Communications* **38**, 353-365.

Gong, X., Wang, Y., Liu, C., Wang, S., Zhao, X., Zhou, M., Li, N., Lu, Y., and Hong, F. (2010b). Effects of manganese deficiency on spectral characteristics and oxygen evolution in maize chloroplasts. *Biological trace element research* **136**, 372-382.

Grossmann, K., Kwiatkowski, J., and Tresch, S. (2001). Auxin herbicides induce H(2)O(2) overproduction and tissue damage in cleavers (Galium aparine L.). *J Exp Bot* **52**, 1811-6.

Grossmann, K., Niggeweg, R., Christiansen, N., Looser, R., and Ehrhardt, T. (2010). The herbicide saflufenacil (Kixor™) is a new inhibitor of protoporphyrinogen IX oxidase activity. *Weed Science* **58**, 1-9.

Gunes, A., Inal, A., Alpaslan, M., Eraslan, F., Bagci, E. G., and Cicek, N. (2007). Salicylic acid induced changes on some physiological parameters symptomatic for oxidative stress and mineral nutrition in maize (Zea mays L.) grown under salinity. *Journal of Plant Physiology* **164**, 728-736.

Gupta, U. C. (1980). Boron Nutrition Of Crops. *Advances in Agronomy* **31**, 273-307.

Gupta, U. C. (1983). Boron deficiency and toxicity symptoms for several crops as related to tissue boron levels. *Journal of plant nutrition* **6**, 387-395.

Gyamfi, S., Pfeifer, U., Stierschneider, M., and Sessitsch, A. (2002). Effects of transgenic glufosinate-tolerant oilseed rape (Brassica napus) and the associated herbicide application on eubacterial and Pseudomonas communities in the rhizosphere. *FEMS Microbiology Ecology* **41**, 181-190.

Hajiboland, R., Farhanghi, F., and Aliasgharpour, M. (2012). Morphological and anatomical modifications in leaf, stem and roots of four plant species under boron deficiency conditions/Modificaciones morfológicas y anatómicas en hoja, tallo y raíces de cuatro especies de plantas en deficiencia de boro. *In* "Anales de Biología", pp. 15. Servicio de Publicaciones, Universidad de Murcia.

Hänsch, R., and Mendel, R. R. (2009). Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl). *Current opinion in plant biology* **12**, 259-266.

Hansen, H., and Grossmann, K. (2000). Auxin-induced ethylene triggers abscisic acid biosynthesis and growth inhibition. *Plant Physiology* **124**, 1437-1448.

Hawkesford, M. J., and De Kok, L. J. (2006). Managing sulphur metabolism in plants. *Plant, Cell & Environment* **29**, 382-395.

Hernandez, J., Jimenez, A., Mullineaux, P., and Sevilia, F. (2000). Tolerance of pea (Pisum sativum L.) to long-term salt stress is associated with induction of antioxidant defences. *Plant, Cell & Environment* **23**, 853-862.

Hewitt, E. J., and Bond, G. (1966). The Cobalt Requirement of Non-legume Root Nodule Plants. *Journal of Experimental Botany* **17**, 480-491.

Hirsch, A. M., and Torrey, J. G. (1980). Ultrastructural changes in sunflower root cells in relation to boron deficiency and added auxin. *Canadian Journal of Botany* **58**, 856-866.
Hu, H., Brown, P. H., and Labavitch, J. M. (1996). Species variability in boron requirement is correlated with cell wall pectin. *Journal of Experimental Botany* **47**, 227-232.
Hu, Y., Burucs, Z., and Schmidhalter, U. (2008). Effect of foliar fertilization application on the growth and mineral nutrient content of maize seedlings under drought and salinity. *Soil Science & Plant Nutrition* **54**, 133-141.
Husted, S., Laursen, K. H., Hebbern, C. A., Schmidt, S. B., Pedas, P., Haldrup, A., and Jensen, P. E. (2009). Manganese deficiency leads to genotype-specific changes in fluorescence induction kinetics and state transitions. *Plant physiology* **150**, 825-833.
Ibs, K.-H., Gabriel, P., and Rink, L. (2002). Zinc and the immune system of elderly. *Advances in Cell Aging and Gerontology* **13**, 243-259.
Iturbe-Ormaetxe, I., Escuredo, P. R., Arrese-Igor, C., and Becana, M. (1998). Oxidative damage in pea plants exposed to water deficit or paraquat. *Plant physiology* **116**, 173-181.
Janda, T., Gondor, O. K., Yordanova, R., Szalai, G., and Pál, M. (2014). Salicylic acid and photosynthesis: signalling and effects. *Acta physiologiae plantarum* **36**, 2537-2546.
Jaworski, E. G. (1972). Mode of action of N-phosphonomethylglycine. Inhibition of aromatic amino acid biosynthsis. *Journal of Agricultural and Food Chemistry* **20**, 1195-1198.
Kannan, S., and Charnel, A. (1986). Foliar absorption and transport of inorganic nutrients. *Critical reviews in plant sciences* **4**, 341-375.
Keren, N., Berg, A., Van Kan, P. J., Levanon, H., and Ohad, I. (1997). Mechanism of photosystem II photoinactivation and D1 protein degradation at low light: the role of back electron flow. *Proceedings of the National Academy of Sciences* **94**, 1579-1584.
Khodary, S. (2004). Effect of salicylic acid on the growth, photosynthesis and carbohydrate metabolism in salt-stressed maize plants. *Int. J. Agric. Biol* **6**, 5-8.
Kim, J., Woo, D., and Rees, D. (1993). X-ray crystal structure of the nitrogenase molybdenum-iron protein from Clostridium pasteurianum at 3.0-. ANG. resolution. *Biochemistry* **32**, 7104-7115.
Klepper, L. (1991). NOx evolution by soybean leaves treated with salicylic acid and selected derivatives. *Pesticide Biochemistry and Physiology* **39**, 43-48.
Kobyłecka, J., and Skiba, E. (2008). The effect of phenoxyacetic herbicides on the uptake of copper, zinc and manganese by Triticum aestivum L. *L. Polish Journal of Environmental Studies* **17**, 895-901.
Kok, B., Forbush, B., and McGloin, M. (1970). COOPERATION OF CHARGES IN PHOTOSYNTHETIC O2 EVOLUTION–I. A LINEAR FOUR STEP MECHANISM. *Photochemistry and Photobiology* **11**, 457-475.
Koshiba, T., Kobayashi, M., and Matoh, T. (2009). Boron deficiency. *Plant Signaling & Behavior* **4**, 557-558.
Krämer, U., and Clemens, S. (2005). Functions and homeostasis of zinc, copper, and nickel in plants. *In* "Molecular biology of metal homeostasis and detoxification", pp. 215-271. Springer.
Kremer, R. J., and Means, N. E. (2009). Glyphosate and glyphosate-resistant crop interactions with rhizosphere microorganisms. *European Journal of Agronomy* **31**, 153-161.
Kriedemann, P. E., Graham, R., and Wiskich, J. (1985). Photosynthetic dysfunction and in vivo changes in chlorophyll a fluorescence from manganese-deficient wheat leaves. *Australian journal of agricultural research* **36**, 157-169.

Krieger-Liszkay, A. (2004). Singlet oxygen production in photosynthesis. *Journal of experimental botany* **56**, 337-346.
LaRossa, R. A., and Schloss, J. V. (1984). The sulfonylurea herbicide sulfometuron methyl is an extremely potent and selective inhibitor of acetolactate synthase in Salmonella typhimurium. *Journal of Biological Chemistry* **259**, 8753-8757.
Lermontova, I., and Grimm, B. (2000). Overexpression of plastidic protoporphyrinogen IX oxidase leads to resistance to the diphenyl-ether herbicide acifluorfen. *Plant Physiology* **122**, 75-84.
Lin, C.-W., Chang, H.-B., and Huang, H.-J. (2005). Zinc induces mitogen-activated protein kinase activation mediated by reactive oxygen species in rice roots. *Plant Physiology and Biochemistry* **43**, 963-968.
Liu, Y., Li, E., Yang, C., and Peng, S. (2013). Effects of boron-deficiency on anatomical structures in the leaf main vein and fruit mesocarp of pummelo [Citrus grandis (L.) Osbeck]. *The Journal of Horticultural Science and Biotechnology* **88**, 693-700.
Ljung, K. (2013). Auxin metabolism and homeostasis during plant development. *Development* **140**, 943-950.
Locke, M. A., and Bryson, C. T. (1997). Herbicide-soil interactions in reduced tillage and plant residue management systems. *Weed Science* **45**, 307-320.
Lonhienne, T., Nouwens, A., Williams, C. M., Fraser, J. A., Lee, Y. T., West, N. P., and Guddat, L. W. (2016). Commercial herbicides can trigger the oxidative inactivation of acetohydroxyacid synthase. *Angewandte Chemie* **128**, 4319-4323.
Lunde, C., Zygadlo, A., Simonsen, H. T., Nielsen, P. L., Blennow, A., and Haldrup, A. (2008). Sulfur starvation in rice: the effect on photosynthesis, carbohydrate metabolism, and oxidative stress protective pathways. *Physiologia Plantarum* **134**, 508-521.
Maathuis, F. J. M. (2009). Physiological functions of mineral macronutrients. *Current Opinion in Plant Biology* **12**, 250-258.
Madamanchi, N. R., Yu, X., Doulis, A., Alscher, R. G., Hatzios, K. K., and Cramer, C. L. (1994). Acquired Resistance to Herbicides in Pea Cultivars through Pretreatment with Sulfur Dioxide. *Pesticide Biochemistry and Physiology* **48**, 31-40.
Martín-Rejano, E. M., Camacho-Cristóbal, J. J., Herrera-Rodríguez, M. B., Rexach, J., Navarro-Gochicoa, M. T., and González-Fontes, A. (2011). Auxin and ethylene are involved in the responses of root system architecture to low boron supply in Arabidopsis seedlings. *Physiologia Plantarum* **142**, 170-178.
Matoh, T., and Kobayashi, M. (1998). Boron and calcium, essential inorganic constituents of pectic polysaccharides in higher plant cell walls. *Journal of Plant Research* **111**, 179-190.
Mazur, B. J., and Falco, S. C. (1989). The development of herbicide resistant crops. *Annual Review of Plant Biology* **40**, 441-470.
McKersie, B. D., Chen, Y., de Beus, M., Bowley, S. R., Bowler, C., Inzé, D., D'Halluin, K., and Botterman, J. (1993). Superoxide dismutase enhances tolerance of freezing stress in transgenic alfalfa (Medicago sativa L.). *Plant physiology* **103**, 1155-1163.
McLay, L., and Robson, A. (1992). The effect of chlorsulfuron and diclofop-methyl on the uptake and utilization of zinc by wheat. *Australian Journal of Agricultural Research* **43**, 59-65.
Mendel, R. R., and Hänsch, R. (2002). Molybdoenzymes and molybdenum cofactor in plants. *Journal of Experimental Botany* **53**, 1689-1698.

Mittler, R., Vanderauwera, S., Gollery, M., and Van Breusegem, F. (2004). Reactive oxygen gene network of plants. *Trends in Plant Science* **9**, 490-498.
Monaco, T. A., and Creech, J. E. (2004). Sulfosulfuron effects on growth and photosynthesis of 15 range grasses. *Journal of range management* **57**, 490-496.
Monk, L. S., Fagerstedt, K. V., and Crawford, R. M. (1989). Oxygen toxicity and superoxide dismutase as an antioxidant in physiological stress. *Physiologia Plantarum* **76**, 456-459.
Morab, H., Koti, R., Chetti, M., Patil, P., and Nalini, A. (2003). Role of nutrients in inducing rust resistance in soybean. *Indian journal of plant physiology* **8**, 85-88.
Nava, I., Gonçalves Jr, A., Schwantes, D., Coelho, G., Stangarlin, J., and Leismann, E. (2015). Foliar application rates of manganese in phenological stages of roundup ready soybean.
Nicholas, D., Maruyama, Y., and Fisher, D. (1962a). The effect of cobalt deficiency on the utilization of nitrate nitrogen in Rhizobium. *Biochimica et biophysica acta* **56**, 623-626.
Nicholas, D. J. D., Kobayashi, M., and Wilson, P. W. (1962b). Cobalt Requirement for Inorganic Nitrogen Metabolism in Microorganisms. *Proceedings of the National Academy of Sciences of the United States of America* **48**, 1537-1542.
Nikiforova, V. J., Kopka, J., Tolstikov, V., Fiehn, O., Hopkins, L., Hawkesford, M. J., Hesse, H., and Hoefgen, R. (2005). Systems rebalancing of metabolism in response to sulfur deprivation, as revealed by metabolome analysis of Arabidopsis plants. *Plant physiology* **138**, 304-318.
Nosek, L., Semchonok, D., Boekema, E. J., Ilík, P., and Kouřil, R. (2017). Structural variability of plant photosystem II megacomplexes in thylakoid membranes. *The Plant Journal* **89**, 104-111.
O'neill, M. A., Eberhard, S., Albersheim, P., and Darvill, A. G. (2001). Requirement of borate cross-linking of cell wall rhamnogalacturonan II for Arabidopsis growth. *Science* **294**, 846-849.
Ogawa, K. i., Kanematsu, S., Takabe, K., and Asada, K. (1995). Attachment of CuZn-superoxide dismutase to thylakoid membranes at the site of superoxide generation (PSI) in spinach chloroplasts: detection by immuno-gold labeling after rapid freezing and substitution method. *Plant and Cell Physiology* **36**, 565-573.
Oliveira, R., Koskinen, W., and Ferreira, F. (2001). Sorption and leaching potential of herbicides on Brazilian soils. *Weed Research* **41**, 97-110.
Osborne, L., and Robson, A. (1992). Duration of zinc uptake inhibition by chlorsulfuron in wheat. *Australian Journal of Agricultural Research* **43**, 1169-1174.
Palit, S., Sharma, A., and Talukder, G. (1994). Effects of cobalt on plants. *The botanical review* **60**, 149-181.
Pfeffer, H., Dannel, F., and Römheld, V. (1998). Are there connections between phenol metabolism, ascorbate metabolism and membrane integrity in leaves of boron-deficient sunflower plants? *Physiologia Plantarum* **104**, 479-485.
Piñol, R., and Simón, E. (2009). Effect of 24-epibrassinolide on chlorophyll fluorescence and photosynthetic CO2 assimilation in Vicia faba plants treated with the photosynthesis-inhibiting herbicide terbutryn. *Journal of plant growth regulation* **28**, 97-105.
Pollard, A. S., PARR, A. J., and LOUGHMAN, B. C. (1977). Boron in relation to membrane function in higher plants. *Journal of Experimental Botany* **28**, 831-841.
Prashanth, S., Sadhasivam, V., and Parida, A. (2008). Over expression of cytosolic copper/zinc superoxide dismutase from a mangrove plant Avicennia marina in indica rice var Pusa Basmati-1 confers abiotic stress tolerance. *Transgenic research* **17**, 281-291.

Radwan, D. E. M. (2012). Salicylic acid induced alleviation of oxidative stress caused by clethodim in maize (Zea mays L.) leaves. *Pesticide Biochemistry and Physiology* **102**, 182-188.

Rahimizadeh, M., Habibi, D., Madani, H., Mohammadi, G. N., Mehraban, A., and Sabet, A. M. (2007). THE EFFECT OF MICRONUTRIENTS ON ANTIOXIDANT ENZYMES METABOLISM IN SUNFLOWER (Helianthus annuus L.) UNDER DROUGHT STRESS/INFLUENCIA DE MICRONUTRIENTES EN METABOLISMO DE ENZIMAS ANTIOXIDANTES EN GIRASOL (Helianthus annaus L.) BAJO LA INFLUENCIA DEL ESTRÉS, CAUSADO POR SEQUÍA/EFFET DES MICRONUTRIMENTS SUR LES ENZYMES ANTIOXYDANTS DANS LE TOURNESOL (Helianthus annuus L.) SOUS LE STRESS DE LA SÉCHERESSE. *Helia* **30**, 167-174.

Rajasekaran, L., and Blake, T. (1999). New plant growth regulators protect photosynthesis and enhance growth under drought of jack pine seedlings. *Journal of plant growth regulation* **18**, 175-181.

Rao, M. V., Paliyath, G., Ormrod, D. P., Murr, D. P., and Watkins, C. B. (1997). Influence of Salicylic Acid on H2O2 Production, Oxidative Stress, and H2O2-Metabolizing Enzymes (Salicylic Acid-Mediated Oxidative Damage Requires H2O2). *Plant Physiology* **115**, 137-149.

Rausch, T., and Wachter, A. (2005). Sulfur metabolism: a versatile platform for launching defence operations. *Trends in Plant Science* **10**, 503-509.

Ray, T. B. (1984). Site of Action of Chlorsulfuron. *Inhibition of Valine and Isoleucine Biosynthesis in Plants* **75**, 827-831.

Rengel, Z. (1995). Sulfhydryl groups in root-cell plasma membranes of wheat genotypes differing in Zn efficiency. *Physiologia Plantarum* **95**, 604-612.

Rengel, Z. (1997). Root exudation and microflora populations in rhizosphere of crop genotypes differing in tolerance to micronutrient deficiency. *Plant and Soil* **196**, 255-260.

Robson, A., and Snowball, K. (1989). The effect of 2-(4-2′, 4′-dichlorophenoxy-phenoxy)-methyl propanoate on the uptake and utilization of zinc by wheat. *Australian Journal of Agricultural Research* **40**, 981-990.

Roth-Bejerano, N., and Itai, C. (1981). Effect of boron on stomatal opening in epidermal strips of Commelina communis. *Physiologia Plantarum* **52**, 302-304.

Rutherford, A. W., and Krieger-Liszkay, A. (2001). Herbicide-induced oxidative stress in photosystem II. *Trends in biochemical sciences* **26**, 648-653.

Saito, K. (2004). Sulfur assimilatory metabolism. The long and smelling road. *Plant Physiology* **136**, 2443-2450.

Schlicke, H., Richter, A., Rothbart, M., Brzezowski, P., Hedtke, B., and Grimm, B. (2015). Function of Tetrapyrroles, Regulation of Tetrapyrrole Metabolism and Methods for Analyses of Tetrapyrroles. *Procedia Chemistry* **14**, 171-175.

Schon, M. K., Novacky, A., and Blevins, D. G. (1990). Boron induces hyperpolarization of sunflower root cell membranes and increases membrane permeability to K+. *Plant Physiology* **93**, 566-571.

Schroth, M. N., and Hancock, J. G. (1982). Disease-suppressive soil and root-colonizing bacteria. *Science* **216**, 1376-1381.

Schwarz, G., and Mendel, R. R. (2006). Molybdenum cofactor biosynthesis and molybdenum enzymes. *Annu. Rev. Plant Biol.* **57**, 623-647.

Schwarz, G., Mendel, R. R., and Ribbe, M. W. (2009). Molybdenum cofactors, enzymes and pathways. *Nature* **460**, 839.
Scroggs, D. M., Miller, D. K., Stewart, A. M., Leonard, B. R., Griffin, J. L., and Blouin, D. C. (2009). Weed response to foliar coapplications of glyphosate and zinc sulfate. *Weed Technology* **23**, 171-174.
Seefeldt, L. C., Hoffman, B. M., and Dean, D. R. (2009). Mechanism of Mo-dependent nitrogenase. *Annual review of biochemistry* **78**, 701-722.
Serecon Management Consulting Inc (October 2011). "Application of Sustainable Agriculture Metrics to Selected Western Canadian Field Crops," Edmonton, Alberta.
Sexton, P., Batchelor, W., and Shibles, R. (1997). Sulfur availability, rubisco content, and photosynthetic rate of soybean. *Crop science* **37**, 1801-1806.
Sharma, P., and Ramchandra, T. (1990). Water relations and photosynthesis in mustard plants subjected to boron deficiency. *Indian Journal of Plant Physiology* **33**, 150-154.
Shimizu, T., Nakayama, I., Nagayama, K., Miyazawa, T., and Nezu, Y. (2002). Acetolactate Synthase Inhibitors. *In* "Herbicide Classes in Development: Mode of Action, Targets, Genetic Engineering, Chemistry" (P. Böger, K. Wakabayashi and K. Hirai, eds.), pp. 1-41. Springer Berlin Heidelberg, Berlin, Heidelberg.
Simpson, D. J., and Robinson, S. P. (1984). Freeze-fracture ultrastructure of thylakoid membranes in chloroplasts from manganese-deficient plants. *Plant physiology* **74**, 735-741.
Singh, B., and Usha, K. (2003). Salicylic acid induced physiological and biochemical changes in wheat seedlings under water stress. *Plant Growth Regulation* **39**, 137-141.
Skiba, E., Kobyłecka, J., and Wolf, W. M. (2017). Influence of 2, 4-D and MCPA herbicides on uptake and translocation of heavy metals in wheat (Triticum aestivum L.). *Environmental Pollution* **220**, 882-890.
Slooten, L., Capiau, K., Van Camp, W., Van Montagu, M., Sybesma, C., and Inzé, D. (1995). Factors affecting the enhancement of oxidative stress tolerance in transgenic tobacco overexpressing manganese superoxide dismutase in the chloroplasts. *Plant Physiology* **107**, 737-750.
Spurr, A. R. (1957). The effect of boron on cell-wall structure in celery. *American Journal of Botany*, 637-650.
Statistics Canada (2013). Farm Environmental Management Survey (2011). (E. A. a. S. Division, ed.). Minister of Industry, Ottowa, Canada.
Steinrücken, H. C., and Amrhein, N. (1980). The herbicide glyphosate is a potent inhibitor of 5-enolpyruvylshikimic acid-3-phosphate synthase. *Biochemical and Biophysical Research Communications* **94**, 1207-1212.
Swarup, R., Perry, P., Hagenbeek, D., Van Der Straeten, D., Beemster, G. T., Sandberg, G., Bhalerao, R., Ljung, K., and Bennett, M. J. (2007). Ethylene upregulates auxin biosynthesis in Arabidopsis seedlings to enhance inhibition of root cell elongation. *The Plant Cell* **19**, 2186-2196.
Tanaka, R., and Tanaka, A. (2007). Tetrapyrrole biosynthesis in higher plants. *Annu. Rev. Plant Biol.* **58**, 321-346.
Terry, N. (1976). Effects of Sulfur on the Photosynthesis of Intact Leaves and Isolated Chloroplasts of Sugar Beets. *Plant Physiology* **57**, 477-479.
Thompson, I. A., and Huber, D. M. (2007). Manganese and plant disease. *Mineral nutrition and plant disease*, 139-153.

Thompson, L., and Cobb, A. (1987). selectivity of clopyralid in sugar beet; studies on ethylene evolution. *In* "Proceedings of the British Crop Protection Conference-Weeds".

Umena, Y., Kawakami, K., Shen, J.-R., and Kamiya, N. (2011). Crystal structure of oxygen-evolving photosystem II at a resolution of 1.9 Å. *Nature* **473**, 55.

Van Eerd, L. L., Hoagland, R. E., Zablotowicz, R. M., and Hall, J. C. (2003). Pesticide metabolism in plants and microorganisms. *Weed science* **51**, 472-495.

Vauclare, P., Kopriva, S., Fell, D., Suter, M., Sticher, L., Von Ballmoos, P., Krähenbühl, U., Den Camp, R. O., and Brunold, C. (2002). Flux control of sulphate assimilation in Arabidopsis thaliana: adenosine 5′-phosphosulphate reductase is more susceptible than ATP sulphurylase to negative control by thiols. *The Plant Journal* **31**, 729-740.

Wakabayashi, K., and Böger, P. (2004). Phytotoxic sites of action for molecular design of modern herbicides (Part 1): The photosynthetic electron transport system. *Weed biology and management* **4**, 8-18.

Wang, Y.-J., Zhou, D.-M., Sun, R.-J., Cang, L., and Hao, X.-Z. (2006). Cosorption of zinc and glyphosate on two soils with different characteristics. *Journal of hazardous materials* **137**, 76-82.

Wang, Y., Ying, Y., Chen, J., and Wang, X. (2004). Transgenic Arabidopsis overexpressing Mn-SOD enhanced salt-tolerance. *Plant Science* **167**, 671-677.

Weiland, R. T., Noble, R. D., and Crang, R. E. (1975). Photosynthetic and chloroplast ultrastructural consequences of manganese deficiency in soybean. *American Journal of Botany*, 501-508.

Welch, R., Webb, M., and Loneragan, J. (1982). Zinc in membrane function and its role in phosphorus toxicity. *In* "Plant nutrition 1982: proceedings of the ninth International Plant Nutrition Colloquium, Warwick University, England, August 22-27, 1982/edited by A. Scaife". Slough, UK: Commonwealth Agricultural Bureaux, c1982.

Welch, R. M., and Norvell, W. A. (1993). Growth and nutrient uptake by barley (Hordeum vulgare L. cv Herta): studies using an N-(2-Hydroxyethyl) ethylenedinitrilotriacetic acid-buffered nutrient solution technique (II. Role of zinc in the uptake and root leakage of mineral nutrients). *Plant Physiology* **101**, 627-631.

Wimmer, M. A., and Eichert, T. (2013). Mechanisms for boron deficiency-mediated changes in plant water relations. *Plant science* **203**, 25-32.

Wimmer, M. A., Goldberg, S., and Gupta, U. C. (2015). 8 Boron. *Handbook of Plant Nutrition*, 305.

Wu, G., Wilen, R. W., Robertson, A. J., and Gusta, L. V. (1999). Isolation, chromosomal localization, and differential expression of mitochondrial manganese superoxide dismutase and chloroplastic copper/zinc superoxide dismutase genes in wheat. *Plant physiology* **120**, 513-520.

Yachandra, V. K. (2005). The catalytic manganese cluster: organization of the metal ions. *In* "Photosystem II", pp. 235-260. Springer.

Zelko, I. N., Mariani, T. J., and Folz, R. J. (2002). Superoxide dismutase multigene family: a comparison of the CuZn-SOD (SOD1), Mn-SOD (SOD2), and EC-SOD (SOD3) gene structures, evolution, and expression. *Free Radical Biology and Medicine* **33**, 337-349.

Zhang, J., and Kirkham, M. (1994). Drought-stress-induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. *Plant and Cell Physiology* **35**, 785-791.

Zhao, D., and Oosterhuis, D. M. (2003). Cotton growth and physiological responses to boron deficiency. *Journal of plant nutrition* **26**, 855-867.

Zhao, W., Guo, Q., and Zhao, J. (2007). A membrane-associated Mn-superoxide dismutase protects the photosynthetic apparatus and nitrogenase from oxidative damage in the cyanobacterium Anabaena sp. PCC 7120.

Zobiole, L. H. S., Bonini, E. A., de Oliveira, R. S., Kremer, R. J., and Ferrarese-Filho, O. (2010). Glyphosate affects lignin content and amino acid production in glyphosate-resistant soybean. *Acta Physiologiae Plantarum* **32**, 831-837.

Zobiole, L. H. S., de Oliveira Junior, R. S., Bonato, C. M., Muniz, A. S., CASTRO, C. d., de Oliveira, F. A., Constantin, J., and OLIVEIRA JUNIOR, A. d. (2009). Effect of increasing doses of glyphosate on water use efficiency and photosynthesis in glyphosate-resistant soybeans. *In* "Embrapa Soja-Artigo em anais de congresso (ALICE)". In: WORLD SOYBEAN RESEARCH CONFERENCE, 8., 2009, Beijing. Developing a global soy blueprint for a safe secure and sustainable supply: proceedings. Beijing: Chinese Academy of Agricultural Sciences: Institute of Crop Science, 2009. Poster. WSRC 2009. 1 CD-ROM. Editado por Lijuan Qiu, Rongxia Guan, Jian Jin, Qijan Song, Shuntang Guo, Wenbin Li, Yuanchao Wang, Tianfu Han, Xiaobing Liu, Deyue Yu, Lianzhou Jiang, Deliang Peng.