MANGANESE NUTRITION AND PHOTOSYNTHESIS IN NAD-MALIC ENZYME C-4 PLANTS

A Dissertation

presented to the Faculty of the Graduate School

at the University of Missouri-Columbia

In Partial Fulfillment

of the Requirements for the Degree

Doctor of Philosophy

By

MARU KIPLETING KERING

Dr. Dale Blevins, Dissertation Advisor

August, 2008

The undersigned, appointed by the Dean of the Graduated School, have examined the dissertation entitled

MANGANESE NUTRITION AND PHOTOSYNTHESIS IN NAD-MALIC ENZYME C-4 PLANTS

presented by Maru Kipleting Kering

a candidate for the degree of Doctor of Philosophy

and hereby certify that, in their opinion, it is worthy of acceptance.

Dr. Dale Blevins

Dr. Jerry C. Nelson

Dr. Robert Kallenbach

Dr. David Emerich

Dr. Stephen Pallardy

DEDICATION

A special dedication goes to my loving parents

"*Kongoi got-tab Ketele Kapchepsuge* "

ACKNOWLEDGEMENTS

First, I am grateful to my academic advisor, Dr. Dale Blevins for his initial acceptance as well as for the guidance thereafter, the scientific input, and for his ability to untangle complex scientific phenomena with the potency to hook and keep young, aspiring scientists like me thinking and dreaming science. It is this unique ability, his sharp scientific mind, and his tolerance to keep up with me as I lose and regain my balance during the tortuous wading through the intricacy of plant nutrition and physiology, that got me this far. I will forever be indebted to him. I am also grateful to my committee members, Dr. Jerry Nelson, Dr. Robert Kallenbach, Dr. David Emerich, and Dr. Stephen Pallardy for their academic input and support.

I am also indebted Dr. Krystyna Lukaszewski, whose pleasant polish greeting "Cześć Jak się masz" lightened up my days and kept me moving forward towards the finish line. To you Krystyna, I am grateful for your fun-laced scientific advice, numerous scientific suggestions, and editorial abilities which refined my research result write-up. I will miss both the Polish greeting and the fun scientific comments.

I am indebted to my colleagues Dr. William Edward McClain II, Melissa Ann Remley, and Elizabeth Jo Hamilton, for their patience, support, and willingness to help whenever they could. They were my 'adopted' siblings for the duration of my study and allowed me to be a complete me. From this triad, I got a brother's smile and a sister's hug. I was happy, I was mad, I was indifferent, and I

exercised my right to fight over nothing, as is among siblings. Their ability to learn and practice Swahili and Nandi phrases, the constant “Habari yako, mkenya?”, “Tuonane kesho!”, “Chamgei Maru”, kept me constantly conscious or subconscious of an attachment to my homeland, Kenya. You were three incredible ‘Wazungu’ and I will forever be indebted to you. I also want to acknowledge all undergraduate students who by virtue of their work in Dr. Blevins’ laboratory, assisted in the numerous chores associated with my research. You all deserve a pat on the back and I say thanks to all of you.

Lastly, I want to acknowledge my loving parents, who allowed me to go with my dreams, missed me in the process, but supported me nonetheless. I dedicate this dissertation to them because they are the two most instrumental people in what I am today. There has not been a time until now to let them know that they are the reason I keep taking life-changing and successful missions. During these life-gambling decisions, I got so much from them and could not have asked for more.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

MANGANESE NUTRITION AND PHOTOSYNTHESIS IN NAD-MALIC ENZYME C-4 PLANTS

Maru Kipleting Kering

Dr. Dale Blevins, Dissertation Advisor

ABSTRACT

Based on their photosynthetic pathways, plants can be divided into three major groups: C-3, CAM, and C-4. In C-4 plants, the release of CO_2 for Calvin cycle reactions in bundle sheath cells (BSC) involves one of the three principal enzymes: NADP-malic enzyme (NADP-ME), PEP-carboxykinase enzyme (PEP-CK), and NAD-malic enzymes (NAD-ME). Of these three decarboxylating enzymes, only the activation of NAD-ME has an absolute requirement for Mn, therefore, leaf Mn concentrations could be critical for maximum NAD-ME activity and the continued supply of CO_2 to bundle sheath cells. The objective of this research was to determine the Mn requirement for optimum photosynthesis and plant biomass production for two agriculturally important NAD-ME C-4 species, pearl millet (*Pennisetum glaucum* L. R. Br) and purple amaranth (*Amaranthus hypochondriacus* L.). These species were examined in parallel with two NADP-ME (no Mn activation required) species, corn (*Zea mays* L.) and sorghum (*Sorghum bicolor* L. Moench), and two C-3 species, wheat (*Triticum aestivum* L. cv. Ernie) and squash (*Cucurbita pepo* L. cv. straighneck) added as controls. Plants were grown in a complete nutrient solution with Mn concentrations ranging

from 0 to 100 μM. Field grown pearl millet and purple amaranth received Mn from two sources, Mn beads and manganese chloride.

Manganese concentration required for optimum photosynthetic rate and biomass production of the C-3 and NADP-ME C-4 species was found to be ~2 μM, which is the concentration commonly used in plant nutrient media. Manganese concentrations above 2 μM had no significant effect on either photosynthetic rate or biomass production of these plants. Also, in C-3 and NADP-ME C-4 species, light saturated photosynthesis (A_{max}) was the highest for plants receiving 2-5 μM Mn and no change was observed with increasing Mn concentration. In contrast, in both NAD-ME species, the optimum growth and photosynthetic responses required Mn concentrations 20-fold higher than those typically used in hydroponic media, and increasing Mn concentration from 10 to 75 μM resulted in a 50% increase in photosynthetic rate in purple amaranth and a 36% increase in pearl millet. NAD-ME plants receiving higher Mn concentrations had greater responses to increasing photosynthetic photon flux density (PPFD), and at saturating light, pearl millet and purple amaranth receiving ≥ 50 μM Mn achieved higher A_{max} than those receiving lower Mn treatments. However, in all plant species, Mn treatment had little effect on the apparent quantum yield (AQY), perhaps indicating that at this range, light rather than Mn was limiting photosynthesis. Interestingly, Mn concentration higher than 2 μM had little effect on stomatal conductance in all six tested species. This strongly implies that increased photosynthetic rates in NAD-ME species with

higher Mn nutrition was a result of a better internal supply/utilization of CO_2 and not of an improved stomatal conductance.

In field experiments, Mn fertilization resulted in slightly increased leaf Mn concentrations and an up to ~20% increase in photosynthetic rate. In general, Mn fertilization had little effect on seed mineral element composition or seed protein and oil content, but resulted in a slight increase in seed yield.

This is, to my knowledge, the first information on the substantial, 20-fold higher Mn requirement for optimum photosynthesis and biomass production of NAD-ME C-4 plants, compared to other plant species. This finding should be considered in future research on NAD-ME C-4 crops, especially under soil conditions that decrease Mn availability for plant uptake. As more information is collected on NAD-ME C-4 plant biochemistry, physiology, and structure, more specific recommendations for nutrient requirements and more specific Mn application strategies can be developed.

CHAPTER 1

LITERATURE REVIEW

The Plant Kingdom: Strategies for classification

The plant kingdom, Plantae, includes a wide range of diverse organisms that vary greatly in size and complexity. It comprises about 260,000 species that are divided into groups based on a number of structural and life-cycle features (Soltis and Soltis 2004). The main classification systems for the plant kingdom include (1) artificial: devised specifically for convenience, focusing on one or a few morphological characteristics, (2) natural: dependent on the total knowledge of plant biology, (3) phylogenic: classication based on evolutionary sequence, reflecting plant genetic relationships (Lawrence 1951).

The artificial system was used by Carolus Linnaeus, who classified plants based on the number of stamens in the flower (Weier et al. 1970). Because his publications, 'Genera Plantarum' (1737) and 'Species Plantarum' (1753), formed the basis for the two name-system of plant taxonomy, Carl Linnaeus is considered the father of the binomial nomenclature (Davis and Heywood 1963). During the era of the natural system of classification, de Jussieu, de Condole, Lamarck, and others grouped plants based on their understanding of correlations in plant biological characters (Davis and Heywood 1963).

The publication of Darwins' work 'The Origin of Species' in 1859, contributed to a more diverse approach to plant taxonomy, and gave more credit to the phylogenic relations (Davis and Heywood 1963). Darwin's idea that species are represented by variable populations was rediscovered in the

principles of Mendelian genetics (Davis and Heywood 1963) and led to the development of population genetics (Wilkins 1962).

Advances in science, the discovery of genes, and the realization that expression of morphological and other traits in plants is under the control of genes encoded in DNA, have led to further improvement of phylogenic classification (Taiz and Zieger 2006). Advances in molecular biology, allozyme comparisons, protein sequencing and DNA/DNA hybridization (Vicky Jr. 1984), and the development of polymerase chain reaction (PCR) for DNA sequencing (Hoch and Stephenson 1995), have helped to determine phylogenic relationships among organisms. In plants, chloroplast DNA is the preferred component for phylogenic comparison because of its small, relatively uniform size, and a highly conserved genome (Doyle et al. 1984, Clegg and Zurawski 1992, and Olmstead and Palmer 1994). In general, current plant classification incorporates both classical taxonomy, as well as new approaches like cytology and DNA hybridization to achieve phylogenetic classification.

It has long been known that environmental factors like temperature, light, soil moisture, aeration, and nutrient content result in plant modification (Morisset and Boutin 1984). Plant genetic composition and the environment interact and cause changes in the expression of morphological and metabolic characters. The response to a specific environmental factor varies among individuals, between populations, and between plant species (Bradshaw 1965).

Differences in plant phenotypic response to the same environmental factor may indicate differences in biochemical and physiological processes, and based

on these responses, plants may be classified into different groups. Therefore, in addition to the phylogenetic relations, plants are divided into different categories based on specific biochemical traits that are unique for different groups and separate them from others. One such example is the evolutionary adaptations of the photosynthetic pathway to environmental conditions.

Plant classification based on different photo-synthetic carbon fixation reactions

Plants are able to utilize solar energy to synthesize organic molecules. Incorporation of inorganic materials into organic compounds occurs during photosynthesis, a process unique to all green plants, some algae, and photosynthetic bacteria. The synthesized organic compounds can be stored, or used to meet immediate plant energy and growth requirements.

Photosynthesis occurs in plant chloroplasts in two main steps: the light reactions (photophosphorylation) and the carbon reduction reactions (Calvin cycle). Calvin cycle reactions are common to all plants, but in response to environmental pressures, some species developed mechanisms for CO_2 concentration. As a result, there is some variation in the photosynthetic pathway between the evolutionarily older and younger plant species. Based on their photosynthetic pathways, plants are grouped into three distinct types: C-3, CAM, and C-4.

C-3 plants

The C-3 pathway of carbon fixation is so named because the first stable organic compound formed during CO_2 reduction is a three carbon (C) molecule, phosphoglyceric acid (PGA) (Benson et al. 1950). In C-3 photosynthesis, the Calvin cycle reactions occur in the chloroplast stroma of mesophyll cells (MC). During Calvin cycle reactions, the pentose sugar, ribulose-1,5-bisphosphate (RuBP), is carboxylated in a reaction catalyzed by ribulose-1,5-bisphosphate carboxylase-oxygenase (Rubisco). The reducing power and energy for these reactions come from reduced nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP) formed during light photophosphorylation reactions. Carboxylation of RuBP results in the formation of two molecules of 3-phosphoglyceric acid (3-PGA). Therefore the first CO_2 fixation reaction in these plants is the Rubisco reaction, where one carbon from CO_2 is added to a five carbon sugar RUBP, resulting in two molecules of a three carbon compound, 3-PGA (Taiz and Zeiger 2006).

Crassulacean acid metabolism (CAM) plants

Crassulacean acid metabolism was first identified in *Crassulacea* (Lawlor 2001). Plants utilizing this photosynthetic pathway are found predominantly in xerophytic environments. To conserve water, CAM plants have higher water retention capacity, thickened cuticles, and low stomatal densities. In addition to Calvin cycle reactions, CAM plants have auxiliary reactions that concentrate CO_2

close to Rubisco in the chloroplast stroma. However, the CO_2 concentrating process and the Calvin cycle reactions are separated in time. During the night, the stomata are opened, CO_2 is taken in and converted to the bicarbonate ion (HCO_3^{-1}) in mesophyll cells by the Zn-containing enzyme, carbonic anhydrase (CA). The HCO_3^{-1} formed is then attached to phospho-enol pyruvate (PEP) by PEP-carboxylase to form oxaloacetate (OA). In the next stage, malate dehydrogenase (MDH) reduces OA to malate, which is moved to vacuoles for storage. During the day, photophosphorylation occurs and ATP and NADPH are synthesized. The stored malate is then released from vacuoles and decarboxylated by nicotinamide-adenine dinucleotide phosphate malic enzyme (NADP-ME) (Edward and Walker 1983, Bhagwat 2005). The CO_2 resulting from malate decarboxylation is used in Calvin cycle reactions and the other product, pyruvate, is phosphorylated to regenerate PEP.

C-4 plants

In the C-4 photosynthetic pathway, initially identified in sugarcane (Kortschak et al. 1965, Hatch and Slack 1966), the first organic compound produced by CO_2 fixation is a four-carbon molecule, OA (Edwards and Walker 1983). Plants belonging to this photosynthetic group are distinguished by the presence of a well developed, thick layer of chloroplast-containing cells called bundle sheath cells (BSC). Like CAM plants, C-4 plants inhabit some of the driest, hottest, and most saline environments (Long 1999), and also concentrate CO_2 before Calvin cycle reactions (Lawlor 2001). In dry environments, water

rather than CO_2 may be the most limiting resource, and C-4 plants have reduced stomatal apertures to control water loss by transpiration. Reduced CO_2 uptake arising from the small stomatal aperture is compensated for by concentrating CO_2 around Rubisco in BSC. In this manner, the carboxylase and oxygenase activities of Rubisco are increased and reduced, respectively. In C-4 plants, the CO_2 concentration pathway begins in the mesophyll cells with the enzymatic hydration of CO_2 by CA to form HCO_3^{-1}. Hydration of CO_2 is followed by the incorporation of HCO_3^{-1} into PEP to form OA, in a PEP-carboxylase catalyzed reaction. Oxaloacetate formed is then reduced to malate by MDH. The fate of malate in the mesophyll cell then depends on the subtype of the C-4 plant (Taiz and Zeiger 2006, Buchanan et al. 2000).

In C-4 plants, unlike the CAM plants, both the CO_2 concentration mechanism and Calvin cycle reactions occur at the same time, but are separated spatially. The CO_2 hydration and incorporation of HCO_3^{-1} into PEP to form OA occurs in the MC (Badge and Price 1994, Burnell 1986), however, the second fixation, a Calvin cycle reaction, occurs in the BSC chloroplast. While decarboxylation of the 4-C organic acid may occur in different cell compartments in different C-4 plants, it always occurs within the BSC. In C-4 plants, the release of CO_2 for Calvin cycle reactions in BSC involves one of three principal enzymes: NADP-ME, nicotinamide-adenine dinucleotide malic enzyme (NAD-ME), or PEP-carboxykinase (PEP-CK).

Intermediates

Although there are distinct differences separating C-3, CAM, and C-4 plants, some species show no specific pattern and are considered intermediate. For example, some CAM plants, depending on environmental conditions have the ability to switch between the CAM and C-3 photosynthetic pathways (Edwards and Walker 1983). There are also plants whose anatomy, biochemistry, and CO_2 compensation points are intermediate between C-3 and C-4 (Brown 1976, Ku et al. 1985, Hattersley et al. 1986). In C_3-C_4 intermediates *Flaveria* and *Moricandia*, the presence of Kranz anatomy is responsible for their low apparent photorespiration as compared to their C-3 relatives (Brown and Hattersley 1989). In a C_3-C_4 intermediate *Amaranthaceae*, higher photosynthetic rates than those found in C-3 plants were observed despite the low levels of C-4 photosynthetic enzymes (Rajendrudu et al. 1986). It was concluded that the low apparent photorespiration due to presence of the Kranz-like anatomy in this C_3-C_4 Amaranthaceae was responsible for the high 'net' photosynthesis.

Macronutrient-use efficiency of C-3 and C-4 plants

The difference in biomass production per unit leaf nutrient concentration differs among plant species. According to many studies, different photosynthetic pathways can be connected with different nutrient-use efficiencies (NUE) of some major plant macronutrients.

Nitrogen

Nitrogen (N) is a critical component of amino acids and proteins, including important photosynthetic and photosynthesis-associated enzymes, as well as other accessory compounds like ATP and NADPH. It is also a structural component of the chlorophyll forming molecule, the porphyrin ring. In all plants, at saturated light levels, leaf photosynthetic capacity is positively correlated with leaf N concentration (Epstein and Bloom 2005). Addition of N to N-deficient plants results in photosynthetic rates that increase linearly with increasing leaf N. However, N requirements differ among plant species, as does the plant N-use efficiency. It has been reported that C-4 plants, using less leaf N, have greater photosynthetic rates and accumulate more biomass than C-3 plants (Bolton and Brown 1978, Brown 1978, Schmitt and Edwards 1981, Blevins 1983). In C-4 corn, higher photosynthetic N-use efficiency as compared to C-3 rice was attributed to low N investment in the C-4 cycle enzymes, allocation of more N to thylakoid components, and a general reduction in amount of Rubisco in leaves (Makino et al. 2003). Even transgenic rice with levels of Rubisco optimal for CO_2-

saturated photosynthesis showed lower N-use efficiency than corn (Makino et al. 2003). A greater N-use efficiency was also found in the C-4 plant pigweed (*Amaranthus retroflexus* L.), than in the ecologically similar C-3 lambsquarters (*Chenopodium album* L.) (Sage and Pearcy 1987).

Phosphorus

Phosphorus (P) is a ubiquitous component in plant metabolism, and photophosphorylation is a critical step during photosynthesis. Photophosphorylation involves an addition of phosphorus to ADP to form ATP, a high energy compound that helps to drive the photosynthetic carboxylation reactions (Lawlor 2001). All cell membranes contain phospholipids, and P is needed for membrane transport processes important for photosynthesis. A thylakoid membrane-located transporter protein co-transports the inorganic phosphate ion (Pi) and triose-3 phosphate (Rychter and Rao 2005). This allows export of triose-3 phosphate into the cytosol and prevents its inhibition of photosynthesis as well as the buildup of extremely high starch in the chloroplast. In C-4 photosynthesis, the CO_2 acceptor compound is a phosphorylated compound PEP, and decarboxylation of OA in PEP-CK C-4 plants involves the high energy P-containing compound, ATP. It is also important to note that the activities of several enzymes depend on phosphorylation or dephosphorylation of amino acid residues on the protein (Nelson and Cox 2005).

Under P deficiency, the photosynthetic rates of two C-4 grasses decreased by 25% while the photosynthesis rate of a C-3 grass under the same

conditions decreased by 50% (Ghannoun and Conroy 2007). However, despite the difference in photosynthetic responses, P deficiency reduced dry biomass equally in all grasses. Halsted and Lynch (1996) found differences in P-use efficiency between monocots and dicots. Low P inhibited branching in dicots to a larger extent than it inhibited tillering in monocots. Monocots, therefore, are able to maintain higher leaf production under P-deficient conditions than dicots. A three year field experiment on P-use efficiency of a C-4 corn and two C-3, sunflower (*Helianthus annuus* L.), and sugarbeet (*Beta vulgaris* L.) showed maize to have a higher P-use efficiency (Jocić and Sarić 1983).

Potassium

Potassium is critical for xylem transport, is involved in control of xylem osmotic potential and in improving water uptake/retention in plants (Mengel and Kirkby 1978). Potassium is a key component in the mechanism of stomatal opening and closing (Fisher and Hisiao 1968, Humble and Raschke 1971) and K deficiency impairs these processes (Terry and Ulrich 1973). Phloem loading and transport of photosynthates also involves K (Asyley and Goodson 1972, Mengel and Kirkby 1980). For example K-deficient corn plants showed defective metabolite transport between mesophyll cells and BSC (Barankiewicz 1978). Furthermore, K plays a critical role in protein and starch synthesis (Evans and Sorger 1966, Koch and Mengel 1974). The roles of K in protein synthesis include transport of organic and inorganic materials, and activation of enzymes involved (Blevins 1985, Marschner 1995). Finally, K helps to maintain thylakoid

depolarization and activates ATP-ase, the critical proton pump, whose activity results in ATP synthesis and photoreduction of NADP during photophosphorylation (Pfündel and Mengel 1972).

Potassium also balances negatively charged amino acids like aspartate and glutamate, and stabilizes protein-water layer interactions (Blevins 1985). Because of the numerous roles of K, and the differences in metabolic and physiological processes observed in plants, there is a wide variation among and between plant species in K requirement. In a study of 10 potato cultivars, the ability to use non-exchangeable K in soil was determined to be the main factor responsible for differences in K-use efficiency among these cultivars (Trehan et al. 2005). In rice, the efficiency of K translocation and distribution within the plant was found to be responsible for differences in cultivars for internal K-use efficiency (Yang et al. 2004). Both inter-specific and intra-specific differences in K utilization were found in cool-season Kentucky bluegrass (*Poa pratensis* L.), perennial ryegrass (*Lolium perenne* L.), and tall fescue (Liu et al. 1995). The fact that the content of Rubisco, the dominant protein in green leaves, is lower in C-4 than in C-3 plants (Schmitt and Edwards 1983), may result in a lower K need to balance the negative charges. In fact, a difference in K-use efficiency was observed between C-4 and C-3 forage grasses, with C-4 grasses achieving higher biomass per unit leaf K than the C-3 grasses (Blevins 1983).

C-4 plants have several beneficial nutritional characterists like high N-, P-, and K-use efficiencies, as well as high photosynthetic efficiency through the CO_2 concentrating mechanism.

Differences in micronutrient profiles between C-3 and C-4 plants

Different photosynthetic pathways in C-3 and C-4 species can be linked with specific micronutrient requirements.

Zinc

Carbonic anhydrase, a Zn-containing enzyme, is required for the initial CO_2 fixation in C-4 plants, but not for CO_2 fixation in C-3 plants. During photosynthesis, all C enters the plant via the stomata as atmospheric CO_2. In C-3 plants, CO_2 is incorporated into RuBP by Rubisco to form two molecules of 3-PGA. However, in C-4 plants, HCO_3^{-1} is initially fixed by addition to PEP by PEP-carboxylase to form OA. The hydration of atmospheric CO_2 to the bicarbonate anion (HCO_3^{-1}) is catalysed by the Zn-containing CA. In C-4 plants, the formation of the 4-C organic acid requires a rapid and constant supply of HCO_3^{-1} to PEP-carboxylase. Because of the use of HCO_3^{-1} by C-4 plants, there is a need for elevated CA activity. Therefore, Zn is required to maintain high photosynthetic rates and to achieve high yields in C-4 plant.

Zinc deficiency in the plant, C-4 corn, resulted in a reduced net photosynthetic rate and stomatal conductance (Wang and Jin 2005), a reduction in maximum quantum efficiency of photosystem II (PS II), and a general depression of PS II activities. In a transgenic C-4 dicot *Flaveria bidentis* (L.) Kuntze expressing low CA activities, reduced photosynthetic activity and poor growth were observed at ambient CO_2 levels (Von Caemmerer et al. 2004). In

Missouri, Zn fertilization improved the yields of sorghum (*Sorghum bicolor* (L.) Moench) and corn but had less impact on C-3 small grains and alfalfa (*Medicago sativa* L.) hay production (Buchholz et al. 1993). In Minnesota, application of 0.2 Kg Zn/ha to a Zn-deficient soil doubled maize grain yield (Rehm 2004). In C-3 plants, where molecular CO_2 is the first reactant in the C fixation reactions, high Zn concentrations may not be critical because CO_2 is favored over HCO_3^{-1} at equilibrium conditions. In C-3 cotton, no direct relationship between CA activity and photosynthesis was observed and optimum photosynthesis was achieved at what would be considered Zn-deficient conditions (Ohki et al. 1976). In wheat, another C-3 plant, there was no relationship between CA activity and seedling growth (Dell and Wilson 1985).

Because of the non-photosynthetic roles of Zn as a structural component and activator of many enzymes, C-3 plants show some response to Zn deficiency. Zinc-deficient common bean (*Phaseolus vulgaris* L.) showed reduced internode length, reduced leaf light-use efficiency, and lower maximum photosynthetic rates (Gianquinto et al. 2000). These plants also had about 3-fold lower saturation irradiance, reduced photosynthesis and decreased seed production. Given the additional role of Zn in C-4 photosynthesis, perhaps the photosynthetic classification of the species should be taken into account when considering Zn supply necessary for optimum plant growth.

Sodium

Although sodium (Na) is not required by C-3 plants, there is evidence for its role in the photosynthetic process in some C-4 plants (Brownwell and Crossland 1974). A Na-dependent ATPase-mediated active transport of amino acids between the MC and BSC has been reported for C-4 plant, amaranth (*Amaranthus paniculatus* L.) (Raghavendra and Das 1978). Sodium fertilization of representative C-4 species reduced leaf alanine concentration by a factor of two, as compared to Na-deficient plants, yet it had no effect on leaf alanine in a Na-deficient C-3 tomato (*Lycopersicum esculentum* Miller) (Nable and Brownell 1984). In other C-4 plants, mexican fireweed (*Kochia trichophylla* L. Schr.) and pearl millet (*Pannicum miliaceum* L.), Na nutrition increased the synthesis of PEP from pyruvate (Brownell and Bielig 1996).

In Na-deficient C-4 *Kochia childsii*, *Chloris gayana*, *Amaranthus edulis*, *Amaranthus tricolor* and *Atriplex spangiosa*, higher concentrations of malate, PEP and 3-PGA, and lower concentration of pyruvate and alanine were found compared to plants receiving sufficient Na (Johnston et al. 1988). Following Na fertilization, five out of seven C-4 grass species, barnyardgrass (*Echinochloa crusgalli* (L.) Beauv), kleingrass (*Panicum coloratum* L.), fall panic grass (*Panicum dichotomiflorum* Michx), guinea grass (*Panicum maximum* Jacq.), and rhodes grass (*Chloris gayana* Kunth), showed positive response in growth and nitrate reductase activity (Ohta et al. 1988), while the dicotyledonous plant pigweed (A*maranthus tricolor* L.) had up to 3-fold increase in dry matter production (Ohta et al. 1989, Matoh et al. 1986).

Manganese

Manganese (Mn) is a micronutrient element required by all plant species for growth and reproduction (Marschner 1995). Inside the plant, Mn is a component of the water splitting protein complex, PS II. It is a constituent of superoxide dismutase (MnSOD), and a key activator of a number of critical metabolic enzymes (Marshner 1995). Specifically, Mn plays a role in nitrogen metabolism by activating arginase and glutamyl transferase enzymes (Burnell 1988) and is critical for maximizing N_2-fixation in soybean under water stress (Vadez et al. 2000, Purcell et al. 2000). It is involved in rhizobial metabolism (Waters and Emerich 2000) and in leaf ureide catabolism in soybean by activating allantoate amidohydrolase (Winkler et al. 1987, Lukaszewski et al. 1992). Manganese is also required for the activation of NAD-malic enzyme, a critical enzyme in the C-4 photosynthetic pathway (Hatch and Kagawa 1974, Burnell 1986). Since it is a constituent of the PSII in all plants, Mn deficiency could significantly affect leaf photosynthetic activity, dry matter accumulation, forage and grain yield of all plants, but C-4 plants with Mn-activated malate decarboxylation reaction may be especially affected.

Because of such specific Mn functions, optimum leaf Mn concentrations differ among plant species. For example, in non-nitrogen fixing C-3 plants like barley, leaf Mn concentrations of 10-15 mg/kg were found to be sufficient for optimum dry weight (Hannam and Hohki 1988). In soybean, Mn concentrations from 21-100 mg/kg were considered sufficient and levels above 250 mg/kg were considered toxic (Hannam and Hohki 1988). However, no research has been

published on the possible impact of the Mn-dependent NAD-ME photosynthetic pathway on Mn requirement in C-4 plants.

C-4 plant diversity based on the principal decarboxylation reactions

In C-4 plants, the release of CO_2 for Calvin cycle reactions in BSC involves decarboxylation of the 4-C organic acid by one of three enzymes: NADP-ME, NAD-ME or PEP-CK. Based on the principal decarboxylase involved in CO_2 release, C-4 plants are divided into three sub-types:, NADP-ME, PEP-CK, and NAD-ME (Edwards and Black 1971, Hatch and Kagawa 1974, Hatch et al. 1982, Burnell 1986). The differences in C-4 plant photosynthetic pathways, the intercellular organic molecule movement, and decarboxylating enzymes involved are presented in Figure 1-1.

NADP-ME C-4 plants

In NADP-ME C-4 plants, OA is reduced to malate by MDH using NADH in the mesophyll cell. Malate is then moved to the BSC where it is decarboxylated by NADP-ME to release CO_2 and pyruvate. Magnesium is the preferred cation for NADP-ME activation, but it may be substituted by Mn (Hatch and Kagawa, 1974). Pyruvate is moved back to the mesophyll cell where it is phosphorylated to form PEP, whereas the CO_2 released is used in the Calvin cycle reactions in chloroplast stroma of the BSC (Lawlor 2001, Bhagwat 2005).

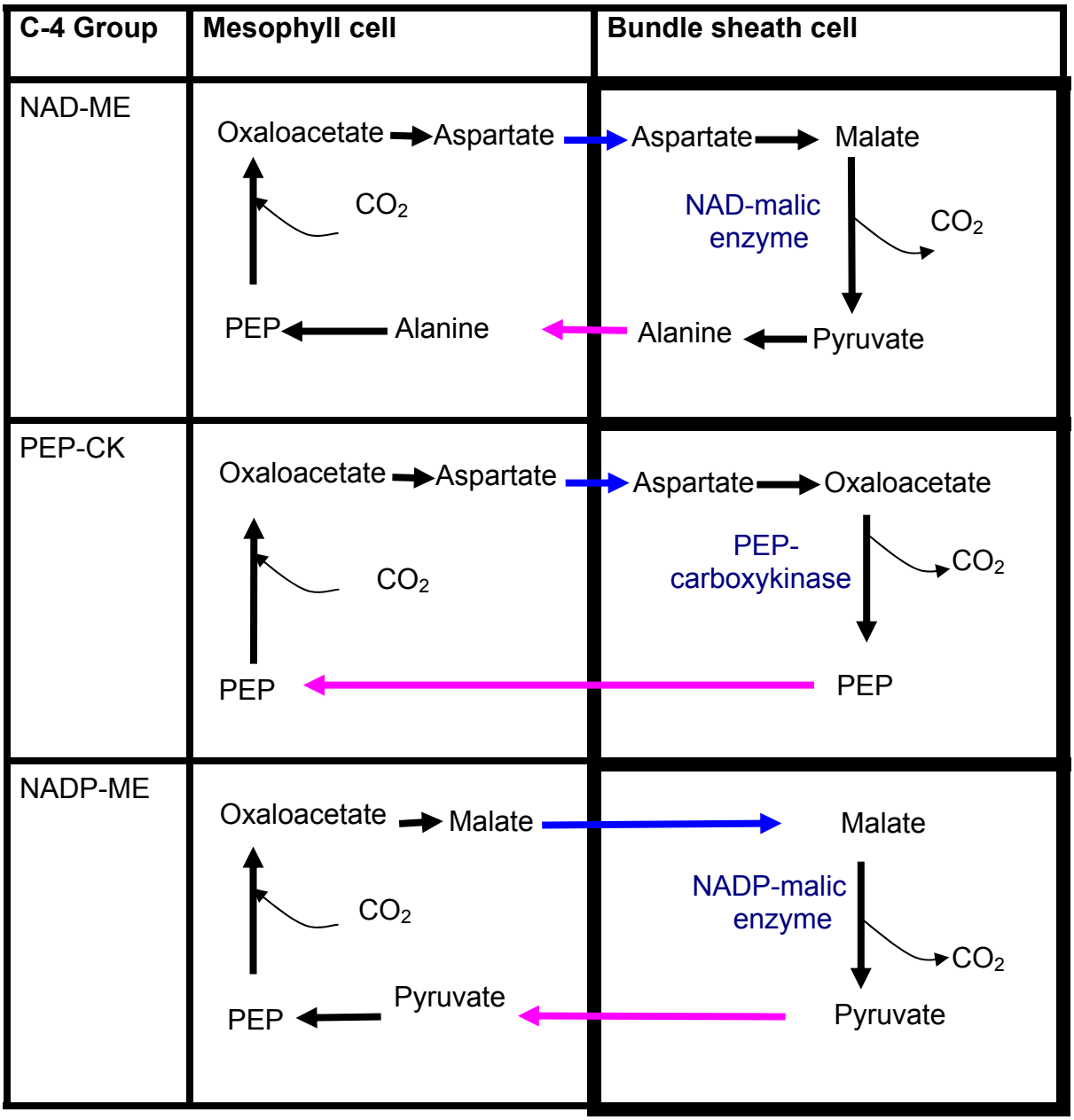


Figure 1-1. Movement of organic compounds between the mesophyll and bundle sheath cells and the specific compound decarboxylated to release CO_2 for Calvin cycle reactions in C-4 plants. Re-drawn from Marschner (1995).

PEP-CK C-4 plants

In PEP-CK C-4 plants, OA is aminated to aspartate by aspartate aminotransferase in mesophyll cells. Aspartate is then moved into BSC, where OA is re-formed. In the BSC, a cytosolic Mn-activated PEP-CK decarboxylates and phosphorylates OA to release PEP and CO_2. The released CO_2 is used for Calvin cycle reactions in BSC chloroplast stroma, whereas PEP is moved to the mesophyll cell to incorporate more HCO_3^{-1}. The PEP-CK is activated by Mn, however, it can also be activated by Mg (Zhi-Hui et al. 2002). The Mg activation may be linked to Mg-ATP, a compound which is brought into position to phosphorylate OA and release PEP with simultaneous release of CO_2 by this enzyme.

NAD-ME C-4 plants

In NAD-ME C-4 plants, OA is aminated to aspartate by aspartate aminotransferase in the mesophyll cell. Aspartate is then moved into BSC, where OA is re-formed and reduced to malate by a cytosolic MDH. Malate is then moved to the BSC mitochondria where it is decarboxylated by a mitochondrial Mn-activated NAD-ME to release pyruvate and CO_2 (Kagawa and Hatch 1975). Pyruvate is converted to alanine which is shuttled back to the mesophyll cells where it is deaminated and phosphorylated to form PEP (Figure 1-1). The released CO_2 is used in the Calvin cycle reaction in BSC chloroplast stroma.

Manganese, which activates both PEP-CK and NAD-ME, may be replaced by Mg in PEP-CK, however, NAD-ME activity has an absolute requirement for

Mn (Burnell 1986). The NAD-ME reaction is critical for the release of CO_2 and pyruvate in BSC of NAD-ME C-4 plants (Dever et al. 1998). An *Amaranthus edulis* (Speg.) mutant deficient in NAD-ME, though able to oxidize malate via the Krebs cycle, could not decarboxylate malate (Dever et al. 1998). It is noteworthy that Mn activated NAD-ME activity in BSC of C-4 plants is about 50 times greater than that normally found in leaves of C-3 plants (Hatch and Carnal, 1992).

Specific nutrient requirements within C-4 plants

The differences in nutrient requirement and/or efficiency are not only found between C-3 and C-4 plants, but also among the different C-4 sub-types. These differences can be linked to the unique steps in the photosynthetic pathways.

Nitrogen

In a study of 27 C-4 grass species under sufficient and deficient N levels, NADP-ME grasses had a higher CO_2 assimilation rate per unit N and a greater total dry weight per total N than NAD-ME grasses (Ghannoun et al. 2005). Compared to NAD-ME grasses, NADP-ME grasses had lower amounts of leaf N, attributed to less soluble protein and a lower amount of Rubisco with faster turnover rates (k_{cat}).

Sodium

Sodium deficient C-4 plants had higher concentrations of malate, PEP, and 3-PGA, and lower concentrations of pyruvate and alanine than Na–sufficient plants (Johnston et al. 1988). However, the levels of pyruvate and alanine in Na-deficient PEP-CK C-4 *Chloris gayana*, and NAD-ME C-4 *Amaranthus edulis*, *Amaranthus tricolor*, and *Atriplex spangiosa* where higher than in NADP-ME C-4 *Kochia childsii*. Since Na is needed for transport of pyruvate into the MC where pyruvate is used for regeneration of PEP (Figure 1-1), it can be expected that within the C-4 plant, the NADP-ME species have higher Na requirement than the NAD-ME and PEP-CK. Following Na fertilization, NAD-ME C-4 kleingrass (*Panicum coloratum* L.) and fall panic grass (*Panicum dichotomiflorum* Michx), as well as PEP-CK C-4 guinea grass (*Panicum maximum* Jacq.) and rhodes grass (*Chloris gayana* Kunth) showed higher nitrate reductase activity, a response not seen in NADP-ME C-4 species barnyardgrass (*Echinochloa crusgalli* (L.) Beauv) and corn (Ohta et al. 1988). In the chloroplasts of NAD-ME C-4 pearl millet, pyruvate uptake was increased by Na application, a case not found in NADP-ME corn and sorghum (Ohnishi et al. 1990). It was concluded that NAD-ME plants had a Na-pyruvate cotransporter, while NADP-ME had a H-pyruvate cotransporter. The involvement of Na in pyruvate transport in NAD-ME plants has been suggested to be the primary function of Na in C-4 plant nutrition.

Manganese

It is well established that the decarboxylation enzymes in BSC of C-4 plants, PEP-CK and NAD-ME, are activated by Mn (Hatch and Kagawa 1974, Burnell 1986), while NADP-ME is activated by Mg (Hatch and Kagawa, 1974). In sudan grass, a C-4 plant that utilizes a Mg-activated NADP-ME, optimum dry weight was achieved at solution Mn concentrations of between 4.5 and 9.0 μM Mn (Bowen, 1972). Shoot tissue Mn concentrations > 200 μg/g were toxic and led to decreased dry matter accumulation. It is possible that high Mn concentrations in plant tissue inhibit the activation of both Rubisco and NADP-ME by competing with Mg, a view proposed by Marschner (1995). If this is so, a potential summative effect on photosynthesis reduction may occur. However, since the NAD-ME activation is totally dependent on Mn (Burnell 1986), the response of NAD-ME C-4 plants to Mn may differ from that of other C-4 species.

Important forage and grain crops with the NAD-ME photosynthetic pathway

Many important cereal and forage crops belong to the NAD-ME C-4 subtype, including amaranth (*Amaranthaceae*), pearl millet (*Pennisetum glaucum* (L.) R. Br), switch grass (*Panicum virgatum* L.), Bermuda grass (*Cynodon dactylon* L.), bufallo grass (*Buchloe dactyloides* L.) and blue grama (*Bouteloua gracilis* L.) (Edwards and Walker 1983).

Pearl millet (*Pennisetum glaucum* (L.) R. Br.), one of the most important cereals in drought-prone areas and a staple primary caloric source for millions of people in semi-arid tropical regions of Africa and Asia, is grown for food worldwide on a total of 40 million ha (FAO 1986, Diouf et al. 2006). Grain from pearl millet has higher protein and essential amino acid content than maize, sorghum, wheat, or triticale grain (Burton et al. 1972, Ejeta et al. 1987, Smith et al. 1989, Haydon and Hobbs 1991). A superior amino acid profile compared to sorghum, the high protein content and high digestible energy found in pearl millet have made it an important nutrient source for poultry and swine production (Singh and Perez-Maldonando 2003). As a result, new dwarf pearl millet hybrids specifically designed for grain production, have been released, (e.g University of Nebraska cultivar HGM 686 (Myers 2002).

Pearl millet grows better on poor soils than most other crops and is estimated to occupy over 600,000 hectares in the US (Andrews et al. 1996). It is widely used as a high quality summer crop for milk and beef production (Wilson, 2004). Pearl millet, unlike the common summer forages, sudan grass and sorghum, is free of prussic acid, a compound toxic to animals at high concentration (Teutsch 2002). Drought and high temperature tolerance make pearl millet a good alternative for hot and/or dry summer grazing, when a “slump” occur in the productivity of popular cool season grasses, like tall fescue, occurs. In fact, pearl millet has been proposed as a good alternate forage crop during late summer and early fall for Missouri farmers (Kallenbach et al. 2004).

The NAD-ME C-4 photosynthetic pathway is also present in some dicots, like *Amaranthaceae*. Amino acid and protein quality of amaranth grain are higher than those of most cereal grains (Becker et al. 1981, Breene 1991) and protein contents range between 13 and 19% (Stordahl et al. 1999, Lehmann 1990, Yue et al. 1987). Amaranth protein has high levels of lysine, cysteine, and methionine, three essential amino acids found to be low in other grains like corn, wheat, and rice (Senft 1979, Bressani et al. 1987). Amaranth seed contains 6 to 10% oil, made mostly of unsaturated fatty acids (76%) including high linolenic acid (Betschart et al. 1981, Lorenz and Hwang 1985, Garcia et al. 1987).

Amaranth can be grown for forage, and when cut at bud stage it has 14-18% protein, 30-40% acid detergent fiber, and 43-53% neutral detergent fiber (Stordahl et al. 1999). Amaranth leaves and stems are higher in undegraded intake protein (UIP) than alfalfa and comfrey (Cheeke and Bronson 1979). High UIP improved feed efficiency and increased body weight gain in heifers (Tomlinson et al. 1997) and milk yield in dairy cows (Vagnoni and Broderick 1997). The UIP values of 230-310 g kg^{-1} of total crude protein were reported for another NAD-ME C-4 plant switch grass, compared to 110-180 g kg^{-1} in C-3 plant smooth brome grass (*Bromus inermis* L.) (Mitchell et al. 1997). It was suggested that the presence of Kranz anatomy contributed to higher UIP values in switch grass.

Because of their Kranz anatomy, higher UIP can be expected in both pearl millet and amaranth. This high UIP potential may improve growth rate and milk

production in animals as observed for other forages with high UIP (Tomlinson et al. 1997, Vagnoni and Broderick 1997).

C-4 photosynthetic pathway and plant adaptability to changing environmental conditions

Native C-4 grasses, inhabiting the extensive prairies and savannas of tropical and temperate regions of the world, account for 18% of global primary productivity (GPP) (Lloyd and Farquhar 1996, Ehleringer et al. 1997). Any changes in C-4 productivity driven by CO_2, temperature, or other climatic perturbations, will likely have an impact on GPP (Wand et al. 1999). Adaptability of these plants to extreme environments may be linked to their C-4 photosynthetic pathway, which concentrates CO_2 around Rubisco, and maintains high photosynthetic rates despite extreme environmental conditions. As a result of the CO_2 concentrating mechanisms, C-4 plants under high temperature and moisture stress conditions may reduce stomatal gaseous fluxes but still maintain Rubisco CO_2 saturation. At similar stomatal gaseous fluxes, C-3 plants may experience low mesophyll CO_2 concentration, which could significantly reduce CO_2 input for Rubisco. Low Rubisco CO_2 levels would result in reduced photosynthetic rates, lower plant growth rates, and consequently reduced yields. In has been reported that at high temperature and elevated CO_2, photosynthetic rates and biomass production were greater in C-4 than C-3 plants (Pearcy et al. 1981).

Cereal grains, like corn, wheat, rice, sorghum, and millet, are a major source of food for millions of people worldwide. However, their productivity under high temperatures and moisture stress could depend on their photosynthetic make-up, with C-4 plants generally being more drought and heat tolerant than C-3 plants (Hattersley 1992). There are also differences among the C-4 plants in their tolerance to extreme conditions, as shown by their geographical distribution (Hattersley 1992, Ellis et al. 1980, Taub 2000). Grasses belonging to different C-4 sub-types have characteristic leaf Kranz anatomy and geographic distributions according to rainfall, as seen in Australia, South Africa and the US. With increasing rainfall, NADP-ME C-4 grasses increase in abundance, whereas NAD-ME grasses become less abundant. Despite some predictions, it is premature to conclude that C-4 plants will lose their competitive advantage over C-3 species in elevated CO_2 (Wand et al. 1999). For example, an increase in CO_2 assimilation with increasing temperature was found in NAD-ME and NADP-ME C-4 plants at both low (42 Pa) and elevated (62 Pa) CO_2 concentrations (Ghannoum et al. 2001). Similar responses to increased CO_2 concentrations have been reported for NADP-ME big blue stem (*Andropogon gerardii* L.) (Sionit and Petterson 1984) and a NAD-ME goose grass (*Eleusine indica* L.) (Knapp et al. 1993). A new study, using an open-air concentrations technology (Leakey et al. 2006), suggests that corn may respond to elevated CO_2 concentrations only under moisture stress conditions. In an open prairie experiencing periodic water stress, elevated CO_2 could improve productivity in all C-4 because of their water-use efficiency (Owensby et al. 1993). Therefore, with increasing desertification,

higher moisture stress could result in an increased response of C-4 plants to future high global CO_2 concentrations.

Under the projection that future global conditions may be drier with lower soil fertility, crops with high nutrient- and water-use efficiencies may be beneficial. The C-4 plants have been found to achieve high photosynthetic rates and biomass with low leaf N, K and Rubisco concentrations (Bolton and Brown, 1978, Brown 1978, Blevins 1985, Ghannoum et al. 1997, Ghannoum and Conroy 1998, Makino et al. 2003). Efficient water-use allows C-4 plants to perform better in dry environments than other plants (Sage and Pearcy 1987). Pig weed (*Amaranthus retroflexus* L.), a C-4 plant, had higher water-use efficiency than lambsquaters (*Chenopodium album* L.), an ecologically similar C-3 plant. Even within C-4 plants, differences in water-use efficiency exist and NAD-ME C-4 grasses have greater water use efficiency than NADP-ME C-4 grasses (Ghannoum et al. 2002). Therefore, grasses belonging to the NAD-ME subtype, like pearl millet, Bermuda grass, and switch grass may be adapted to drier environments with low inherent soil N than other C-4 subtype species.

The ever increasing global temperature and reduced availability of water call for a change of focus in crop production to crops capable of withstanding high temperature and drought, like C-4 plants. Because C-4 plant morphological and photosynthetic mechanisms allow them to withstand moisture and temperature stress, reaching optimum growth and yields could depend on other growth limiting factors like macro- and micronutrient supplies and use-efficiencies.

One of the most important aspects of C-4 photosynthesis is the ability to survive extremely high water stress by utilizing the photosynthetic pathway that concentrates CO_2 around Rubisco in the Calvin cycle reaction. This adaptation in NAD-ME C-4 plants involves a Mn-activated decarboxylation reaction. Yet, very little is known about the leaf tissue Mn concentration needed to optimize this process, or how to provide the conditions needed to optimize NAD-ME reaction.

Manganese in soil

Manganese cation (Mn (II)), the plant available form of Mn in soil solution and exchange sites on soil colloidal surfaces, is usually in equilibrium with solid-phase Mn (Norvell 1988). The equilibrium is under the influence of soil pH, redox conditions, exchange surface characteristics, organic matter content, and the diverse microbe population (Norvell 1988, Ghiorse 1988, Thomson et al. 2005). Human factors can interfere directly or indirectly with the bio-availability of this micronutrient, as well. Fertilizers may supply Mn (II) into the soil where it can react and be oxidized into different forms, including Mn (III) and Mn (VII). Lime application to acidic soil may help alleviate Mn toxicity problems, but in slightly acidic soil, liming may result in Mn deficiency. For example, liming of a fragipan soil in southwest Missouri and a claypan in central Missouri greatly decreased leaf Mn concentration in tall fescue (Hamilton 2006). On the other hand, application of ammonical N fertilizer and sulfur to high pH soils may improve plant availability of Mn by lowering the pH (Norvell 1988).

Other crop production management activities may also affect Mn availability for plant uptake. For example, glyphosate [N- (phosphonomethyl) glycine], commonly known as Roundup®, is a wide spectrum herbicide that kills almost all natural plants, but is ineffective on genetically modified Roundup Ready® plants. It has been shown that Roundup applied to Roundup Ready® crops may chelate some cations including Mn (Bailey et al. 2002, Bernard et al. 2005). Roundup® could impair Fe and Mn nutrition in plants due to formation of poorly soluble glyphosate-metal complexes in plant tissue and/or rhizosphere interactions (Eker et al. 2006). Glyphosate-cation complexes prevent free mobility of Mn and Fe within the plants and could be responsible for the observed chlorosis in glyphosate-treated plants. It is possible, that Roundup Ready® corn and soybean cultivars may require higher Mn fertilization than non-Roundup Ready® cultivars.

Soil Mn availability for plant uptake may also be improved by proper fertilizer management. For example, it has been shown that P application increased Mn uptake in wheat plants (Jackson et al. 1964) and tall fescue (McClain 2007). The active transport of ions using ATP, a compound with three phosphate groups, makes P an important factor in mineral element uptake and transport (Epstein and Bloom 2005). In animals, sequestration rates of Mn, Ca, and Mg in internal vesicles are increased by inorganic P (Brierley 1963). This is another indication of the role of P in transport dynamics of these cations inside the cell, the most likely being the phosphorylation of gated cation channels across membranes.

There are other factors affecting plant available Mn in the soil. In high pH soils, insoluble forms of Mn predominate over the plant available Mn (II). Under these conditions, the beneficial effect of P on Mn uptake is lost since most of the P is complexed into insoluble Ca compounds. In soil with high organic matter content, Mn may be chelated by humic substances and other organic compounds, making it unavailable for plant uptake. Alternative Mn fertilizers or application techniques that prevent oxidation of plant available Mn (II) may be an option to solving Mn deficiency associated with soil redox reactions and improve crop production in soils with low inherent Mn, high organic matter, or high pH.

Objectives

Despite a wealth of information on the critical role of Mn in C-4 photosynthesis, very little is known about Mn requirements of C-4 plants, and in particular about leaf Mn concentrations required for maximum photosynthetic rates and maximum activity of NAD-ME, the Mn-dependent CO_2 decarboxylation enzyme.

Therefore, the first objective of this study was to determine Mn concentrations in nutrient solution that optimize photosynthetic rates and plant growth in two NAD-ME C-4 plants, pearl millet (*Pennisetum glaucum* (L.) R. Br.) and purple amaranth (*Amaranthus hypochondriacus* (L.) cv. plainsman). Manganese requirements of these NAD-ME C-4 species were compared with the requirements of two NADP-ME species, corn (*Zea mays* (L.) cv. FR 697) and sorghum (*Sorghum bicolor* (L.) Moench), and two C-3 species, wheat (*Triticum aestivum* (L.) cv. Ernie) and squash (*Cucubita pepo* (L.) cv. straighneck). The effect of varying light intensity (photosynthetic photon flux density, PPFD) on leaf photosynthetic responses of plants grown with different Mn concentrations was determined.

The second objective of the study was to determine if applied Mn to soil in field plots of two NAD-ME C-4 plants pearl millet and purple amaranth would affect photosynthetic rates, seed yield, and seed protein and oil composition. The study also compared two Mn fertilizer sources, a conventional fertilizer $MnCl_2$,

and Mn fertilizer beads supplied by Dr. Larry Sanders (Specialty Fertilizer Products, Kansas City, MO).

Literature cited

Andrews DJ, Hanna WW, Rajewski JF, and Collins VP 1996 Advances in grain pearl millet: Utilization and production research. In Janick J (Ed) Progress in New Crops. ASHS Press, Alexandria, VA, pp 170-177.

Asyley DA and Goodson RD 1972 Effect of time and plant potassium status on ^{14}C-labeled photosynthate movement in cotton. Crop Sci. 12, 686-690.

Badger MR and Price GD 1994 The role of carbonic anhydrase in photosynthesis. Annu. Rev. Plant Physiol. Plant Mol. Biol. 45, 393-420.

Bailey WA, Poston DH, Wilson HP, and Hines TE 2002 Glyphosate interaction with manganese. Weed Technol. 16, 792-799.

Barankievwicz TJ 1978 CO_2 exchange rate and ^{14}C photosynthetic products of maize leaves as affected by potassium deficiency. Z. PlanzenPhysiol. 89, 11-20.

Becker R, Wheeler EL, Lorenz K, Stafford AE, Grosjean OK, Betschart AA, Saunders R M 1981 A compositional study of amaranth grain. J. Food Sci. 46, 1175-1180.

Bernard ML, Thelen KD, Penner D 2005 Glyphosate efficacy is antagonized by manganese. Weed Technol. 19, 27-34.

Benson AA and Calvin M 1950 Carbon dioxide fixation by green plants. Ann. Rev. Plant Physiol. 1, 25-40.

Betschart, AA, Irving DW, Shepherd AD, and Saunders RM 1981 *Amaranthus cruentus:* Milling characteristics, distribution of nutrients within seed components, and the effects of temperature on nutritional quality. J. Food Sci. 46, 1181-1187.

Bhagwat AS 2005 Photosynthetic carbon assimilation of C_3, C_4, and CAM pathways. In Pessarakli M (Ed) Handbook of Photosynthesis, 2nd Edition. Taylor and Francis Group, New York, pp 367-389.

Blevins DG 1983 Potassium –use efficiency of C4-forage grasses. Curr. Top. Plant Biochem. Physiol. 2, 238.

Blevins DG 1985 Role of potassium in protein metabolism in plants. In Munson RD (Ed) Potassium in Agriculture. American Society of Agronomy, Crop Science Society of America, Soil Science Society of America, Madison, Wisconsin, USA, pp 413-442.

Bolton J and Brown RH 1978 Effects of nitrogen nutrition on photosynthesis and associated characteristics in C_3, C_4 and intermediate grass species. Plant Physiol. 61, 38-45.

Bowen JE 1972 Manganese-silicon interaction and its effects on growth of sudan grass. Plant Soil. 37, 577-588.

Bradshaw AD 1965 Evolutionary significance of phenotypic plasticity in plants. Adv Genet. 13, 115-155.

Breene WM 1991 Food uses of grain amaranth. Cereal Foods World 36, 426-430.

Bressani R, Gonzales JM, Zuniga J, Breuner M and Elias LG 1987 Yield, selected chemical composition and nutritive value of 14 selections of amaranth grain representing four species. J. Sci. Food Agric. 38, 347–356.

Brierley GP 1963 Ion accumulation in earth mitochondria. In Chance B (Ed) Energy-Linked Functions of Mitochondria. Academic Press Inc., New York, pp 237-251.

Brown RH 1976 Characteristics related to photosynthesis and photorespirationof *Pannicum milioides*. In Burris RH and Black CC (Eds) CO_2 Metabolism and Plant Productivity. University Park Press, Baltimore, pp 311-325.

Brown RH 1978 Difference in N use efficiency in C_3 and C_4 plants and its implications in adaptation and evolution. Crop Sci. 18, 93–98.

Brown RH and Hattersley PW 1989 Leaf anatomy of C_3-C_4 species as related to evolution of C_4 photosynthesis. Plant Physiol. 91, 1543-1550.

Brownwell PF and Crossland CJ 1974 Growth responses to sodium by *Bryophyllum tubiflorum* under conditions inducing crassulacean acid metabolism. Plant Physiol. 54, 416-417.

Brownell PF and Bielig LM 1996 The role of sodium in the conversion of pyruvate to phosphoenolpyruvate in mesophyll chloroplasts of C_4 Plants. Aust. J. Plant Physiol. 23, 171 – 177.

Buchanan B, Gruissem W, and Jones RL 2000 Biochemistry and molecular Biology of Plants. American Society of Plant Physiologists, Rockville, Maryland.

Buchholz DD, Brown JR, and Hanson RG 1993 Using Your Soil Test Results. University of Missouri Extension, Columbia, Missouri.

Burnell JN 1986 Purification and properties of phospho*enol*pyruvate carboxykinase from C_4 Plants. Aust. J. Plant Physiol. 13, 577 – 587.

Burnell JN 1988 The biochemistry of manganese in plants. In Graham RD, Hannam R, and Uren NC (Eds) Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht, Netherlands, pp 125-137.

Burton GW, Wallace AT and Rachie KO 1972 Chemical composition and nutritive value of Pearl millet [*Pennisetum typhoide* (Burm.) Staph and E. C. Hubbard] grain. Crop Sci. 12,187-188.

Clegg MT and Zurawski G 1992 Chloroplast DNA and the study of plant phylogeny: present status and future prospects. In Soltis PE, Soltis DE, and Doyle JJ (Eds) Molecular Systematics of Plants. Chapman and Hall, New York, New York, USA. pp 1-13.

Davis PH and Heywood VH 1963 Principles of Angiosperm Taxonomy. D Van Nostrand Company, Inc, Princeton, New Jersey.

Dell B and Wilson SA 1985 Effect of zinc supply on growth of three species of Eucalyptus seedlings and wheat. Plant Soil 88, 377-384.

Dever LV, Pearson M, Ireland RJ, Leegood RC, Lea PJ 1998 The isolation and characterisation of a mutant of the C4 plant, *Amaranthus edulis* deficient in NAD-malic enzyme activity. Planta 206, 649-656.

Diouf FO, Guissé A, Sène M and Diallo N 2006 Characterizing root response to low phosphorous in pearl millet (*Pennisetum glaucum* (L.) R. Br.). Agron. J. 98, 1187-1194.

Dolye JJ, Beachy RN, and Lewis WH 1984 Evolution of rDNA in *Claytonia* polyploidy complexes. In Grant WF (Ed) Plant Biosystematics Academic Press, Canada, pp 321-341.

Edwards GE and Black CC 1971 Photosynthesis in mesophyll cells and bundle sheath cells isolated from *Digitaria sanguinalis* (L). Scop. leaves. In Hatch MD, Osmond CB, Slatyer RO (Eds) Photosynthesis and photorespiration. John Wiley Inc., New York, pp153–158.

Edwards G and Walker D 1983 C_3, C_4: mechanism, and cellular and environmental regulation, of photosynthesis. University of California Press, Berkeley and Los Angeles, California.

Ejeta G, Hassen MM and Mertz ET 1987 In vitro digestibility and amino acid composition of pearl millet (*Pennisetum typhoides*) and other cereals. Proc. Nat. Acad. Sci. (USA) 84, 6016-6019.

Ehleringer JR, Cerling TE, and Helliker BR 1997. C_4 photosynthesis, atmospheric CO_2, and climate. Oecologia 112, 285-299.

Eker S, Ozturk L, Yazici A, Erenoglu B Romheld V and Cakmak I 2006 Foliar-applied glyphosate substantially reduce uptake and transport of iron and manganese in sunflower (*Helianthus annuus* L.) plants. J. Agric. Food Chem. 54, 10019-10025.

Ellis RP, Vogel JC, and Fuls A 1980 Photosynthetic pathways and geographical-distribution of grasses in South-West Africa-Namibia. S. Afr. J. Sci. 76, 307-314.

Evans HJ and Sorger GJ 1966 Role of mineral elements with emphasis on the univalent cations. Annu. Rev. Plant Physiol. 17, 47-76.

Epstein E and Bloom AJ 2005 Mineral Nutrition of Plants. Principles and Perspectives. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

FAO 1986 Production Yearbook. Vol. 40. FAO, United Nations, Rome.

Fisher RA and Hisiao TC 1968 Stomatal opening in isolated epidermal strips of *Vicia faba* II. Response to KCl concentration and the role of potassium absorption. Plant Physiol. 43, 1953-1958.

Garcia LA, Alfaro MA, and Bressani R 1987 Digestibility and nutritional-value of crude-oil from three amaranth species. J. Amer. Oil Chem. Soc. 64, 371-375.

Ghiorse WC 1988 The biology of manganese transforming microorganisms in soil. In Graham RD, Hannam R, and Uren NC (Eds) Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht, Netherlands, pp 75-85.

Ghannoum O, von Caemmerer S, Barlow EWR, Conroy JP 1997 The effect of CO_2 enrichment and irradiance on the growth, morphology and gas exchange of a C3 (*Panicum laxum*) and a C4 (*Panicum antidotale*) grass. Aust. J. Plant Physiol. 24, 227–237.

Ghannoum O and Conroy JP 1998 Nitrogen deficiency precludes a growth response to CO_2 enrichment in C_3 and C_4 *Panicum* grasses. Aust. J. Plant Physiol. 25, 627–636.

Ghannoum O, von Caemmerer S, Conroy JP 2001 Plant water use efficiency of 17 Australian NAD-ME and NADP-ME C_4 grasses at ambient and elevated CO_2 partial pressure. Aust. J. Plant Physiol. 28, 1207-1217.

Ghannoum O, von Caemmerer S, Conroy JP 2002 The effect of drought on plant water use efficiency of nine NAD-ME and nine NADP-ME Australian C_4 grasses. Funct. Plant Biol. 29, 1337–1348.

Ghannoum O, Evans JR, Chow WS, Andrews TJ, Conroy JP, and von Caemmerer S 2005 Faster Rubisco is the key to superior nitrogen-use efficiency in NADP-malic enzyme relative to NAD-malic enzyme C_4 grasses. Plant Physiol. 137, 638-650.

Ghannoum O and Conroy J P 2007 Phosphorus deficiency inhibits growth in parallel with photosynthesis in a C_3 (Panicum laxum) but not two C_4 (*P. coloratum* and *Cenchrus ciliaris*) grasses. Funct. Plant Biol. 34, 72–81.

Gianquinto G, Abu-Rayyan A, Di Tola L, Piccotino D and Pezzarossa B 2000. Interaction effects of phosphorus and zinc on photosynthesis, growth and yield of dwarf bean grown in two environments. Plant Soil 220, 219-228.

Hamilton JE 2006 Elemental concentration changes in soil and stockpiled tall fescue leaves after liming. MS Thesis. University of Missouri-Columbia, Columbia, MO.

Hannam RJ and Ohki K 1988 Detection of manganese deficiency and toxicity in plants. In Graham RD, Hannam R, and Uren NC (Eds) Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht, Netherlands. pp 243-259.

Halsted M and Lynch J 1996 Phosphorus response of C3 and C4 species. J. Exp. Bot. 47, 497-505.

Hatch MD and Kagawa T 1974 Activity, location and role of NAD malic enzyme in leaves with C4 pathway photosynthesis. Aust. J. Plant Physiol. 1, 357-369.

Hatch MD, Tsuzuki M, Edwards GE 1982 Determinination of NAD malic enzyme in leaves of C4 carbon pathway plants. Effects of malate dehydrogenase and other factors. Plant Physiol. 69, 483-491.

Hatch MD and Carnal NW 1992 The role of mitochondria in C4 photosynthesis. In Lambers H and van der Plas LHW (Eds) Molecular, biochemical and physiological aspects of plant respiration. SPB Academic Publisher, Hague, Netherlands, pp 135–148.

Hatch MD and Slack CR 1966 Photosynthesis by sugar cane leaves- a new carboxylation reaction and the pathway of sugar formation. Biochem J. 101, 103-111.

Hattersley PW, Wong SC, Perry S, and Roksandic Z 1986 Comparative ultrastructure and gas exchange characteristics of the C3-C4 intermediate *Neurachne minors* ST Blake (*Poaceae*). Plant Cell Environ. 9, 217-233.

Hattersley PW 1992 C4 photosynthetic pathway variation in grasses (*Poaceae*): its significance for arid and semi-arid lands. In Chapman GP (Ed) Desertified grasslands: their biology and management. Academic Press, London, pp 181–212.

Haydon KD and Hobbs SE 1991 Nutrient digestibilities of soft winter wheat, improved triticale cultivars, and pearl millet for finishing pigs. J. Anim. Sci. 69, 719-725.

Hoch CP and Stephen AG 1995 Experimental and molecular approaches to plant biosystematics. Missouri Botanical Garden.

Humble GD and Raschke K 1971 Stomatal opening quantitatively related to potassium transport. Plant Physiol. 48, 447-453.

Jackson DC, Laudencia PN, and Carson Jr. EW 1964 Effects of phosphorus nutrition on manganese uptake and transport in wheat seedlings. 8Th Intern. Congress of Soil Science, Bucharest, Romania, pp 165-179.

Jocic' B and Saric' MR 1983 Efficiency of nitrogen, phosphorus, and potassium use by corn, sunflower, and sugarbeet for the synthesis of organic matter. Plant and Soil 72, 219-223.

Johnston M, Grof CPL, and Brownell PF 1988 The effect of sodium nutrition on the pool sizes of intermediates of the C_4 photosynthesis pathway. Aust. J. Plant Physiol. 15, 7498-760.

Kagawa T and Hatch MD 1975 Mitochondria as a site of decarboxylation in C4 acid in C4 pathway photosynthesis. Arch. Biochem. Biophys. 167, 687-696.

Kallenbach R, 2004 Missouri Forages and Grassland Council Meeting, Lake of the Ozarks, November 2004.

Knapp AK, Hamerlynck EP, Owensby CE 1993 Photosynthetic and water relations responses to elevated CO_2 in the C-4 grass *Andropogon gerardii* Int. J. Plant Sci. 154, 459-466.

Koch K and Mengel K 1974 The influence of the level of potassium supply to young tobacco plants (Nicotiana tabacum L.) on short-term uptake and utilisation of nitrate nitrogen (^{15}N). J. Sci. Food Agric. 25, 465-471.

Kortschak HP, Hartt CE, Burr GO 1965. Carbon dioxide fixation in sugarcane leaves. Plant Physiol. 40, 209-213.

Ku MSB, Monson RK, Littlejohn Jr. RO, Nakamoto H, Fisher DB, Edward GE 1985 Photosynthesis characteristics of C_3-C_4 intermediate *Flavaria* species. I Leaf anatomy, photosynthetic responses to O_2 and CO_2 and activities of key enzymes of the C_3 and C_4 pathways. Plant Physiol. 71, 944-948.

Long SP 1999 Environmental Response. In Sage RF and Monson RK (Eds) 'C_4 Biology'. Academic Press, San Diego, CA, pp 215-249.

Lowlar DW 2001 Photosynthesis. BIOS Scientific Publishers Ltd, Oxford, UK

Lawrence GHM 1951 Taxonomy of Vascular Plants. The Macmillan Company, New York, New York.

Leakey ADB, Uribelarrea M, Ainsworth EA, Naidu SL, Rogers A, Ort DR, and Long SP 2006 Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. Plant Physiol.140, 779-790.

Lehmann, J W 1990 The potential of grain amaranth in the 1990s and beyond. Proc. 4th National Amaranth Symposium: Perspectives on production, processing, and marketing. Minneapolis, MN. 23–25 Aug. 1990. Univ. Minn., St. Paul, MN. pp. 1–7.

Liu H, Hull RI, and Duff DT 1995 Comparing cultivars of three cool-season turfgrasses for potassium uptake kinetics and potassium recovery in the field. J. Plant Nutr. 18, 467-485.

Lloyd J and Farquhar GD 1996 The CO_2 dependence of photosynthesis, plant growth response to elevated atmospheric CO_2 concentration and their interaction with soil nutrient status. 1. General principles and forest ecosystems. Funct. Ecol. 10, 4-32.

Lorenz K and Hwang YS 1985 Lipids in amaranths. Nutrition Reports International 31, 83-89.

Lukaszewski K, Blevins DG, and Randall DD 1992 Asparagine and boric acid cause allantoate accumulation in soybeans leaves by inhibiting manganese-dependent allantoate amidohydrolase. Plant Physiol. 99, 1670-1676.

Makino A, Sakuma H, Sudo E and Mae T 2003 Differences between maize and rice in N-use efficiency for photosynthesis and protein allocation. Plant and Cell Physiol. 44, 952-956.

Marshner H 1995 Mineral Nutrition of Higher Plants. Academic Press New York, USA.

Matoh T, Ohta D and Takahashi E 1986 Effect of sodium application on growth of *Amaranthus tricolor* L. Plant Cell Physiol. 27, 187- 192.

McClain II WE 2007 Phosphorus Nutrition in Tall Fescue: From Stockfiling to Seed Production. Ph.D Dissertation, University of Missouri-Columbia, Columbia, MO

Mengel K and Kirkby EA1978 Principles of Plant NutritionInternational Potash Institute, Bern, Switzerland.

Mengel K and Kirkby E 1980 Potassium in crop production. Adv. Agron. 33, 59–110.

Mitchell, RB, Redfearn DD, Moser LE, Grant RJ, Moore KJ, and Kirch BJ 1997. Relationships between in situ protein degradability and grass developmental morphology. J. Dairy Sci. 80, 1143–1149.

Morisset P and Boutin C 1984 The biosystematic importance of phenotypic plasticity. In WF Grant (Ed) Plant Biosystematics. Academic Press, Ontario, Canada, pp 293-306.

Myers R 2002 Alternative crop guide. National Edition. Jefferson Institute Columbia, MO.

Nable RO and Brownell PF 1984 Effect of sodium nutrition and light upon the concentration of alanine in leaves of C_4 plants. Austr. J. Plant Physiol. 11, 319-324.

Nelson DL and Cox MM 2005 Principles of Biochemistry. 4th Edn. W, H Freeman and Company, New York.

Norvell WA 1988 Inorganic reactions of manganese in soils. In Graham RD, Hannam RJ, and Uren NC (Eds) Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht, Netherlands, pp 37-58.

Ohnishi J, Flügge UI, Heldt HW, and Kanai R 1990 Involvement of Na^+ in active uptake of pyruvate in mesophyll chloroplast of some C_4 plants. Na^+/pyruvate cotransport. Plant Physiol. 94, 950-959.

Ohki K 1976 Effect of Zn nutrition on photosynthesis and carbonic anhydrase activity in cotton. Physiol. Plant. 38, 300-308.

Ohta D, Matsui J, Matoh T and Takahashi E 1988 Sodium requirement of monocotyledonous C_4 plants for growth and nitrate reductase activity. Plant Cell Physiol. 29, 1429-1432.

Ohta D, Matsui J, Matoh T and Takahashi E 1989 Sodium stimulates growth of Amaranthus tricolor L. plants through enhance nitrate assimilation. Plant Physiol. 89, 1102-1105.

Olmstead RG and Palmer JD 1994 Chloroplast DNA systematics. A review of methods and data-analysis. Amer. J. Bot. 81, 1205-1224.

Owensby CE, Coyne PI, Ham JH, Auen LM, and Knapp AK 1993 Biomass production in a tall grass prairie ecosystem exposed to ambient and elevated CO_2. Ecol. Appl. 3, 644-653.

Pearcy WR, Tomosa N, and Williams K 1981 Relationships between growth, photosynthesis and competitive interactions for C3 and C4 plants. Oecologia 48, 371-376.

Pflüger R and Mengel K 1972 The photochemical activity of chloroplast obtained from plants with different potassium nutrition. Plant Soil 36, 417-425.

Purchell LC, King CA, and Ball RA 2000 Soybean cultivar differences in ureides and the relationship to drought tolerant nitrogen fixation and manganese nutrition. Crop Sci. 40, 1062-1070.

Raghavendra AS and Das VSR 1978 (Na^+-K^+)-stimulated ATPase in leaves of C_4 plants: possible involvement in active transport of C4 acids. J. Exp. Bot. 29, 39-47.

Rajendrudu G, Prasad JSR, and Das Rama VS 1986 C_3-C_4 intermediate species in *Alternanthera* (*Amaranthaceae*). Leaf anatomy, CO_2 compensation point, net CO_2 exchange and activities of photosynthetic enzymes. Plant Physiol. 80, 409-414.

Rehm G 2004 Zinc Fertilization in Minnesota. A Review. University of Minnesota Extension.http://www.extension.umn.edu/cropenews/2004/04MNCN32.htm.

Rychter AM and Rao IM 2005 Role of phosphorus in photosynthetic carbon metabolism. In Pessarakli M (Ed) Handbook of Photosynthesis, 2nd Edn. Taylor and Francis Group, New York, pp 123-148.

Sage RF and Pearcy RW 1987 The nitrogen use efficiency of C_3 and C_4 plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium Album* (L.) and *Amaranthus retroflexus* (L.). Plant Physiol. 84, 959-963.

Schmitt MR and Edward GE 1981 Photosynthetic capacity and nitrogen use efficiency of maize, wheat, and rice: a comparison between C3 and C4 photosynthesis. J. Exp. Bot. 32, 459-466.

Senft, J 1979 Protein quality of amaranth grain. Proc. 2nd Amaranth Conf., Rodale Research Center, Kutztown, PA. 13–14 Sept. 1979. Rodale Press, Emmaus, PA, pp. 43–47.

Singh DN and Prez-Maldonado R 2003 Pearl millet as an alternative feed grain for pigs and poultry. Asia Pac. J. Clin. Nutr. 12 Suppl. S40.

Sionit N and Patterson DT 1984. Responses of C-4 grasses to atmospheric CO_2 enrichment. I. Effect of irradiance. Oecologia 65, 30-34.

Smith RL, Jensen LS, Hoveland CS and Hanna WW 1989 Use of pearl millet, sorghum, and triticale grain in broiler diets. J. Prod. Agric. 2, 78-82.

Soltis PS and Soltis DE 2004 The origin and diversification of angiosperms. Amer. J. Bot. 91, 1614-1626.

Stordahl, JL, Sheaffer CC, and DiCostanzo A 1999 Variety and maturity affect amaranth forage yield and quality. J. Prod. Agric. 12, 249–253.

Taiz L and Zeiger E 2006 (Eds.) Plant Physiology, 4th Edition. Sinauer Associates, Inc., Publishers, Sunderland, Massachusetts.

Taub DR 2000 Climate and the US distribution of C_4 grass sub-families and decarboxylation variants of C_4 photosynthesis. Amer. J. Bot. 87, 1211-1215.

Terry N and Ulrich A 1973 Effect of potassium deficiency on the photosynthesis and respiration of leaves of sugar beet. Plant Physiol. 51, 783-786.

Teutsch C 2002 Warm-Season Annual Grasses for Summer Forage. Extension Agronomist, Southern Piedmont AREC; Virginia Tech. Publication number 418-004.

Thomson IA, Huber DM, Guest CA, and Schulze G 2005 Fungal manganese oxidation in a reduced soil. Environ. Microbiol. 7, 1480-1487.

Todd CD and Polacco JC 2004 Soybean cultivars 'Williams 82' and 'Maple Arrow' produce both urea and ammonia during ureide degradation. J Exp. Bot. 55, 867-877.

Tomlinson DL, James RE, Bethard GL, and McGilliard ML 1997 Influence of undegradability of protein in the diet on intake, daily gain, feed efficiency, and body composition of Holstein heifers. J. Dairy Sci. 80, 943–948.

Trehan SE, El Dessougi H, and Claassen L 2005 Potassium efficiency of 10 potato cultivars as related to their capability to use nonexchangeable soil potassium by chemical mobilization. Commun. in Soil Sci. and Plant Anal. 36, 1809-1822.

Von Caemmerer S, Quinn V, Hancock NC, Price GD, Furbank RT and Ludwig M 2004 Carbonic anhydrase and C_4 photosynthesis: a transgenic analysis. Plant Cell Environ. 27, 697-703.

Vadez V, Sinclair TR, Serraj R, and Purcell L.C 2000 Manganese application alleviates the water deficit-induced decline of N_2 fixation. Plant Cell Environ. 23, 459-505.

Vagnoni, DB, and GA Broderick 1997 Effects of supplementation of energy or ruminally undegraded protein to lactating cows fed alfalfa hay or silage. J. Dairy Sci. 80, 1703–1712.

Vicky Jr. RK 1984 Biosystematics 1983. In Grant FW (Ed) Plant Biosystematics. Academic Press Inc., Orlando, Florida, pp 1-24.

Wand SJE, Midgley GF, Jones MH and Curtis P 1999 Response of wild C4 and C3 grass (*Poaceae*) species to elevated atmospheric CO_2 concentration: a meat-analytic test of current theories and perceptions. Global Change Biology 5, 732-741.

Wang H and Jin J 2005 Photosynthetic rate, chlorophyll fluorescence parameters, and lipid peroxidation of maize leaves as affected by zinc deficiency. Photosynthetica 43, 591-596.

Waters B and Emerich D 2000 Transport of metabolites to and from symbiosomes and bacteroids. In Triplett EW (Ed) Procaryotic Nitrogen Fixation: A Model System for the Analysis of a Biological Process. Horizon Scientific Press, Norfork, England, pp 549-558.

Weier TE, Stocking CR and Barbour M 1970 Botany: An introduction to plant biology. John Wiley & Sons, Inc., New York.

Wilkins JS 1962 Some reasons for rediscovery of Mendel's work in the first years of the present century. Brit. J. Sci. 1, 5-17.

Wilson, JP 2004 Breeding Pearl Millet For Improved Stability, Performance, And Pest Resistance. International Sorghum And Millet Crsp 2004 Annual Report, Intsormil Publication 03-06 Pg. 63-67. (Technical Report).

Winkler RG, Blevins DG, Polacco JC, Randall DD. 1987 Ureide catabolism of soybeans II. Pathway of catabolism in intact leaf tissue. Plant Physiol. 83, 585–591.

Yang XE, Liu JX, Wang WM, Ye ZQ and Luo AC 2004 Potassium internal use efficiency relative to growth vigor, potassium distribution, and carbohydrate allocation in rice genotypes. J. Plant Nutr. 27, 837-852.

Yue SX, Sun HL, Chang BY, Chen ZP, and Zuo JW 1987 The nutritional composition of grain amaranth and its potential for utilization. Acta Agron. Sin. 13, 151–156.

Zhi-Hui C, Walker RP, Acheson RM, and Leegood RC 2002 Phosphoenolpyruvate carboxykinase assayed at physiological concentrations of metal ions has a high affinity for CO_2. Plant Physiol. 128, 160-164.

CHAPTER 2

MANGANESE REQUIREMENT FOR OPTIMUM PHOTOSYNTHESIS AND GROWTH IN NAD-ME C-4 PLANTS IN COMPARISON TO NADP-ME C-4 AND C-3 PLANTS

Introduction

Manganese (Mn) is a micronutrient required by all plant species for growth and reproduction (Marschner 1995). Inside the plant, Mn is a component of the water splitting protein complex PS II, a constituent of superoxide dismutase (MnSOD), and a key activator in a number of critical metabolic enzymes (Marshner 1995). Manganese plays a role in nitrogen metabolism by activating arginase and glutamyl transferase enzymes (Burnell 1988). In C-4 plants, Mn activates the four carbon organic acid decarboxylating enzymes in BSC, NAD-ME and PEP-CK (Hatch and Kagawa 1974, Burnell 1986). Though Mn can be substituted by Mg in PEP-CK, it is absolutely required for NAD-ME (Burnell, 1986).

Because of the specific Mn requirements for different metabolic processes, the optimum leaf Mn concentrations vary among plant species. For example, in the non-nitrogen fixing C-3 plant barley, leaf Mn concentrations of 10-15 mg/kg were found to be sufficient for optimum dry weight (Hannam and Ohki 1988), while in soybean, leaf Mn concentration was relatively higher, 21-100 mg/kg, which may be linked to the Mn-requiring ureide degrading enzyme, allantoate amidohydrolase (Winkler et al. 1987, Lukaszewski et al. 1992). There is little information on the leaf Mn required by C-4 species. It has been reported that in sudan grass, a C-4 plant that utilizes a Mg-activated NADP-ME during photosynthesis, optimum dry weight was achieved at solution Mn concentrations between 4.5 and 9.0 μM (Bowen, 1972), and that shoot tissue Mn concentrations > 200 mg/kg dry weight were toxic, causing decreased dry matter accumulation.

It may be that high Mn concentration in NADP-ME C-4 plant tissue inhibits the activation of both Rubisco and NADP-ME by competing with Mg, as proposed by Marschner (1995). No research has been published on other C-4 species, but considering the crucial role of Mn in the enzymatic release of CO_2 for Calvin cycle reactions in NAD-ME and PEP-CK C-4 plants, it is conceivable that higher Mn nutrition can be required for these plants. To test this hypothesis, Mn requirement for the optimum performance of two NAD-ME C-4 species pearl millet and purple amaranth, two NADP-ME C-4 species corn and sorghum, and two C-3 species wheat and squash, were established with a series of hydroponic experiments. All plants were grown in a complete nutrient solution supplied with increasing Mn concentrations. Manganese impact on root and shoot growth, photosynthesis, stomatal conductance, relative chlorophyll content, and root and shoot tissue mineral element composition, was determined.

Materials and methods

Plant materials and growth conditions

Two pearl millet (*Pennisetum glaucum* (L.) R. Br.) cultivars were tested in this experiment: a forage type hybrid, PP102M, obtained from MFA Incorporated (Columbia, MO), and a grain type, HGM 686, obtained from Crosbyton Seed Company (Crosbyton, TX). Purple amaranth (*Amaranthus hypochondriacus* (L.) cv. plainsman) obtained from Albert Lea Seed House (Albert Lea, MN), corn (*Zea mays* (L.) cv. FR 697) kindly supplied by the Georgia Davis laboratory, University

of Missouri-Columbia, and grain sorghum (*Sorghum bicolor* (L.) Moench) obtained from Plainview First Company (Plainsview, TX) were also included. Wheat (*Triticum aestivum* L. cv. Ernie) from University of Missouri Foundation Seed (Columbia, MO) and squash (*Cucubita pepo* L. cv straighneck) from Ferry-Morse Seed Company (Fulton, KY) were used as C-3 control plants.

Pearl millet, purple amaranth, and sorghum seeds were germinated in an aerated 1 L beaker of 0.2 mM $CaCl_2$ solution for a period of 48 hours (pearl millet and sorghum) or 72 hours (purple amaranth). Corn and squash were germinated for 96 hours in upright germination paper rolls soaked and maintained in 0.2mM $CaCl_2$ solution. Seedlings were grown hydroponically in complete nutrient solution consisting of: 2.0 mM $CaCl_2.2H_2O$; 0.5 mM $MgCl_2.6H_2O$; 2.0 mM KNO_3; 0.4 mM KH_2PO_4; 0.13 mM $FeCl_2.4H_2O$; 2.3 µM H_3BO_3; 0.6 µM $ZnSO_4.7H_2O$; 0.10 µM $NaMoO_4.2H_2O$; 0.11 µM $NiCl_2.6H_2O$; 0.01 µM $CoCl_2.6H_2O$; 0.15 µM $CuSO_4.5H_2O$; 0.1µM $Si(OH)_4$ (Reinbott and Blevins 1999), containing varying Mn concentrations. The solution pH was adjusted to 5.6 with KOH and nutrient solutions changed every other day to minimize the change in pH. Depending on the species, plants received Mn at concentrations of 0, 2, 5, 10, 15, 25, 30, 50, 75 or 100 µM. Before treatments were applied, Mn from seed was the only source for plant growth. The Mn treatments were started after at least one photosynthetic leaf had developed.

The C-4 plants were placed in a growth chamber with a PPFD of 550 µmol m^{-2} s^{-1} in a 16 hr, 29 ^oC light period and 8 hr, 20 ^oC dark period. The C-3 plants where placed in a growth chamber under similar conditions except that

light and dark temperatures were 25 ^{o}C and 18 ^{o}C, respectively. Plant parameters of interest were measured prior to harvest, 10-14 days after the start of Mn treatments.

Growth measurements

Shoots and roots were harvested after completion of photosynthesis and stomatal conductance measurements. Roots were rinsed repeatedly with DI water and all harvested material was oven dried at 70^{o} C to a constant weight and then ground.

Relative chlorophyll content

Relative chlorophyll content, determined as leaf greenness on each leaf used for the photosynthesis measurements, was measured with a SPAD-502 chlorophyll meter (Konica Minolta, Osaka, Japan).

Photosynthetic rate and stomatal conductance

Leaf photosynthetic rate and stomatal conductance were determined simultaneously on the most fully expanded leaf 10-14 days after Mn treatments were initiated. In wheat, pearl millet, corn and sorghum, measurements were obtained from the mid-point between the blade tip and leaf sheath. In amaranth and squash, measurements were done on the fourth leaf down from the shoot

apex and away from the midrib. Measurements were taken between 11:00 a.m and 3:00 p.m at 550 µmol quanta m^{-2} s^{-1} light levels, identical to the growth chamber radiation condition. Photosynthesis and stomatal conductance were determined using a LI-COR 6400 Portable Photosynthesis System (LICOR Inc., Lincoln, Nebraska, USA) with a 6400-02 LED light source curvette. Leaf cuvette conditions were: CO_2 flow rate of 500 µmol s^{-1}, sample CO_2 concentrations of 400 µmol mol^{-1}, PPFD of 550 µmol quanta m^{-2} s^{-1}, leaf temperature of 27 ± 0.5 ^{o} C, and vapor pressure deficit that range from 2.5 to 3.2 kPa.

Nutrient analysis

Ground plant material was digested in nitric acid with a microwave accelerated digestion system (MARSXPress by CEM, Matthews, NC). Digested samples were filtered, diluted, and analyzed for macro- and micronutrient elements. Phosphorus was determined colorimetrically (Murphy and Riley, 1962) and Ca, Mg, K, Mn, Fe and Zn were determined by atomic absorption.

Statistical analysis

Each experiment was a randomized complete block with three replicates per Mn treatment. Experiments were repeated three times. The PROC GLM model in SAS version 9.2 (SAS, 2004) was used to test the effect of Mn on measured parameters. All effects significant at $P<0.05$ were separated using Fisher's Protected Least Significant Difference.

Results

Manganese effect on shoot and root dry weight

Preliminary experiments with a range of 0-12.5 μM Mn in nutrient solutions showed a linear response of growth for NAD-ME C-4 plants, pearl millet and amaranth. Therefore, the range of Mn concentration was greatly expanded to include 0, 2, 5, 10, 25, 50, 75 and 100 uM for NAD-ME C-4 plants, pearl millet and purple amaranth, and for NADP-ME C-4 plants, maize and sorghum. For C-3 plants, only wheat received the same Mn concentration series. In squash, preliminary studies showed leaf necrosis at Mn concentrations ≥ 50 μM (Figure 2-1), therefore squash was grown in solutions with 0, 2, 5, 10, 15, 20, 25 or 30 μM Mn.

All plants grown without Mn grew poorly and had the lowest shoot and root dry weight (Table 2-1 & 2-2). In C-3 plants wheat and squash, shoot and root dry weight reached the optimal level at 2 μM Mn, and remained at this level at all higher Mn concentrations (Table 2-1 & 2-2). Squash plants grown with 25 and 30 μM Mn concentrations became necrotic by harvest time. Similar to C-3 plants, NADP-ME C-4 corn and sorghum shoot and root weight were relatively unchanged between 2 and 100 μM Mn (Table 2-1 & 2-2).

In contrast, NAD-ME C-4 pearl millet and purple amaranth required much higher Mn for optimum growth (Figure 2-2) and reached maximum shoot dry weight between 50 and 100 μM Mn (Table 2-1). Purple amaranth shoot dry weight at 50 or 75 μM Mn was ~3-fold higher than at 2 μM Mn, a Mn

concentration commonly used in nutrient solutions. Pearl millet plants grown with ≥ 50 μM Mn had greater dry weights than those receiving < 25 μM Mn.

Figure 2-1. Squash plants after one week of growth in complete nutrient solution with different Mn concentrations. Note the symptoms of Mn toxicity displayed by leaves of plants treated with Mn concentration ≥ 50 µM.

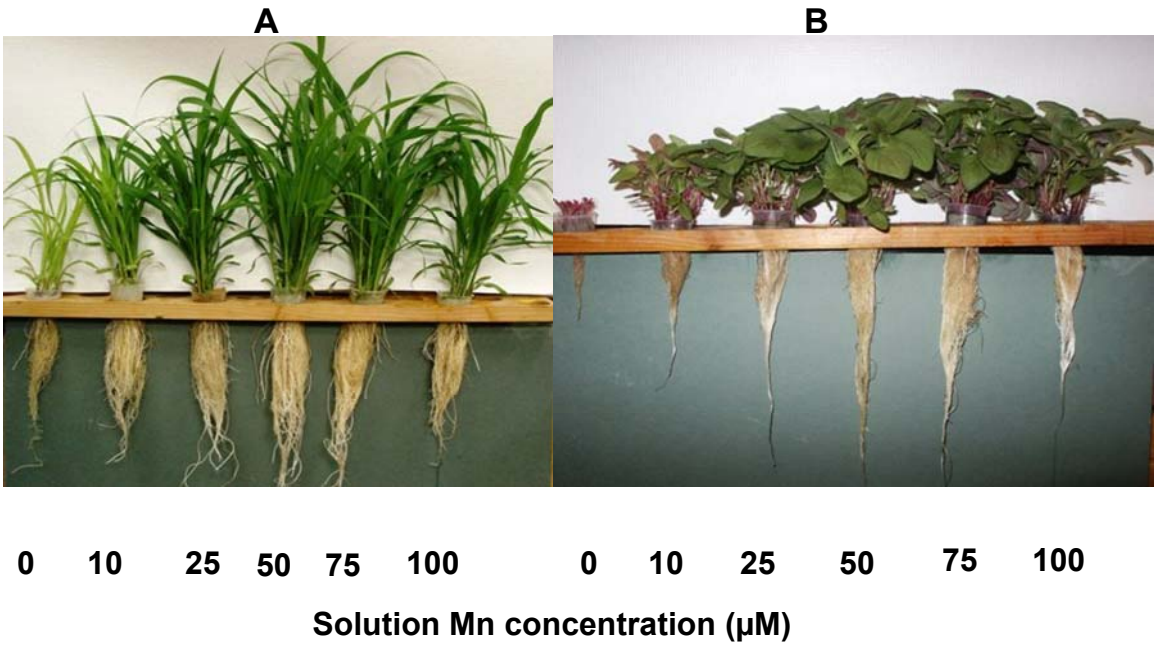


Figure 2-2. Pearl millet ‘hybrid HGM 686’ (A) and purple amaranth (B) plants after 14 days of growth in complete nutrient solution with different Mn concentrations. Note the maximum shoot and root growth at ~50 and 75 µM Mn concentrations.

Table 2-1. Shoot dry weight of two NAD-ME, two NADP-ME C-4 plants, and two C-3 plants grown hydroponically in complete nutrient solution with different Mn concentrations.

Manganese	C-4 Plants					C-3 Plants	
(μM)	NAD-ME C-4			NADP-ME C-4			
	Pearl millet		Purple				
	HGM 686	PP102M	amaranth	Corn	Sorghum	Wheat	Squash
	Shoot dry weight (mg/plant)						
0	44c[Ŧ]	38c	10d	187c	21c	45b	389c
2	118b		37c	309a	65b	56a	518abc
5	119b		40c	203b	80a	57a	537ab
10	130b	128b	42c	234ab	86a	54a	508bc
15	–	–	–	–	–	–	474bc
20	–	–	–	–	–	–	500bc
25	143ab	148ab	60bc	234ab	86a	54a	481bc
30	–	–	–	–	–	–	499bc
50	157a	166a	113a	224b	90a	58a	–
75	154a	167a	107a	223b	79a	58a	–
100	150a	173a	78b	264ab	78a	60a	–

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$

Table 2-2. Root dry weight of two NAD-ME, two NADP-ME C-4 plants, and two C-3 plants grown hydroponically in complete nutrient solution with different Mn concentrations.

Manganese	C-4 Plants					C-3 Plants	
(μM)	NAD-ME C-4			NADP-ME C-4			
	Pearl millet		Purple				
	HGM 686	PP102M	amaranth	Corn	Sorghum	Wheat	Squash
	Shoot dry weight (mg/plant)						
0	18c[Ŧ]	17b	2c	65a	2b	23a	102b
2	44b	_	12b	101a	49a	26a	138a
5	47ab	_	13b	74a	55a	27a	142a
10	42b	55a	11b	79a	61a	25a	126a
15	_	_	_	_	_	_	117ab
20	_	_	_	_	_	_	105b
25	50ab	56a	12b	76a	52a	27a	98b
30	_	_	_	_	_	_	103b
50	53a	57a	23a	84a	56a	26a	_
75	50ab	61a	21ab	82a	47a	28a	_
100	48ab	61a	13b	99a	47a	29a	_

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$

Relative chlorophyll content

In C-3 plants wheat and squash, and in NADP-ME plants, corn and sorghum, chlorophyll meter readings were optimum at 2 μM Mn and were unaffected by higher Mn concentrations (Table 2-3). However, in the NAD-ME species, pearl millet (HGM 686) and purple amaranth, plants receiving 50 and 75 μM Mn had higher chlorophyll readings than those receiving 2, 5, 10 and 25 μM Mn (Table 2-3). In pearl millet (PP102M), all plants receiving ≥ 25 μM had similar readings which where higher than those for plants receiving ≤ 10 μM. Except for squash, plants grown with 0 μM Mn had significantly lower chlorophyll readings than those receiving Mn treatment.

Table 2-3. SPAD-502 chlorophyll meter readings for two NAD-ME, two NADP-ME C-4 plants and two C-3 plants grown hydroponically in complete nutrient solution with different Mn concentrations.

Manganese (μM)	C-4 plants					C-3 Plants	
	NAD-ME C-4			NADP-ME C-4			
	Pearl millet		Purple				
	HGM 686	PP102M	amaranth	Corn	Sorghum	Wheat	Squash
	SPAD-502 Meter Reading						
0	22.7d[Ŧ]	16.1a	11.4d	28.3b	21.3b	39.3c	30.2a
2	31.3c	_	13.2c	38.0a	36.3a	45.3b	32.5a
5	33.9c	_	16.2c	42.0a	40.3a	48.3a	31.5a
10	35.9bc	24.5b	14.7c	38.3a	38.1a	47.8a	32.4a
15	_	_	_	_	_	_	32.8a
20	_	_	_	_	_	_	31.4a
25	37.9b	34.3c	21.2b	40.0a	40.1a	48.3	31.6a
30	_	_	_	_	_	_	32.6a
50	38.3a	36.7c	28.0a	40.6a	41.9a	47.9a	_
75	38.7a	37.7c	29.3a	41.5a	41.7a	47.9a	_
100	38.2ab	37.1c	28.7a	40.2a	40.7a	44.7b	_

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$

Photosynthetic rate

In squash (C-3), photosynthetic rate peaked at Mn concentration of 2 μM Mn, but sharply declined at concentrations above 15 μM Mn (Figure 2-3), indicating Mn toxicity. Photosynthetic rate in wheat (C-3) also peaked at 2 μM Mn and remained close to optimum at higher Mn concentrations (Figure 2-4). Like in wheat, the photosynthesis in NADP-ME C-4 plants peaked at 2 μM Mn and remained constant at higher Mn concentrations (Figure 2-4).

In contrast, the highest photosynthetic rates in NAD-ME plants were observed with 50, 75, or 100 μM Mn (Figure 2-5). Photosynthetic rates of plants supplied with < 25 μM Mn were much lower than the optimum (Figure 2-3). In pearl millet, photosynthetic rate was the highest in plants grown with 50 and 75 μM Mn and dropped with 100 μM Mn, while in amaranth, the increase in photosynthetic rate continued up to 100 μM Mn (Figure 2-5).

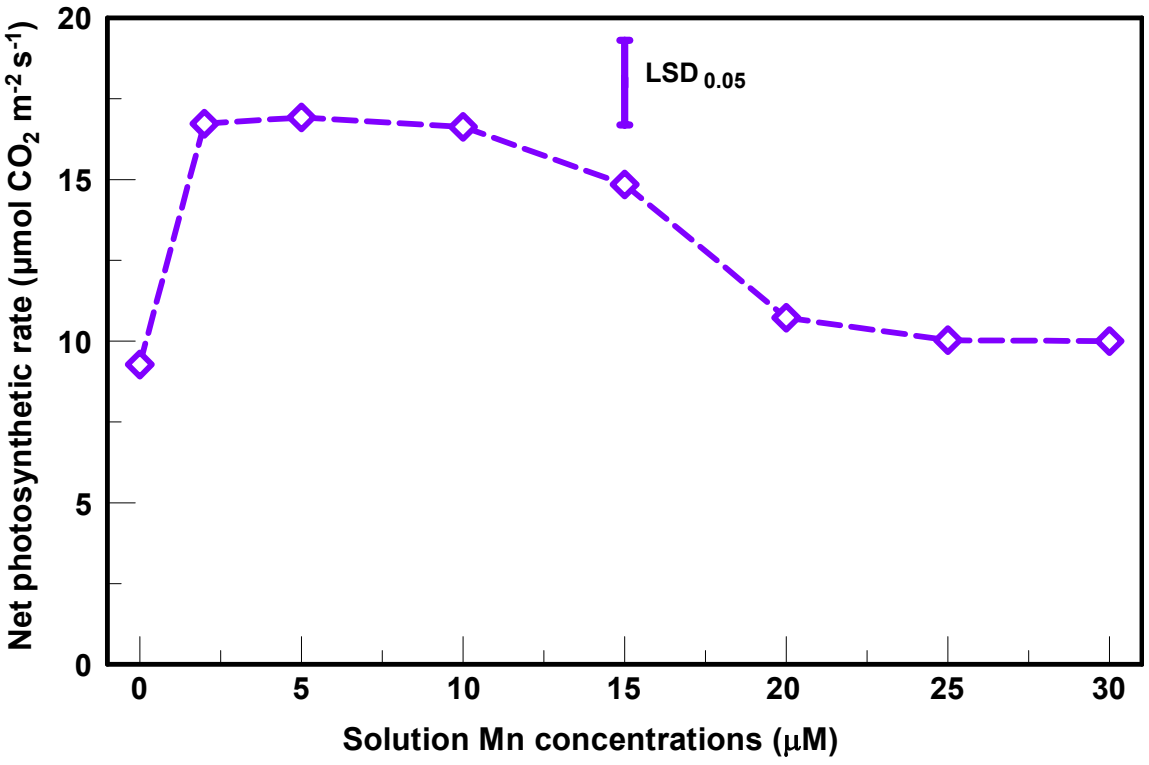


Figure 2-3. Leaf photosynthetic rate in leaves of the C-3 plant squash, grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means (n=9).

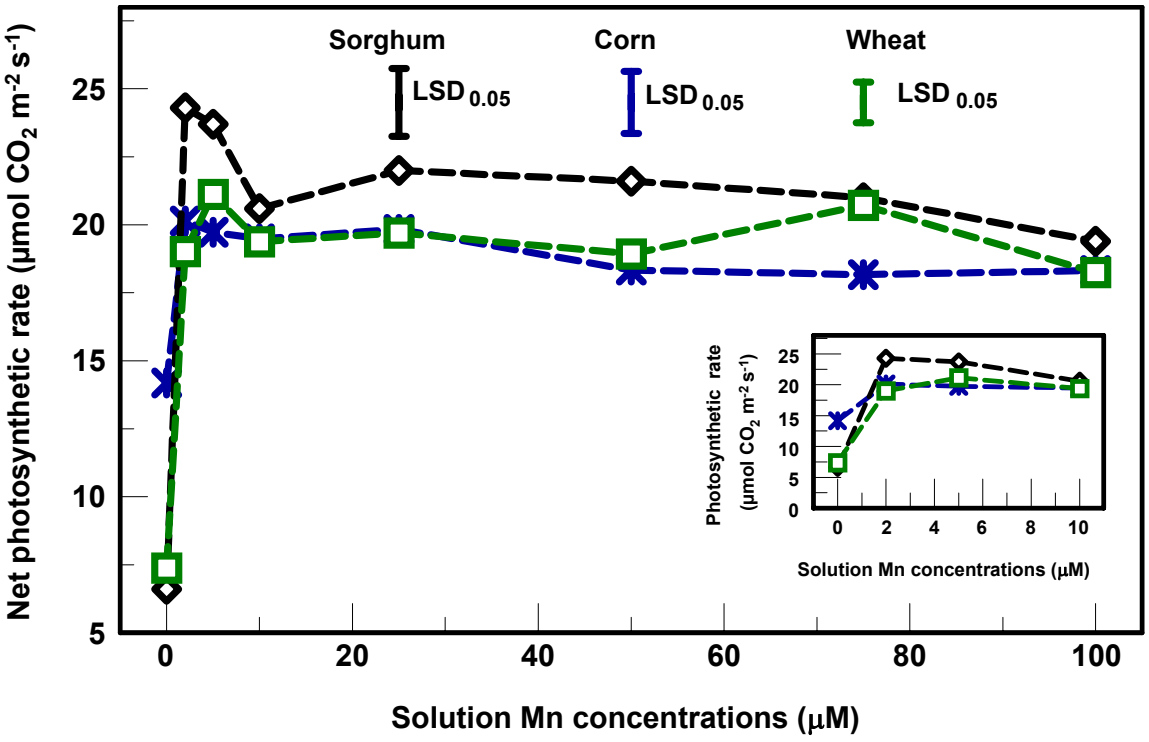


Figure 2-4. Leaf photosynthetic rates of the C-3 plant wheat, and two NADP-ME C-4 plants, corn and sorghum grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means (n=9). Inset shows that photosynthetic rates saturate at ~ 5 µM Mn, a concentration at which photosynthetic rates in NAD-ME C-4 plants are below saturation.

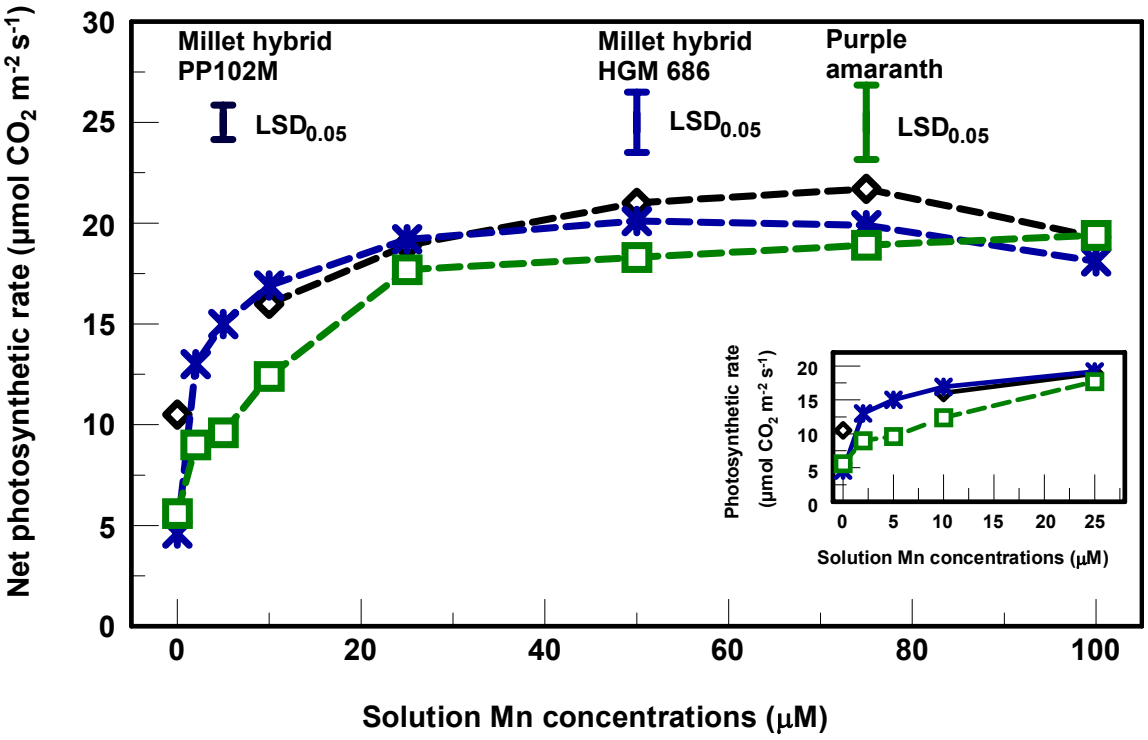


Figure 2-5. Leaf photosynthetic rates of the NAD-ME plants, pearl millet Hybrids 'PP102M and HGM 686' and purple amaranth grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means (n=9). Inset shows a positive slope for photosynthetic rates even at 25 µM Mn, a concentration at which photosynthetic rates in NADP-ME and C-3 plants is either saturated or decreasing.

Leaf stomatal conductance

In squash (C-3), stomatal conductance of plants receiving >15 μM Mn was lower than that of plants grown with < 15 μM Mn, indicating Mn toxicity (Table 2-4). In C-3 wheat, the stomatal conductance was relatively unchanged across the range of Mn treatment and only plants grown without Mn had lower values (Table 2-4). In both NADP-ME C-4 species corn and sorghum, the stomatal conductance remained relatively close over the range of Mn treatments (Table 2-4). Lower values were observed for plants grown with 0 μM Mn. Also in NAD-ME C-4 species, pearl millet and purple amaranth, values obtained for 0 μM Mn were the lowest, and Mn concentrations higher than 2 μM had little effect on stomatal conductance (Table 2-4).

Table 2-4. Leaf stomatal conductance of two NAD-ME, two NADP-ME C-4 plants, and two C-3 plants grown hydroponically in complete nutrient solution with different Mn concentrations.

Manganese	C-4 plants					C-3 Plants	
(µM)	NAD-ME C-4			NADP-ME C-4			
	Pearl millet		Purple				
	HGM 686	PP102M	amaranth	Corn	Sorghum	Wheat	Squash
	(mol H_2O m^{-2} s^{-1})						
0	0.04c[Ŧ]	0.047c	0.089b	0.075a	0.051b	0.296c	0.592b
2	0.076b	_	0.144a	0.076a	0.13a	0.605ab	0.756abc
5	0.088ab	_	0.118ab	0.09a	0.138a	0.635a	0.734abc
10	0.081ab	0.063b	0.112ab	0.079a	0.105a	0.555ab	0.8198a
15	_	_	_	_	_	_	0.610c
20	_	_	_	_	_	_	0.324d
25	0.095ab	0.082a	0.128a	0.089a	0.115a	0.601ab	0.332d
30	_	_	_	_	_	_	0.249d
50	0.100ab	0.073a	0.124a	0.089a	0.106a	0.485b	_
75	0.105a	0.087a	0.112ab	0.094a	0.105a	0.579ab	_
100	0.088ab	0.081a	0.13a	0.088a	0.101a	0.542ab	_

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$

Nutrient element concentration

As expected, in all species used in these experiment, Mn concentrations in shoot and root tissues increased in parallel with Mn treatment (Table 2-5 & 2-6). It is noteworthy that squash showed the highest shoot Mn concentration of all tested plants (Table 2-7). At solution Mn concentration ≥ 50 μM, pearl millet hybrid PP102M, and NADP-ME species corn and sorghum showed high root Mn concentration.

In all species macronutrient elements P, K, Mg, and Ca and micronutrients Fe and Zn in shoots and roots remained relatively unchanged across the spectrum of 2-100 μM Mn (Appendix Table 2-1, 2-2 & 2-3). Squash, a C-3 dicot, had up to 9-fold higher Ca and 4-fold higher Mg concentration in leaves than wheat, a C-3 monocot. Purple amaranth, a C-4 dicot, had 3-fold higher Ca and Mg concentrations in leaves than its photosynthetically similar C-4 monocot, pearl millet.

Table 2-5. Shoot Mn concentrations of two NAD-ME, two NADP-ME C-4 plants, and two C-3 plants grown hydroponically in complete nutrient solution with different Mn concentrations.

Manganese (µM)	C-4 plants					C-3 Plants	
	NAD-ME C-4			NADP-ME C-4			
	Pearl millet		Purple				
	HGM 686	PP102M	amaranth	Corn	Sorghum	Wheat	Squash
	µg/g						
0	18e[Ŧ]	33d	12f	22c	28c	12f	8h
2	58d	_	81e	82c	53c	42ef	93g
5	84d	_	157de	66c	78c	60de	180f
10	104cd	247dc	253cd	108c	196c	79cd	307e
15	_	_	_	_	_	_	404d
20	_	_	_	_	_	_	512c
25	189c	360c	393c	124cb	249abc	119c	716b
30	_	_	_	_	_	_	784a
50	294b	485bc	750b	235b	322ab	183b	_
75	372b	600ba	1056ab	350a	442a	194b	_
100	470a	687a	1424a	413a	531a	261a	_

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Table 2-6. Root Mn concentrations of two NAD-ME, two NADP-ME C-4 plants and two C-3 plants grown hydroponically in complete nutrient solution with different Mn concentrations.

Manganese (μM)	C-4 plants					C-3 Plants	
	NAD-ME C-4			NADP-ME C-4			
	Pearl millet		Purple				
	HGM 686	PP102M	amaranth	Corn	Sorghum	Wheat	Squash
	μg/g						
0	0.611d[Ŧ]	36d	29e	20d	6d	15e	6e
2	132d	_	76d	489d	190cd	150de	83de
5	331cd	_	148cd	834cd	519bcd	243d	182cd
10	647c	1133c	228bcd	748cd	1450bcd	360cd	225c
15	_	_	_	_	_	_	346b
20	_	_	_	_	_	_	433b
25	1067b	2122b	296bc	1560bc	2346bcd	570bc	576a
30	_	_	_	_	_	_	517ab
50	1323ab	2683ab	487ab	1621abc	3869ab	810a	_
75	1635ab	3058a	760ab	2474ab	4783ab	913a	_
100	1974a	3246a	1275a	3935a	6070a	937a	_

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Table 2-7. Comparisons of shoot Mn concentrations of two NAD-ME C-4 , two NADP-ME C-4 plants and two C-3 plants species grown hydroponically in complete nutrient solution with the same Mn concentrations.

Plant species	**Solution Mn concentrations (µM)**				
	0	**2**	**5**	**10**	**25**
	Shoot tissue Mn (µg/g)				
Pearl millet	18a[Ŧ]	58a	84ab	104c	189d
Purple amaranth	12a	81a	157ab	253ab	393b
Corn	22a	82a	66b	108c	124d
Sorghum	28a	53a	78b	196b	249c
Wheat	12a	42a	60b	79c	119d
Squash	8a	93a	180a	307a	716a

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Discussion

The C-3 and NADP-ME C-4 species reached optimum growth with 2-5 μM Mn in nutrient solution, the amount commonly used in plant nutrient media (Epstein and Bloom 2005). However, the concentration of Mn needed for optimum performance of NAD-ME C-4 pearl millet and purple amaranth was about 20-fold higher and ranged between 50-100 μM Mn. In all six species used in this experiment, C-3 wheat and squash, NADP-ME C-4 corn and sorghum, and NAD-ME C-4 pearl millet and purple amaranth, shoot tissue Mn concentrations increased in parallel with Mn concentration in nutrient solution. Except for squash where toxic symptoms were observed, high leaf Mn concentration had no visible impact on wheat and NADP-ME C-4 species. However, in NAD-ME plants, increasing leaf Mn concentrations were correlated with a large, up to 100%, increase in the photosynthetic rate and chlorophyll reading.

The higher photosynthetic rates and dry matter production with higher Mn treatments of NAD-ME C-4 plants may have been due to increased Mn-activation of mitochondrial NAD-ME and the resulting higher CO_2 supply for Calvin cycle reactions. It has been shown that mitochondrial density in BSC of NAD-ME leaves is higher than that in MC (Hatch et al. 1975, Dengler et al. 1996) or of BSC of other C-4 groups (Hatch et al. 1975). This large number of mitochondria in the presence of higher Mn could allow the plant to achieve a higher summative effect on NAD-malic enzyme activity. For example, a 100-fold dilution during

preparation and assays, addition of 200 μM Mn had no effect on NAD-ME activity of roots of plants from solution culture with 10 ppm Mn, but caused a 10-fold increase in those plants receiving 0.25 ppm Mn (Anderson and Evans 1956). In another study, the concentration of Mn in isolated mitochondria bathed in 1000 μM Mn increased from 20 to 640 μM Mn. The NAD-ME activity in isolated mitochondria was 2.5-fold higher in assay solution containing 500 μM Mn than that containing 2.0 μM Mn (Rustin and Lance 1989). In yeast, the presence of K a cation activator of pyruvate kinase in the vicinity of this enzyme, was found to be critical not only enzyme activity but its stability as well (Sorger and Evans 1966). Therefore, in my study, it is possible plants grown with higher Mn treatment concentrations had higher Mn in the mitochondria that maintained stability and activity of NAD-ME better than plants grown with less Mn could not.

Although there was no attempt to identify the cellular location of Mn or to assess the relative amount in soluble form, it is possible that plants with higher tissue Mn concentrations have more Mn in BSC, where the enzymatic decarboxylation of malate by NAD-ME occurs. Manganese has been found to accumulate in tissues with high numbers of mitochondria (Underwood 1977). It has also been reported that rat mitochondria, compared to other organelles, had higher Mn influxes (Maynard and Cotzias 1954).

It is of importance that in my study the increase in photosynthesis and shoot growth occurred without a corresponding increase in stomatal conductance. This strongly indicates that the higher photosynthetic rate and dry

matter production with higher Mn nutrition could be a result of increased Mn-dependent CO_2 decarboxylation.

Perhaps the most significant finding in this work is that the Mn requirement for optimum performance of pearl millet and purple amaranth is up to 20-fold higher than that in C-3 and NADP-ME C-4 plants. This finding should be considered in future research, as well as in future cultivation of NAD-ME C-4 crops.

Summary

The objective of this research was to determine the effect of Mn nutrition on leaf Mn concentrations, photosynthetic rate, and biomass production of two NAD-ME species, pearl millet and purple amaranth, two NADP-ME (no Mn activation required) species, corn and sorghum, and two C-3 plant species, wheat and squash. Plants were grown in a complete nutrient solution with Mn concentrations ranging from 0 to ≤ 100 μM.

Mn treatment required for optimum photosynthesis and growth rates of C-3 and NADP-ME C-4 plants, corn and sorghum, was ~ 2 μM, a concentration found in common nutrient media. In contrast, NAD-ME species C-4 species required a 20-fold higher Mn concentration for optimum photosynthetic rate and growth. Because of a lack of Mn treatment effect on stomatal conductance above 2 μM, better internal supply/utilization of CO_2 for photosynthesis maintained a deep CO_2 concentration gradient between air and leaf intercellular compartment in NAD-ME species receiving higher Mn treatments.

These results, to my knowledge, are the first experimental evidence demonstrating a significantly higher Mn requirement for NAD-ME C-4 plant maximum photosynthesis, growth and yield.

Literature cited

Anderson I and Evans HJ 1956 Effect of manganese and certain metal cations on isocitric dehydrogenase and malic enzyme activities in *Phaseolus vulgaris*. Plant Physiol. 31, 22-28.

Burnell JN 1986 Purification and properties of phospho*enol*pyruvate carboxykinase from C_4 plants. Aust. J. Plant Physiol. 13, 577-587.

Burnell JN 1988 The biochemistry of manganese in plants. In Graham RD, Hannam R, and Uren NC (Eds) Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht, Netherlands, pp 125-137.

Bowen JE 1972 Manganese-silicon interaction and its effects on growth of sudan grass. Plant and Soil 37, 577-588.

Dengler N, Donnelly PM, and Dengler RE 1996 Differentiation of bundle sheath, mesophyll and distinctive cells in the C4 grass *Arundinella hirta* (Poacea). Amer. J. Bot. 83, 1391-1405.

Epstein E and Bloom AJ 2005 Mineral Nutrition of Plants. Principles and Perspectives. Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts.

Hannam RJ and Ohki 1988. Detection of manganese deficiency and toxicity in plants. In Graham RD, Hannam R , and Uren NC (Eds) Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht, Netherlands, pp 243-259

Hatch MD and Kagawa T 1974 Activity, location and role of NAD (Nicotinamide Adenine Dinucleotide) malic enzyme in leaves with C4 (acid decarboxylation) pathway photosynthesis. Aust. J. Plant Physiol. 1, 357-369.

Hatch MD, Kagawa T, and Graig S 1975 Subdivision of C_4-pathway species based on differing C_4 acid decarboxylating systems and ultrastructural features. Aust. J. Plant Physiol. 2, 111-128.

Lukaszewski K, Blevins DG, and Randall DD 1992 Asparagine and boric acid cause allantoate accumulation in soybeans leaves by inhibiting manganese-dependent allantoate amidohydrolase. Plant Physiol. 99, 1670-1676.

Marshner H 1995 Mineral Nutrition of Higher Plants. Academic Press New York, USA.

Maynard LS and Cotzias AC 1954 The partitioning of manganese among organs and intracellular organelles of the rat. J. Bio. Chem. 214, 489-495.

Minolta Camera CO, Ltd., Minolta Radiometric Instrument Division. Osaka Japan.

Murphy J and Riley JP 1962 A modified single solution method for determination of phosphate in natural waters. Anal. Chim. Acta 27, 31-36.

Reinbott TM and Blevins DG 1999 Phosphorus nutritional effects on root hydraulic conductance, xylem water flow and flux of magnesium and calcium in squash. Plant and Soil 209, 263-273.

Rustin P and Lance C 1989 The divalent cation pump and its role in the regulation of malic enzyme activity in purified mitochondria from potato tuber. Plant Cell Physiol. 30, 505-512.

SAS Institute 2004 The SAS system, version 9.1, SAS Institute Inc., Cary, NC.

Sorger GJ and Evans HJ 1966 Effect of univalent cations on the properties of yeast NAD^+ acetaldehyde dehydrogenase. Biochim. Biophys. Acta. 118, 1-8.

Underwood EJ 1977 Trace Elements in Human Nutrition, Manganese, 4^{th} Edn. Academic Press, New York.

Winkler RG, Blevins DG, Polacco JC, and Randall DD. 1987 Ureide catabolism of soybeans II. Pathway of catabolism in intact leaf tissue. Plant Physiol. 83, 585–591.

CHAPTER 3

LIGHT RESPONSE AND APPARENT QUANTUM YIELD OF C-3, NADP-ME C-4, AND NAD-ME C-4 PLANTS GROWN WITH A RANGE OF MANGANESE TREATMENTS

Introduction

Manganese plays a crucial role in more than one aspect of the photosynthetic process. In all plants, Mn is a structural and functional metal component of the water-splitting light-reaction protein complex, PS II (Marshner 1995). During photolysis of water, Mn atoms in PS II act as electron acceptors and ensure continued reduction of water and $P680^+$ (Marschner 1995). However, it has been reported that under high light intensities, Mn moved into the thylakoid lumen and depleted the Mn pool in PS II in pea (*Pisum sativum* L.) and squash (*Cucubita pepo* L.). This movement decreased the ability of PSII to reduce activated $P680^+$ (Hakala et al. 2005). Possibly, higher leaf tissue Mn concentration could prevent the depletion of Mn in PS II and sustain optimal photosynthetic rates at higher light intensities.

Manganese also activates the BSC mitochondrial-located enzyme controlling malate decarboxylation and thus the concentration of CO_2 for the Calvin cycle in the NAD-ME C-4 photosynthetic pathway (Hatch and Kagawa 1974, Burnell 1986). Manganese accumulates in the mitochondria, which are found in particularly large quantities in BSC (Hatch and Kagawa 1974). Possibly, higher Mn nutrition could result in higher mitochondrial Mn concentration and provide sufficient Mn for the NAD-ME reaction.

The Mn impact on light responses in C-4 plants has received little attention. However, it has been reported that in C-4 plant *Amaranthus edulis* L., increasing light by a factor of two increased photosynthetic rates by 4-fold (El-Sharkawy et al. 1968). In *Amaranthus hypochondriacus* L., increased illumination

caused a 2- to 5-fold increase in the velocity (V_{max}) of PEPC activity and a 3- to 4-fold increase in K_i for malate (Parvathi et al. 2000). This change in PEPC properties was reported to be responsible for the faster incorporation of HCO_3^{-1} into PEP with illumination. The incorporation of HCO_3^{-1} into PEP results in the formation of a 4-C organic acid, which, in all C-4 plants, is moved to the BSC and decarboxylated to release CO_2 for the Calvin cycle reactions. So, under higher light intensities, increasing leaf Mn concentration could not only prevent the loss in reducing power of PS II, but also support the NAD-ME decarboxylation activity, providing more CO_2 to the Calvin cycle. Fast decarboxylation would also allow increased synthesis of alanine required for regeneration of PEP to meet the light-associated increase in PEPC activity.

In order to evaluate the effect of light intensity on the NAD-ME photosynthetic pathway at different leaf Mn concentrations, photosynthetic rates and apparent quantum yield where measured across a range of PPFD in plants supplied with 0-100 µM Mn. NAD-ME C-4 plants pearl millet and purple amaranth were evaluated in parallel with NADP-ME C-4 plants, corn and sorghum, and with C-3 plants, wheat and squash.

Materials and methods

Plant materials and growth conditions

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) grain hybrid cv. HGM 686 obtained from Crosbyton Seed Company (Crosbyton, TX), purple amaranth (*Amaranthus hypochondriacus* (L.) cv. plainsman) obtained from Albert Lea Seed House (Albert Lea, MN), corn (*Zea mays* (L.) cv. FR 697) kindly supplied by the Georgia Davis laboratory, University of Missouri-Columbia and grain sorghum (*Sorghum bicolor* (L.) Moench) obtained from Plainview First Company (Plainsview, TX) were tested in this experiment. Wheat (*Triticum aestivum* (L.) cv. Ernie) from University of Missouri Foundation Seed (Columbia, Missouri) and squash (*Cucubita pepo* (L.) cv. straightneck) from Ferry-Morse Seed Company (Fulton, KY) were used as C-3 controls.

Pearl millet, purple amaranth, and sorghum seeds were pre-germinated in an aerated 1 L beaker of 0.2 mM $CaCl_2$ solution for a period of 48 hours (Pearl millet and sorghum) or 72 hours (purple amaranth). Corn and squash were pre-germinated for 96 hours in upright germination paper rolls soaked and maintained in 0.2mM $CaCl_2$ solution. Seedlings were grown hydroponically in complete nutrient solution consisting of: 2.0 mM $CaCl_2.2H_2O$; 0.5 mM $MgCl_2.6H_2O$; 2.0 mM KNO_3; 0.4 mM KH_2PO_4; 0.13 mM $FeCl_2.4H_2O$; 2.3 µM H_3BO_3; 0.6 µM $ZnSO_4.7H_2O$; 0.10 µM $NaMoO_4.2H_2O$; 0.11 µM $NiCl_2.6H_2O$; 0.01 µM $CoCl_2.6H_2O$; 0.15 µM $CuSO_4.5H_2O$; 0.1µM $Si(OH)_4$ (Reinbott and Blevins 1999), containing varying Mn concentrations. The solution pH was adjusted to 5.6 with KOH and nutrient solutions changed every other day to minimize the

change in pH. Depending on species, plants received Mn at concentrations of 0, 2, 5, 10, 15, 25, 30, 50, 75 or 100 µM. The Mn treatments were started after at least one photosynthetic leaf had developed. The C-4 plants were placed in a growth chamber with PPFD of 550 µmol quanta m^{-2} s^{-1} in a 16 hr, 29 ^{o}C light period and 8 hr, 20 ^{o}C dark period. The C-3 plants where placed in a growth chamber under similar conditions except that light and dark temperatures were 25 ^{o}C and 18 ^{o}C, respectively. Plant parameters of interest were measured prior to harvest, 10-14 days after the start of Mn treatments.

Leaf photosynthesis response to photosynthetic photon flux density (PPFD)

Photosynthetic measurements, internal CO_2 concentrations and stomatal conductance were determined 10 -14 days after Mn treatments were initiated. Measurements were taken from the most recently fully expanded leaf. In wheat, pearl millet, corn and sorghum, measurements were obtained from the mid-point between the leaf apex and leaf sheath. In amaranth and squash, the fourth leaf down from the shoot apex was used for measurements. Plants were kept under laboratory PPFD (~120 µmols quanta $m^{-2}s^{-1}$) for at least 30 minutes before light responses were determined. In all species, PPFD of 0, 25, 50, 75, 100, 300, 550, 800 and 1000 µmol quanta m^{-2} s^{-1} were used, except the levels of 25 and 50 µmol quanta $m^{-2}s^{-1}$ in purple amaranth. Photosynthetic rates in response to PPFD were determined in a descending manner (Cheng et al. 2001) starting with highest PPFD of 1000 µmol quanta m^{-2} s^{-1}. Photosynthetic rate and stomatal

conductance response to light were determined using a LI-COR 6400 Portable Photosynthesis System (LICOR Inc., Lincoln, Nebraska, USA) with a 6400-02 LED light source cuvette. Leaf cuvette conditions were: CO_2 flow rate of 500 μmol s^{-1}, sample CO_2 concentration of 400 μmol mol^{-1}, leaf temperature was 27 ± 0.5 ^{o} C and varied with PPFD by < 1.0^{o} C, while vapor pressure deficit range from 2.5 to 3.2 kPa.

Apparent quantum yield

Leaf apparent quantum yield was determined using photosynthetic rates obtained at all or some of these PPFD levels; 0, 25, 50, 75 or 100 μmols quanta m^{-2} s^{-1}. Light intensities of 0, 50, and 100 μmols quanta m^{-2} s^{-1} were used for amaranth. In squash, wheat, sorghum, corn, and pearl millet, light intensities of 0, 25, 75 and 100 μmols quanta m^{-2} s^{-1} were used. The quantum efficiency was determined by regression of photosynthetic rates and PPDF using PROC GLM procedures in SAS version 9.2 (SAS, 2004).

Statistical analysis

Each experiment was a randomized complete block with three replicates per block. In NAD-ME C-4 species, experiments were repeated three times, while only two were done for other species. The PROC GLM procedures in SAS version 9.2 (SAS, 2004) was used to test the effect of Mn on measured

parameters. All effects significant at $P < 0.05$ were separated using Fisher's Protected Least Significant Difference.

Results

Leaf photosynthetic response to progressively greater light (PPFD) and to increasing Mn supply

In all species used in this experiment, plants grown without Mn had severely depressed photosynthetic rates across the range of PPFD. In wheat and squash (C-3), photosynthetic rates were highest with 2 and 5 μM Mn, respectively (Figure 3-1 & 3-2). The photosynthetic response in squash was more sensitive to Mn, and was severely depressed at 20 μM Mn, while wheat showed little change in photosynthetic response up to 100 μM Mn. Both NADP-ME C-4 species, corn and sorghum, also showed little Mn effect on photosynthetic response across the range of PPFD (Figure 3-3 & 3-4). It may be noteworthy that in corn grown without Mn, the photosynthetic rate was only slightly reduced, while in sorghum the photosynthetic rate at 0 Mn was very low. The light saturated photosynthesis occurred at ~ 800 PPFD for corn and ~ 1000 PPFD for sorghum.

In contrast to C-3 and NADP-ME C-4 species, Mn had a large impact on the photosynthetic response of NAD-ME plants (Figure 3-5 & 3-6). In pearl millet, the highest photosynthetic rate across the PPFD range was recorded for plants supplied with 50 μM Mn (Figure 3-5), while the highest response in purple amaranth required 100 μM Mn (Figure 3-6). In both NAD-ME C-4 species, photosynthetic rate observed with a 50-110 μM Mn supply was ~40% higher than that obtained with 2 μM Mn. In all tested species, internal CO_2 concentration

correlated with the photosynthetic rates at specific PPFD (Appendix Figure 3-1, 3-2, 3-3, 3-4, 3-5 & 3-6).

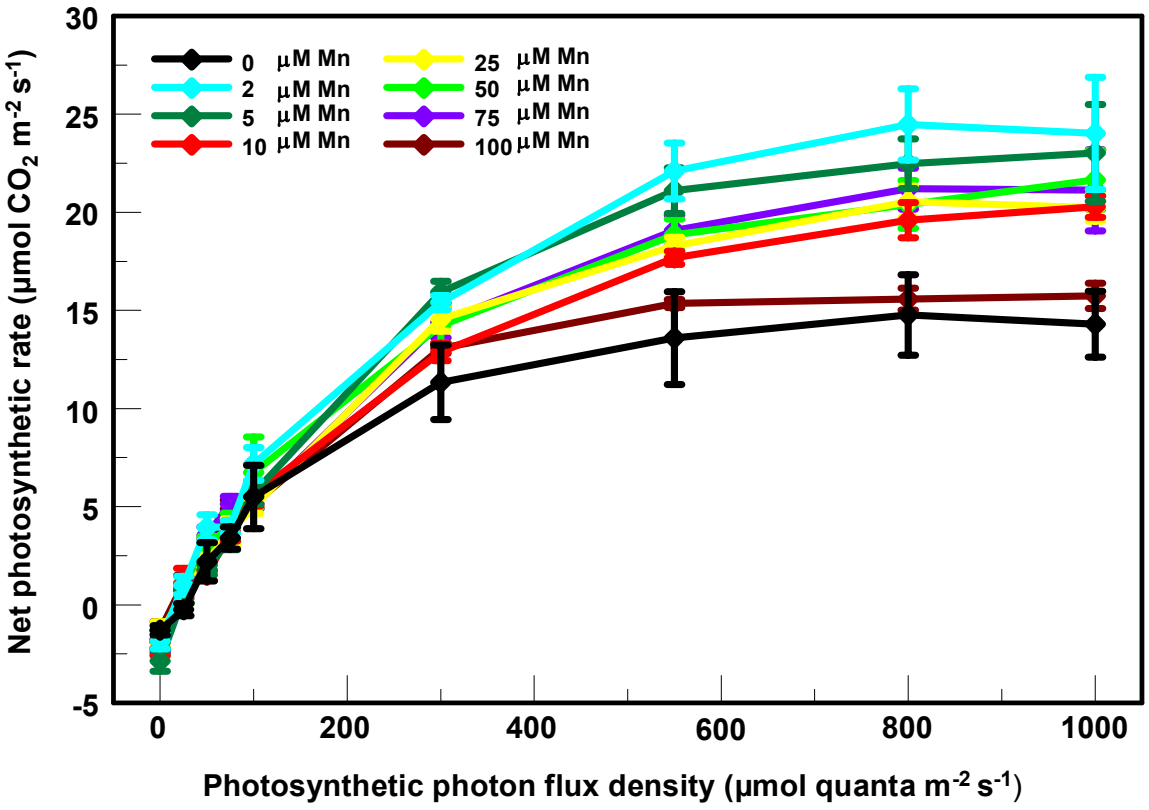


Figure 3-1. Photosynthetic response of the C-3 plant, wheat, to changing PPFD when grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means ± SE (n=6).

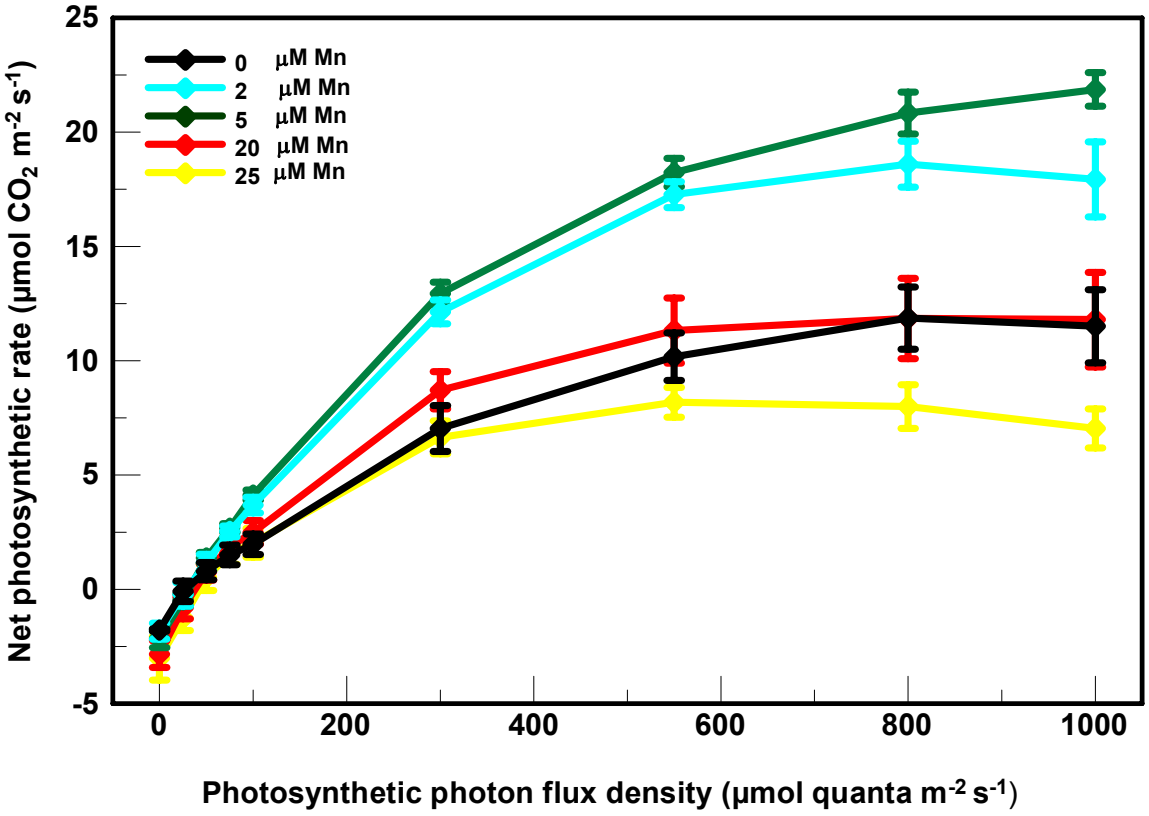


Figure 3-2. Photosynthetic response of the C-3 plant, squash, to changing PPFD when grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means ± SE (n=6).

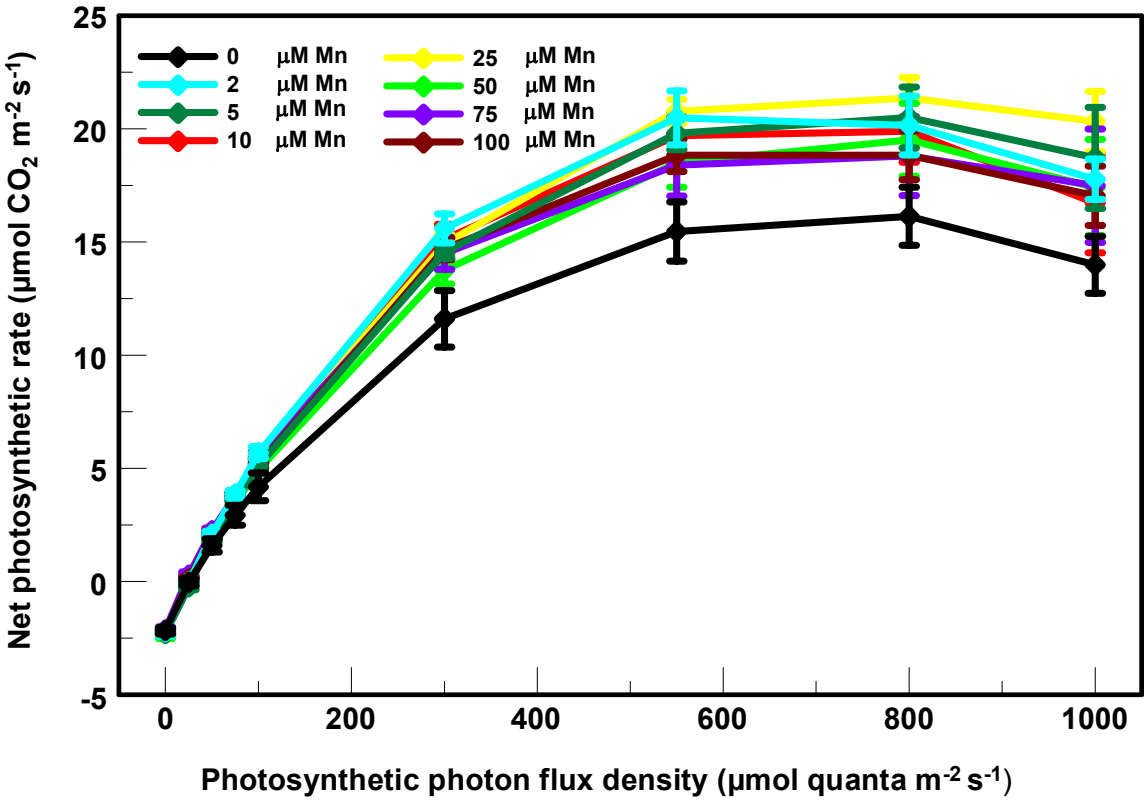


Figure 3-3. Photosynthetic response of the NADP-ME C-4 plant, corn, to changing PPFD when grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means ± SE (n=6).

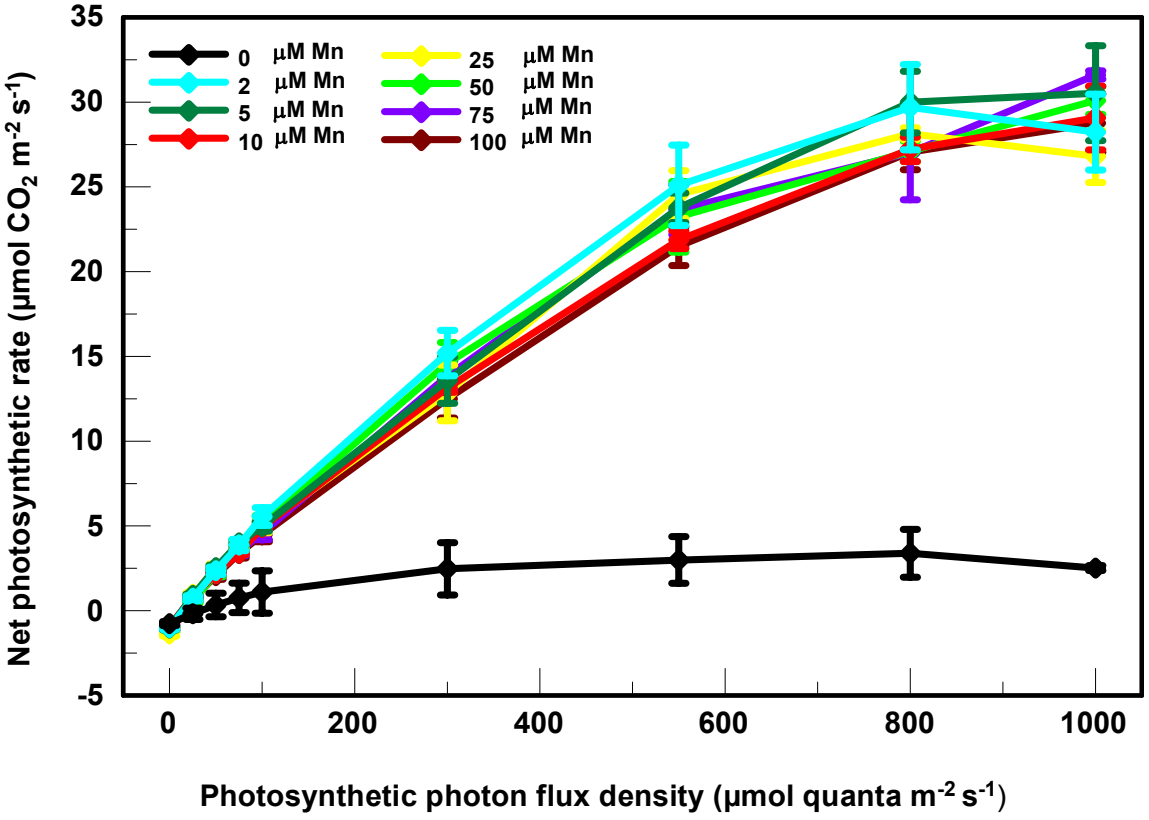


Figure 3-4. Photosynthetic response of the NADP-ME C-4 plant, sorghum, to changing PPFD when grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means ± SE (n=6).

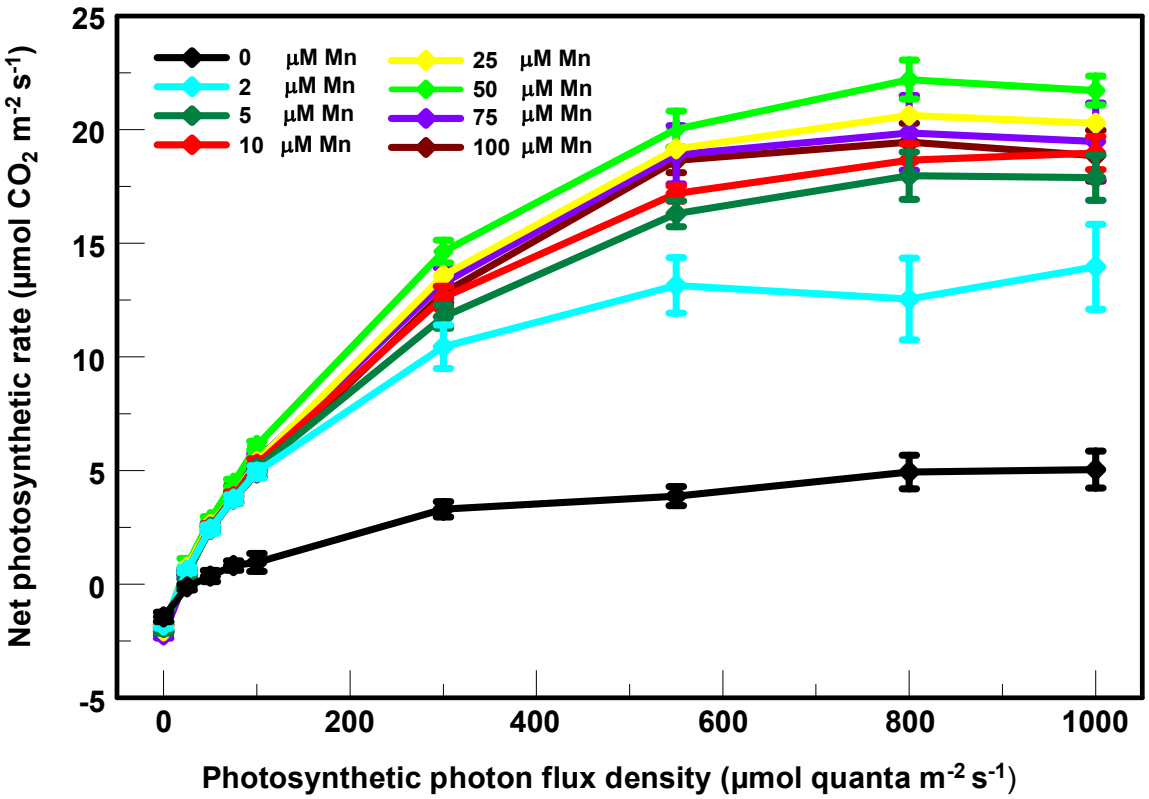


Figure 3-5. Photosynthetic response of the NAD-ME C-4 plant, pearl millet, to changing PPFD when grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means ± SE (n=9).

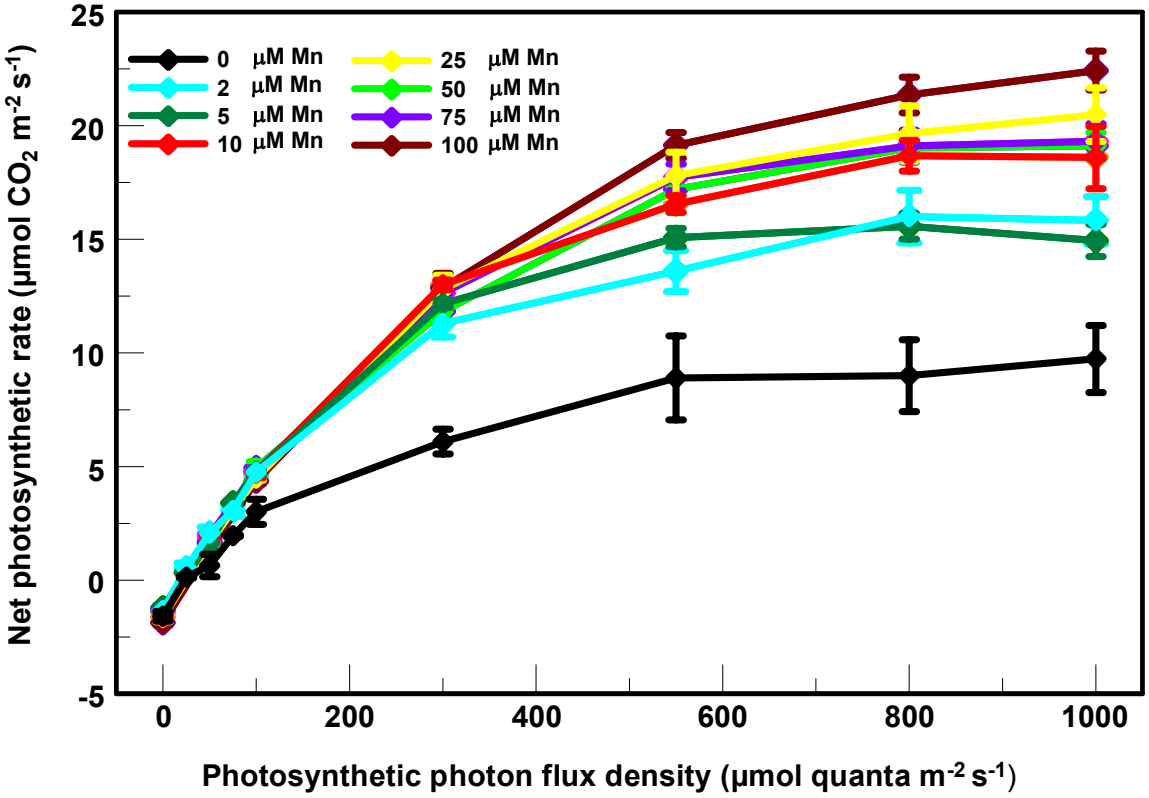


Figure 3-6. Photosynthetic response of the NAD-ME C-4 plant, purple amaranth, to changing PPFD when grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means ± SE (n=9).

Manganese effect on leaf apparent quantum yield and light (PPFD) saturated photosynthesis

Apparent quantum yield

Plants grown without Mn had, in general, the lowest apparent quantum yield (AQY) (Table 3-1). In both C-3 species, wheat and squash, the values were the highest for 2 and 5 µM Mn. In both NADP-ME C-4 species corn and sorghum, plants receiving Mn had similar AQY. Also in the NAD-ME C-4 species purple amaranth, AQY values were similar across the range of Mn treatments. The only exception was the NAD-ME C-4 pearl millet, which showed differences in AQY that reflected the increasing Mn treatments.

Light (PPFD) saturated photosynthesis

Across the plant species, the lowest photosynthetic rates at light saturation were recorded for plants grown with 0 µM Mn. In the C-3 species, wheat, saturated photosynthesis was obtained at ~ 800 µmols quanta m^{-2} s^{-1} (Table 3-2). Plants receiving 0 or 100 µM Mn had the lowest A_{max} of 14.76 and 16.10 µmols CO_2, respectively (Table 3-2). The highest photosynthetic rate of 24.90 µmols CO_2 m^{-2} s^{-1} was found in plants receiving 2 µM Mn. In the C-3 species, squash, plants receiving 5 µM Mn had the highest A_{max} of 21.87 µmols CO_2 m^{-2} s^{-1} (Table 3-2). Squash plants receiving 25 µM Mn had the lowest A_{max} at 7.07 µmols CO_2 m^{-2} s^{-1} (Table 3-2).

In the NADP-ME C-4 species corn, all treatments had saturated photosynthetic rates at 550 µmols quanta m^{-2} s^{-1} (Figure 3-2). All corn plants

receiving Mn treatment had similar A_{max} ranging from 18 to 20 μmols CO_2 m^{-2} s^{-1} (Table 3-2). In sorghum, all Mn treated plants saturated photosynthesis at > 800 μmols quanta m^{-2} s^{-1} (Figure 3-4) and had similar A_{max} of between 28 and 31 μmols CO_2 m^{-2} s^{-1} (Table 3-2).

In the NAD-ME species pearl millet, saturated photosynthesis was obtained at 550 μmols quanta m^{-2} s^{-1} except in plants receiving 50 μM Mn which saturated at 800 μmols quanta m^{-2} s^{-1}. Plants receiving 25 and 50 μM Mn attained A_{max} of 20.2 and 21.5 μmols CO_2 m^{-2} s^{-1}, respectively (Table 3-2). The photosynthetic rates of plants receiving 50 μM Mn were highest at 21.5 μmols CO_2 m^{-2} s^{-1}. In the NAD-ME species C-4, purple amaranth, A_{max} rates of all Mn treated plants were lower than those of plants receiving 100 μM Mn. Plants receiving 25 μM Mn had higher A_{max} than plants receiving 10 μM Mn, but similar to A_{max}'s to those of plants receiving 50 and 75 μM Mn (Table 3-2).

Table 3-1. Apparent quantum yield of two NAD-ME, two NADP-ME C-4 plants, and two C-3 plants grown hydroponically in complete nutrient solution with different Mn concentrations.

Manganese (µM)	C-4 plants				C-3 plant	
	NAD-ME		NADP-ME			
	Pearl millet	Purple amaranth	Sorghum	Maize	Wheat	Squash
	µmol CO_2 per µmol quanta					
0	0.024d[Ŧ]	0.044b	0.020b	0.062b	0.068a	0.036bc
2	0.066c	0.058a	0.065a	0.078a	0.084a	0.055a
5	0.069bc	0.059a	0.060a	0.075a	0.085a	0.063a
10	0.072abc	0.063a	0.057a	0.076a	0.071a	–
15	–	–	–	–	–	–
20	–	–	–	–	–	0.053a
25	0.072abc	0.062a	0.063a	0.076a	0.063a	0.052ab
30	–	–	–	–	–	–
50	0.079a	0.063a	0.065a	0.071a	0.068a	–
75	0.076ab	0.061a	0.055a	0.076a	0.071a	–
100	0.068bc	0.063a	0.055a	0.072a	0.065a	–

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Table 3-2. Optimum photosynthesis rate at saturating PPFD of two NAD-ME, two NADP-ME C-4 plants, and two C-3 plants grown hydroponically in complete nutrient solution with different Mn concentrations.

Plant species	Solution Mn concentration (µM)								
	0	**2**	**5**	**10**	**20**	**25**	**50**	**75**	**100**
Pearl millet	5.13e[Ŧ]	8.50d	17.90c	18.90bc		20.20ab	21.50a	19.30b	18.70bc
Purple amaranth	9.70e	13.97d	14.97d	18.58c		20.47b	19.58bc	19.32bc	22.40a
Corn	15.32b	20.51a	19.82a	19.72a		20.78a	18.51a	18.42a	18.85a
Sorghum	2.52b	28.23a	30.53a	29.94a		26.94a	30.11a	31.67a	28.77a
Wheat	14.76d	24.89a	22.47b	19.59c		20.18c	20.39c	19.50c	15.99d
Squash	11.51c	17.93b	21.87a		11.8c	7.07d			

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$

Discussion

Plants have the ability to convert light energy into chemical energy via electron transport processes that are mediated by several metal containing proteins in the thylakoid membrane of leaf chloroplasts (Marshner 1995). Since light is the source of energy used to generate electron flow and synthesize reducing compounds needed for CO_2 fixation (Marschner 1995, Lawlor 2001, Lambers et al. 1998), it is not surprising that in my experiments the photosynthetic rate increased with increasing light intensity (PPFD) in all species, across all the Mn treatments. The increase in photosynthetic rates at the lower PPFD range, where light is the major limiting factor, may be attributed to the effect of light intensity on PS II reactions, like reduction of water and generation of electrons, or to increased Rubisco activity (Marschner 1995), increased PEP carboxylase, and other Calvin cycle reactions (Pons et al. 1992), but not to Mn. However, differences in photosynthetic response between Mn treatments at higher PPFD range, could reflect the role Mn plays in the malate decarboxylation reactions in NAD-ME plants. The effect of increasing PPFD on photosynthetic enzyme activities and photosynthesis rates in C-4 species has been reported before. For example, in *Flavaria brownie* L., PPFD of 1150 µmols quantum m^{-2} s^{-1} caused higher activities of Rubisco and C-4 photosynthetic enzymes than in plants receiving low PPFD of 240 µmol quantum m^{-2} s^{-1} (Cheng et al. 1989).

In my study, the responses of C-3 species and NADP-ME C-4 species to increasing light intensity reflected their Mn requirement for optimum photosynthesis, 2-5 µM (Chapter 2, Figure 2-3 and 2-4). Photosynthetic response

in these plants increased in parallel with PPFD, but showed no effect of Mn, even at the higher PPFD range. Since these species do not require Mn for malate decarboxylation, the entire increase in photosynthetic rate with higher PPFD is not related to CO_2 release for Calvin cycle reactions. However, in NAD-ME C-4 plants, the increase in photosynthetic response due to increased PPFD strongly correlated to Mn supply. In these plants, at a given PPFD, the activation of Rubisco, PEP-carboxylase, and other Calvin cycle reactions was similar to that in other species. Therefore, higher photosynthetic rates in NAD-ME plants receiving higher Mn was likely the result of higher activation of the Mn-dependent decarboxylating enzyme, NAD-ME, and consequently higher CO_2 assimilation rates.

In all plant species, the low AQY in leaves of plants not receiving Mn may be attributed to reduced efficiency of PS II and other Mn-associated physiological processes. Because AQY was determined at PPFD up to 100 μmol quanta m^{-2} s^{-1}, it may be argued that PPFD, and not Mn, was the main factor limiting photosynthesis at these PPFD levels, hence the lack of differences due to Mn treatment. Even in NAD-ME C-4 plants, this point is validated by the fact that differences in photosynthetic rates between Mn treated plants were observed only at PPFD levels above the 100 μmol quanta m^{-2} s^{-1} used to determine AQY.

In the C-3 wheat and squash, and NADP-ME C-4 species, corn and sorghum, 2 - 5 μM Mn was enough to achieve PPFD saturated A_{max}, probably because these plants did not require Mn for CO_2 release. The lower photosynthetic response in squash at ≥ 20 μM Mn was likely a result of Mn

toxicity. Manganese toxicity in common bean (*Phaseolus vulgaris* L.) (a C-3 plant) was found to be exacerbated at high PPFD (Gonzalez et al. 1998). Bean plants showing Mn toxicity had leaf Mn concentration of ~800 mg kg^{-1}, a value comparable to that in squash plants showing Mn toxicity symptoms in this study (Chapter 2, Table 2-7). The Mn toxicity in beans was associated with reduced levels of free radical scavenging compounds like ascorbate, a situation which may have occurred because of the role of Mn in activating ascorbate oxidase (Gonzalez et al. 1998). Activated ascorbate oxidase could reduce the leaf ability to detoxify free radicals, leading to a photo-oxidative destruction. Another mechanism of Mn toxicity could be related to the possible Mn-substitution for Mg in Mg-activated enzymes, like Rubisco (Marshner 1995). Both possibilities are likely, given that across the range of Mn treatments, squash plants had the highest leaf Mn concentrations of all species in my study.

NAD-ME C-4 plants treated with high Mn concentrations had higher photosynthetic rates at saturating PPFD, which could result from higher activity of Mn-dependent NAD-ME. The improved activity of NAD-ME would lead to better decarboxylation of malate in BSC mitochondria, increased CO_2 supply to Calvin cycle reactions, and subsequently increased photosynthetic rates. Because Mn is required for continued high rates of CO_2 reduction in NAD-ME C-4 plants, its deficiency may result in reduced utilization of light energy, increased PS II photo-damage, and a decline in A_{max}. This could explain the lower maximum photosynthetic rate of plants receiving low concentration of Mn in this study. In addition, Mn treated plants had higher SPAD 502 chlorophyll readings (Chapter

2, Table 2-3). Therefore, high Mn treatment may have produced leaves which had higher chlorophyll and possibly PS II contents and higher light absorption. This may have resulted in increased thylakoid energy generation, electron flow, leaf reducing power, and increased fixation of CO_2.

Summary

The objective of this research was to determine the impact of Mn nutrition on leaf apparent quantum yield (AQY) and light saturated photosynthesis A_{max}. Two NAD-ME species, pearl millet and purple amaranth, two NADP-ME (no Mn activation required) species, corn and sorghum, and two C-3 plant species, wheat and squash were tested. Plants were grown in a complete nutrient solution with Mn concentrations ranging from 0 to ≤ 100 µM, and photosynthetic rates were determined at increasing PPFD up to 1000 µmol quanta m^{-2} s^{-1}.

Except for pearl millet, Mn treatment had no effect on AQY. In C-3 and NADP-ME C-4 species, the highest reponses to increasing PPFD and high A_{max} were in plants receiving 2 or 5 µM Mn. Squash receiving >10 µM Mn concentration showed poor response to increasing PPFD and low A_{max} likely due to Mn toxicity. However, in pearl millet and purple amaranth highest response to increasing PPFD and A_{max} were in plants receiving 50-100 µM Mn. Because Mn treated plants showed better response, it is conceivable that the canopy of Mn rich plants may be better at utilizing the penetrating low levels of PPFD than Mn-deficient plants.

Literature cited

Burnell JN 1986 Purification and properties of phospho*enol*pyruvate Carboxykinase from C_4 Plants. Aust. J. of Plant Physiol. 13, 577 – 587.

Burnell JN, Suzuki I and Sugiyama T 1990 Light induction and effect of nitrogen status upon the activity of carbonic anhydrase in maize leaves. Plant Physiol. 94, 384-387.

Cheng S-H, Moore Bd, Wu J, Edwards GE, and Ku MSB 1989 Photosynthetic plasticity in *Flavaria browni*. Growth irradiance and the expression of C_4 photosynthesis. Plant Physiol. 89, 1129-1135.

El-Sharkawy MA, Loomis RS and Williams WA 1968 Photosynthesis and respiratory exchange of carbon dioxide by leaves of the grain amaranth. J. Appl. Ecol. 1, 243-251.

Gonzalez A, Steffen KL, and Lynch JP 1998. Light and excess manganese-implication for oxidative stress in common bean. Plant Physiol. 118, 493-504.

Hakala M, Tuomine L, Keränen M, Tyystjärvi T, and Tyystjärvi E 2005 Evidence for the role of the oxygen-evolving manganese complex in photoinhibition of photosystem II. Biochim. Biophys. Acta 1706, 68-80.

Hatch MD and Kagawa T 1974 Activity, location and role of NAD malic enzyme in leaves with C4-pathway photosynthesis. Aust. J. Plant Physiol. 1, 357-369.

Hatch MD 1978 Regulation of enzyme in C-4 photosynthesis. In BL Horecker, ER Stadtman (Eds) Current Topics in Cellular Regulation, Vol 14 Academic Press, New York, pp 1-27.

Lambers H, Chapin III FS, and Pons TL 1998 Plant Physiological Ecology. Springer-Verlag, Inc., New York, USA.

Lowlar DW 2001 Photosynthesis. BIOS Scientific Publishers Ltd, Oxford, UK.

Marshner H 1995 Mineral Nutrition of Higher Plants. Academic Press New York, USA.

Parvathi K, Baghwat AS, Ueno Y, Izui K and Raghavendra AS 2000 Illumination increases the affinity of phoshoenolpyruvate carboxylase to bicarbonate in leaves of C_4 photosynthetic plants. Plant Cell Physiol. 41, 905-910.

Pons TL, Pearcy RW, and Seemann JR 1992 Photosynthesis in flash light in soybean leaves grown in different conditions. 1. Photosynthetic induction state and regulation of ribulose-1,5-bisphosphate carboxylase activity. Plant Cell Environ. 15, 569-576.

CHAPTER 4

THE EFFECT OF FIELD-APPLIED MANGANESE ON GROWTH AND SEED YIELD OF PEARL MILLET AND PURPLE AMARANTH

Introduction

Adaptation of many plant species to extreme environmental conditions, like hot temperatures and dry soils, may be linked to their C-4 photosynthetic pathway (Lloyd and Farquhar 1996, Ehleringer et al. 1997). The unique CO_2 concentration mechanism of C-4 plants allows for sufficient Rubisco saturation and high photosynthetic rates even under moisture stress that reduces stomatal gaseous fluxes. The efficient water-use helps C-4 plants to photosynthesize and withstand dry environments (Sage and Pearcy 1987). It has also been reported that C-4 plants respond better to elevated CO_2 under hot and dry conditions. For example, corn (C-4) responded better to elevated CO_2 concentrations under moisture stress conditions (Leakey et al. 2006). Also other C-4 plants subjected to periodic water stress in an open prairie, with elevated CO_2 increased photosynthetic productivity due to better water-use efficiency (Owensby et al. 1993). Therefore, C-4 plants may be better at adapting to the expected rise in atmospheric CO_2 concentrations, because they are also adapted to handling increasing moisture stress caused by increased global desertification.

Cereal grains, like corn, wheat, rice, sorghum, and millet are major sources of food for millions of people worldwide. These cereal crops need high N fertilizer for high yields (Hirel et al. 2007, Triplett et al. 2007), especially wheat and rice, that have low N-use efficiency (Zhu 2000, Raun and Johnson 1999, Zhu 2000). Recently, it has been reported that photosynthesis under high CO_2 was limited by N (Reich et al. 2006). Under projected global conditions that may be dry with low soil fertility and high CO_2 concentration, high nutrient- and water-use

efficiency crops would be more likely to provide sustained high yields and continued food supply. Therefore, there is a need to focus on C-4 plants that show both high N- and water-use efficiencies.

Pearl millet (*Pennisetum glaucum* (L.) R. Br), a NAD-ME C-4 plant, grows better than most cereal crops on poor soils and drier environments (Ejeta et al. 1987). It is estimated to occupy 26 million hectares worldwide (Andrews and Bramel-Cox 1994), and over 600,000 hectares in the US (Andrews et al. 1996). Some pearl millet cultivars produce high quality summer forage for milk and beef production (Wilson 2004, Kallenbach et al. 2004). The high protein and essential amino acid content (Burton 1972, Ejeta et al. 1987, Smith et al. 1989, Haydon and Hobbs 1991) of pearl millet seed (Singh and Perez-Maldonando 2003) make it a major feed supplement for poultry and swine production.

Amaranth (*Amaranthus hypochondriacus* L.), a dicot with the NAD-ME C-4 photosynthetic pathway, is a hardy plant tolerant to drought (Sleugh et al. 2001, Laovoravit et al. 1985, Stordahl 1999). Amaranth seed protein concentrations range between 13 and 19% (Stordahl et al. 1999, Lehamann 1990, Yue et al. 1987) with high lysine, cysteine, and methionine, the three essential amino acids for diet of monogastric animals found to be deficient in other grains, like corn, wheat, and rice (Senft 1979, Bressani et al. 1987).

Based on the results of my previous Chapters (Chapter 2 and 3), Mn is an element that could be critical for NAD-ME C-4 plant photosynthesis and productivity. Unfortunately, little research is published in this area. There is a report of an increased shoot biomass production in NADP-ME C-4 corn following

Mn fertilization (Fagaria 2002). The hydroponic studies presented in Chapters 2 and 3 showed that Mn increased photosynthesis, light response, and shoot biomass in NAD-ME C-4 species pearl millet and purple amaranth. The proposed reason for this increase was related to improved Mn-activation of NAD-ME and CO_2 release for Calvin cycle reactions. These studies show that potential exists for improved crop production with proper Mn nutrition. To test this possibility, the impact of Mn fertilization on leaf photosynthetic rate, nutrient concentrations, and seed yield of pearl millet and purple amaranth was examined in a field experiments. Two sources of Mn fertilizer were compared in this study.

Materials and Methods

Experimental Site

The experiments were carried out at the Rollins Bottom Experiment Site located near the University Golf Course on Hinkson Creek on the University of Missouri-Columbia campus. The alluvial soil, a Moniteau silt loam, has 15% sand, 65% silt, and 20% clay. Soil samples were collected each year before the start of the experiment. The chemical composition of the soil was analyzed by the University of Missouri Soil and Plant Testing Laboratory (Table 4-1). The experiments were each a randomized complete block design with six replicates of each treatment and seven Mn treatments. Each experimental unit was 0.0023 hectares (10 x 25 ft).

Planting

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) hybrid cv. HGM 686 seed was purchased from Crosbyton Seed Company (Crosbyton, TX), and purple amaranth (*Amaranthus hypochondriacus* (L.) cv. plainsman) seed was purchased from Albert Lea Seed House (Albert Lea, MN). Seeds were planted in a prepared seed bed at seeding rates of 10 kg/ha (9 lbs/acre) for pearl millet and 4.5 kg/ha (4 lbs/acre) for purple amaranth. Seeding depth was approximately 1-2 cm and each experimental plot consisted of seven rows spaced 42 cm apart. Data were collected from the middle five rows in each plot. Initial planting was done on June 6th and June 1st in 2006 and 2007, respectively. However, due to poor plant emergence, replanting of purple amaranth was done ~ 2 weeks later in

both years. After planting, Mn from two different sources was applied to appropriate plots at rates of 0, 4.5, 9.0 and 18 kg Mn/ha (0, 4.0, 8.0 and 16 lb Mn/acre). The two Mn sources were conventional fertilizer, $MnCl_2$, and Mn beads supplied by Dr. Larry Sanders (Specialty Fertilizer Products, Kansas City, MO). Nitrogen fertilizer was applied each season at rates of 110 kg/ha (100 lb/acre) for both crops, three weeks after crop emergence.

Table 4-1. Soil chemical composition at the growth site of each plant species during both growing seasons.

Season	Plant species	Soil chemical composition								
		pH	OM %	Bray I P	Bray II P	Ca	Mg	K	CEC (meq/100 g)	Mn (ppm)
				kg/ha						
2006	**Pearl millet**	7.0	1.5	122	527	3439	266	332	9.0	19.2
	Purple amaranth	6.8	1.1	117	482	3185	254	271	8.4	20.0
2007	**Pearl millet**	7.2	1.4	127	684	4476	242	322	11.3	12.0
	Purple amaranth	7.0	1.0	104	572	3577	224	266	9.1	14.0

Crop management

Weed and pest control

In both species, mechanical and chemical methods were used for weed control two weeks after plant emergence. In pearl millet, 2, 4 D Lo-V Ester (2,4 - dichlophenoxyacetic acid, ai 65.1%) was applied to kill broadleaved weed species. The application rate was 1.2 L /ha (½ pint/ acre) with a spray rate of 140 L/ha (15 gallons/acre). In amaranth, Select® herbicide was applied to kill grass weeds. Application rate was 1.2 L /ha (½ pint/ acre) with a spray rate of 140 L/ha. Tilling was done following chemical control to remove any weeds tolerant to the chemicals used. Subsequent weeds were controlled by either manual uprooting or with a tilling implement as the need arose. There were no major insect infestations, therefore, no insect control measures were undertaken. However, there was a late season bird infestation of pearl millet each year.

Irrigation

Prolonged dry periods with no or very little precipitation were experienced during both years. During the period of extreme moisture stress, plants were irrigated. In all cases, a single irrigation operation supplied water equivalent to approximately 25 mm (1 inch) of rainfall. This was done thrice and twice in the first and second season, respectively.

Leaf photosynthetic measurements

Leaf photosynthetic measurements were taken in the field at the time of maximum vegetative growth, just before head emergence in pearl millet and at the time of formation of inflorescence in purple amaranth. Six representative leaves were identified within the middle five rows of each plot, each on a separate plant, and photosynthetic rates were determined using a LI-COR 6400 Portable Photosynthesis System (LICOR Inc., Lincoln, Nebraska, USA) with a 6400-02 LED light source cuvette. Leaf cuvette conditions were: CO_2 flow rate of 500 µmol s^{-1}, sample CO_2 concentration of 400 µmol mol^{-1}, vapor pressure deficit ranged from 2.3 to 3.7 kPa, and leaf temperature varied and ranged between 32 and 37^{o} C depending on ambient air-temperature. Before determining photosynthetic rates, solar radiation levels between 10.00 a.m and 2.30 p.m central daylight time were established. The LICOR- 6400 light source was then set to match the average solar radiation during this time and instantaneous photosynthesis was measured in the chosen leaves. In pearl millet, measurements were taken mid-way between the leaf sheath and the leaf blade tip, on one side of the mid-rib. In purple amaranth, measurements were obtained from the center of the leaf and away from large veins. A photosynthetic value was locked in after the rate of CO_2 assimilation stabilized, about 3-5 minutes after clamping the LICOR sensor head on to the leaf.

Leaf sampling

During the course of each experiment, leaf samples were collected for macro- and micronutrient analysis. A total of 20 leaves were randomly obtained from each experimental plot. The second leaves below the head were collected in pearl millet, while in purple amaranth the most recently matured leaves were picked from the main stem. Leaves were oven dried at 70^0 C to a constant weight.

Seed harvesting

At maturity, when seed moisture content was approximately 12%, the middle five rows of each plot were harvested with a small plot combine and the harvested seed weight was determined.

Macro-and micronutrient analysis

Dried leaves were ground to a fine powder with a modified coffee grinder and the ground material was stored at 5 ^{0}C. For macro-and micronutrient element analysis, 0.25 g sample of the ground material was placed in a 50 ml microwave digestion tube. Samples were digested in 10 ml of nitric acid solution (5 ml of double de-ionized water and 5 ml of concentrated nitric acid (6 N HNO_3)). Digestion was done with an accelerated microwave digestion system (MARSXPress by CEM, Matthews, NC). The digested material was brought to a volume with double deionized water, filtered, and stored at 5 ^{0}C, pending macro-

and micronutrient element analysis by Inductively Couple Plasma Absorption Emission Spectroscopy (ICP-AES, Varian, Inc. Palo Alto, Ca). Elements analyzed were macronutrients P, K, Mg, and Ca and micronutrients Fe, Mn, Zn, Cu, Na, and Mo.

Statistical analysis

Statistical analysis was conducted using PROC MIX program in SAS version 9.2 (SAS 2004). The effect of Mn treatment was tested in both species, separately for each year of study. Means with significant differences ($P<0.05$) were separated using Fisher's Protected Least Significant Difference.

Results

Photosynthetic rate

In both years, pearl millet plants that received Mn fertilizer had higher photosynthetic rates than the untreated controls (Figure 4-1). The lowest Mn rate of 4.5 kg Mn/ha, increased photosynthetic rates by ≥ 25%. No differences were observed between Mn sources.

Manganese fertilization of purple amaranth also resulted in an increase in photosynthesis (Figure 4-2). The increase between the control and the lowest Mn treatment was ≥ 16%. There was little difference between Mn sources.

Leaf stomatal conductance

During both years, pearl millet and purple amaranth plants that received Mn fertilizer had higher stomatal conductance than the untreated controls (Figures 4-3 and 4-4).

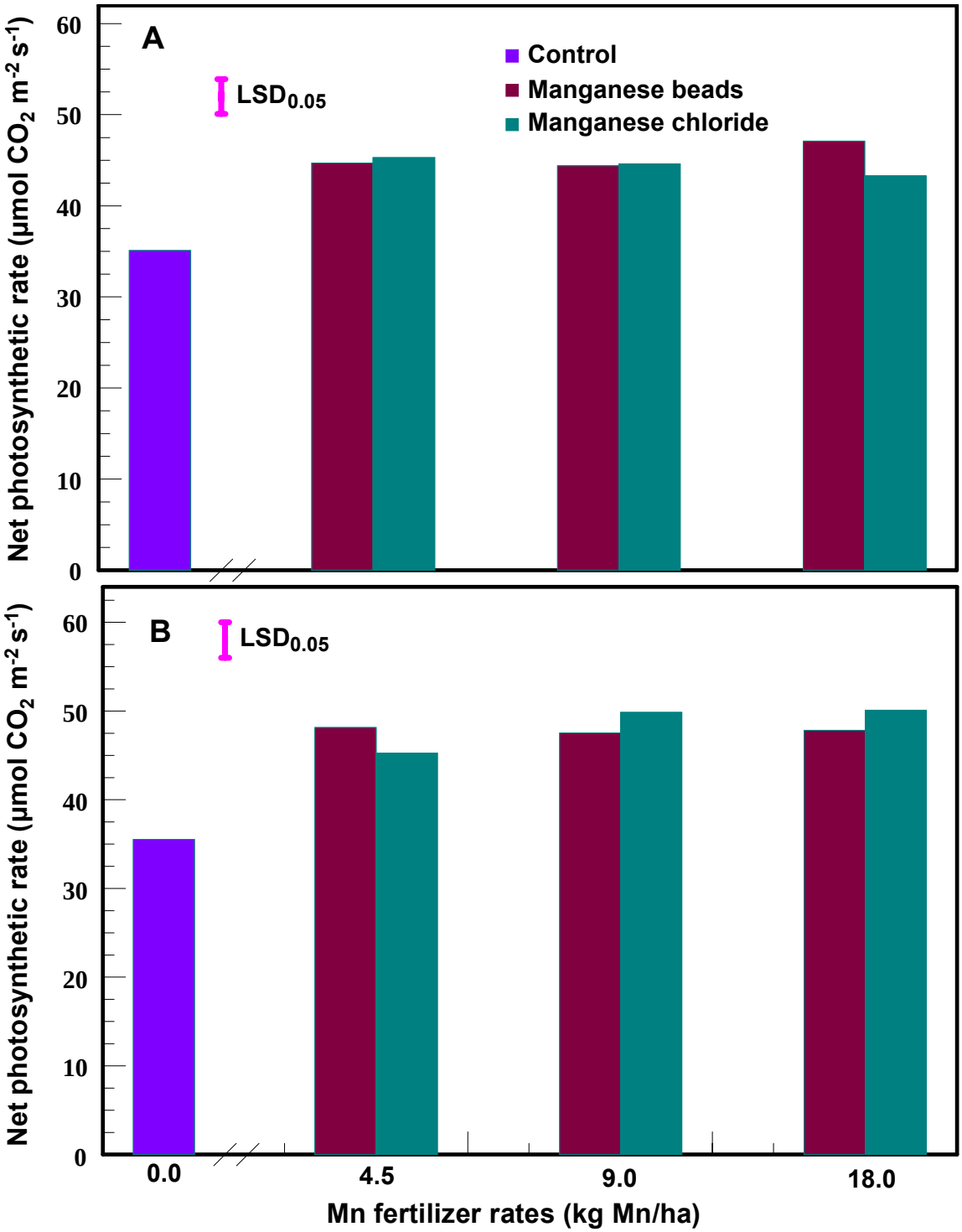


Figure 4-1. Leaf photosynthetic rate in field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

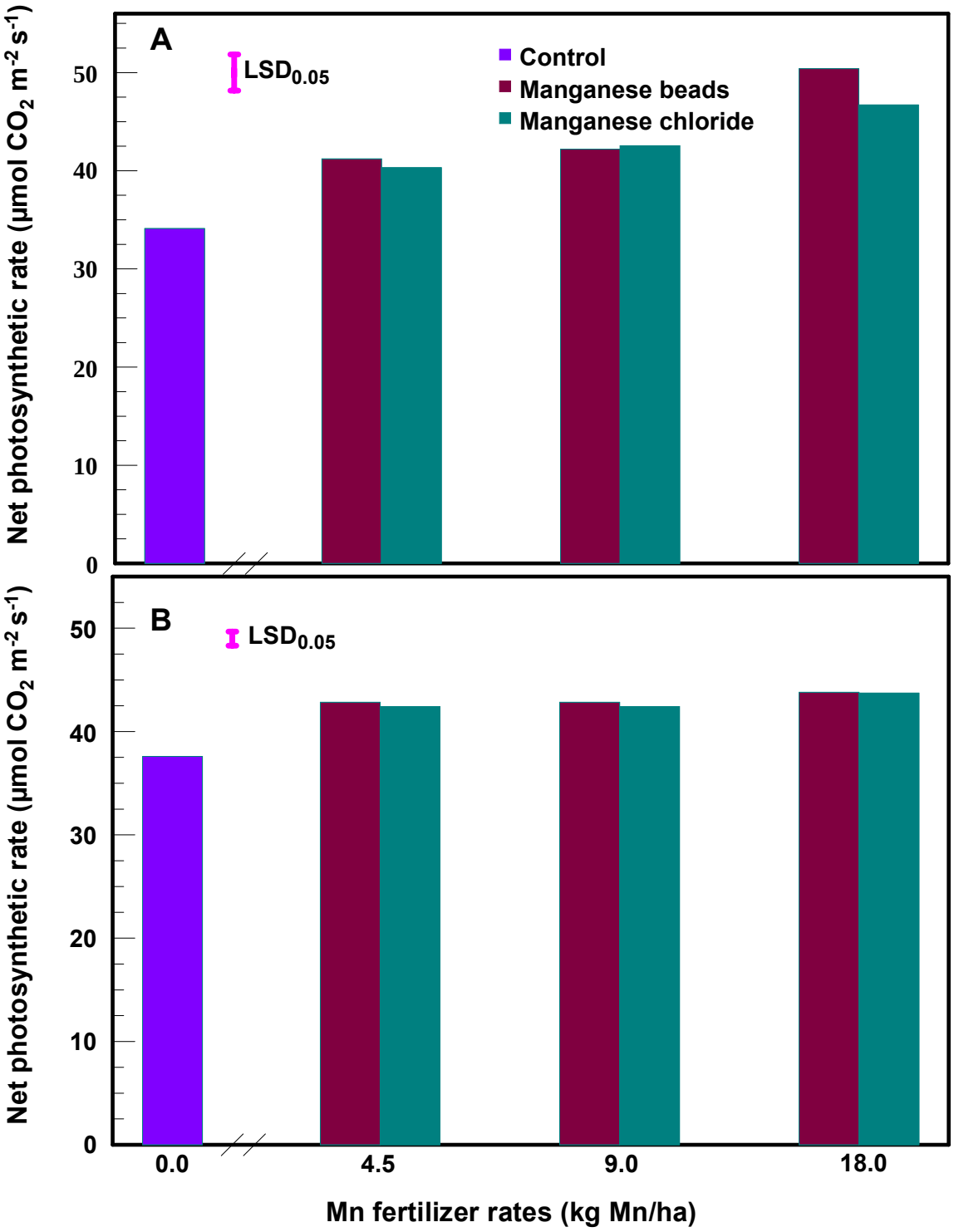


Figure 4-2. Leaf photosynthetic rate in field grown NAD-ME C-4 plant, purple amaranth, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

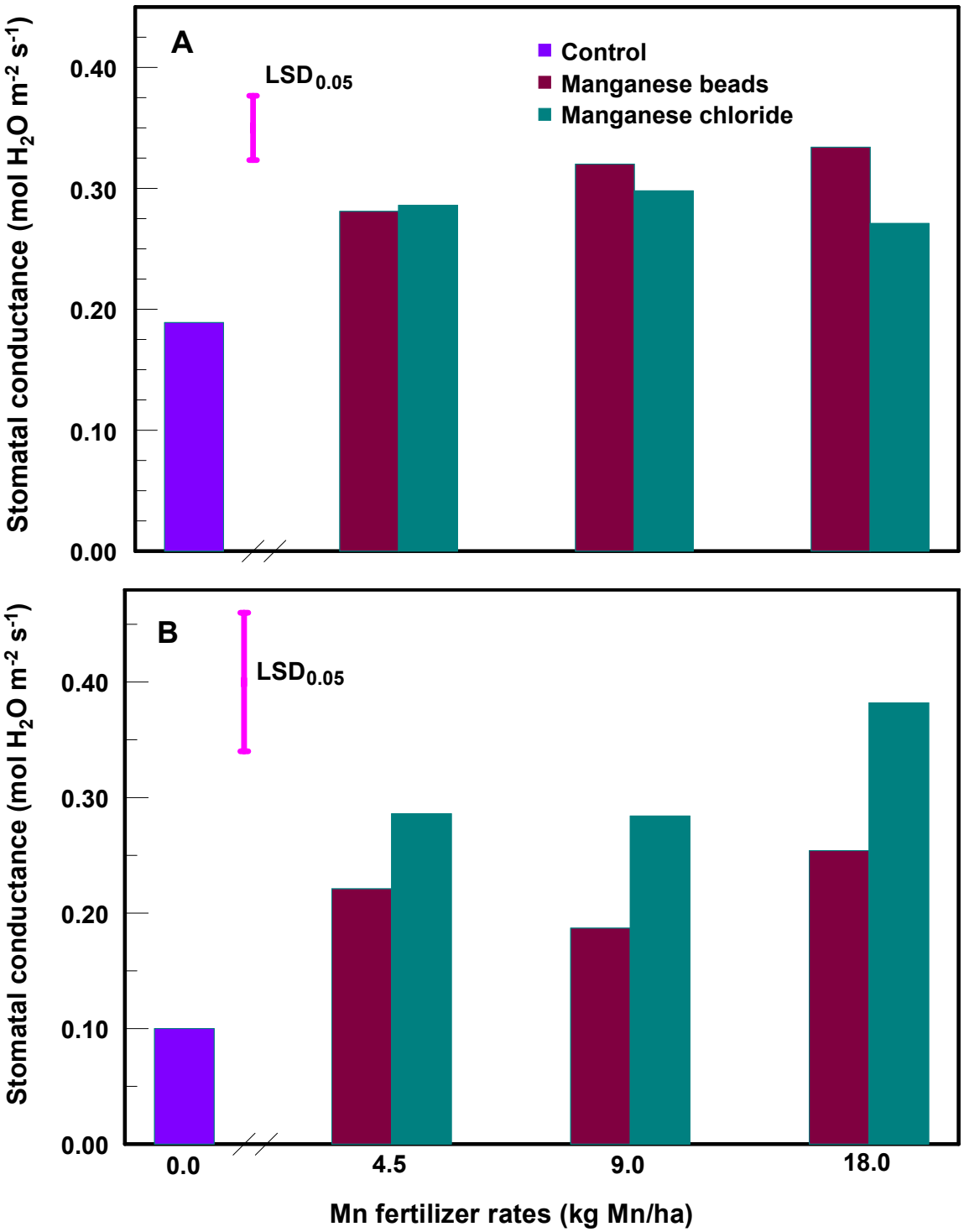


Figure 4-3. Leaf stomatal conductance of field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

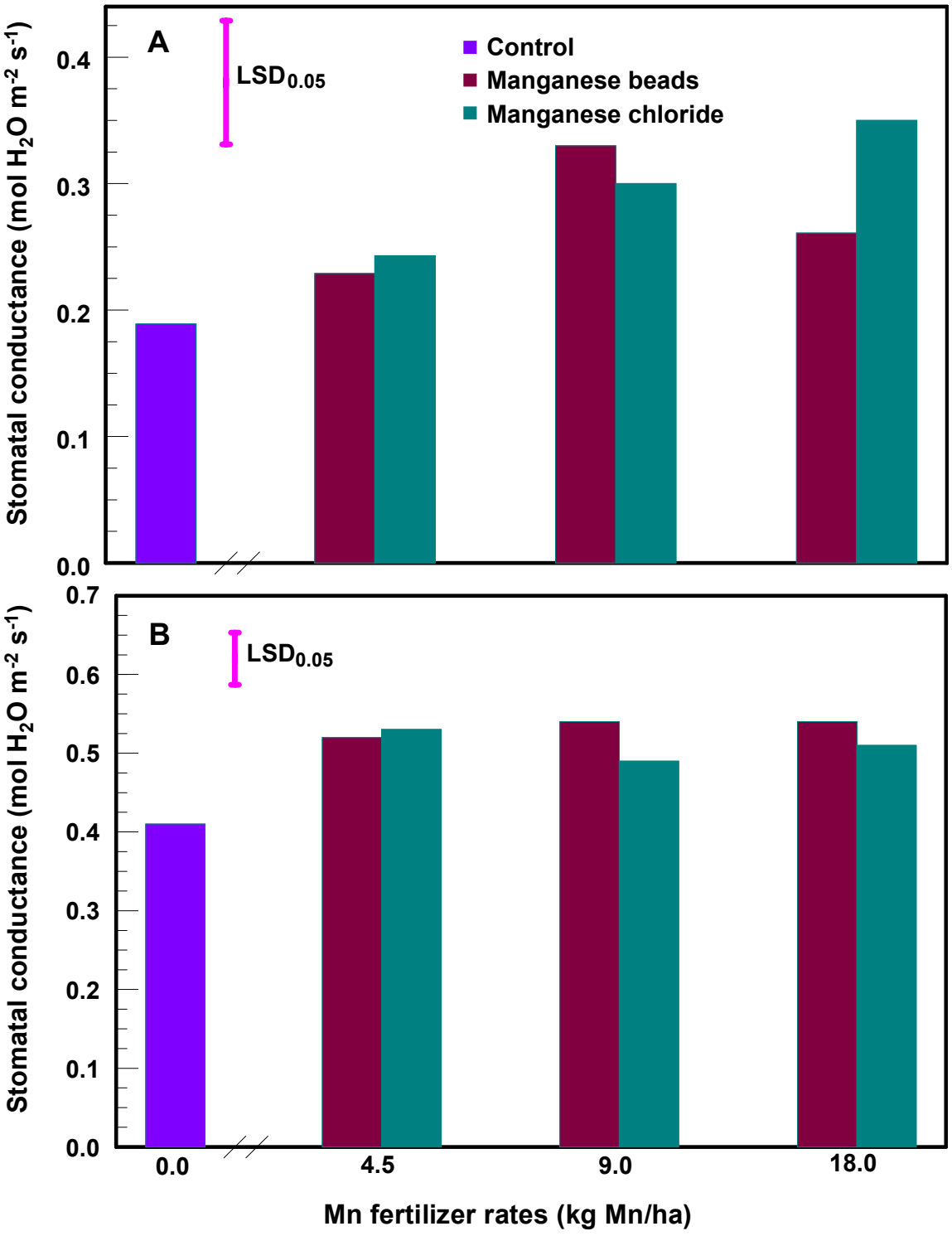


Figure 4-4. Leaf stomatal conductance of field grown NAD-ME C-4 plant, purple amaranth, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

Effect of Mn fertilization on leaf macro-and micronutrient element concentration

Leaf macronutrient concentrations

During both years of the study, leaf macronutrient concentrations in pearl millet and purple amaranth were not altered by Mn fertilization (Tables 4-2 A and B). It is interesting to note that amaranth leaf K, Mg, and Ca concentrations were about 2- to 3-fold higher than those of pearl millet (Tables 4-2 A and B).

Leaf micronutrient concentrations

Manganese

Manganese fertilization significantly increased leaf Mn concentration in pearl millet in the first year, but for both years there was a trend of increasing leaf Mn concentrations with higher rate of Mn fertilizer (Figure 4-5). A similar trend occurred for purple amaranth (Figure 4-6).

Molybdenum

In both years, application of Mn beads significantly reduced leaf Mo concentrations in both pearl millet and purple amaranth. There was very little impact of $MnCl_2$ on leaf Mo concentration (Figure 4-7 and 4-8).

Table 4-2 A. Leaf macronutrient concentrations of field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer. Values are means (n=6)

Mn source	Mn rates	Macronutrient elements							
		2006				2007			
		P	K	Mg	Ca	P	K	Mg	Ca
		%							
None	**0.0**	0.43a[Ŧ]	2.38a	0.28a	0.99a	0.47a	2.18a	0.27a	1.29ab
Manganese beads	**4.5**	0.44a	2.38a	0.30a	1.02a	0.45a	2.19a	0.27a	1.35a
	9.0	0.42a	2.26b	0.28a	0.98ab	0.47a	2.22a	0.28a	1.22b
	18.0	0.45a	2.33ab	0.28a	1.01a	0.46a	2.19a	0.29a	1.27ab
Manganese chloride	**4.5**	0.45a	2.33ab	0.27a	0.92b	0.45a	2.26a	0.28a	1.21b
	9.0	0.45a	2.32ab	0.28a	0.94b	0.47a	2.22a	0.28a	1.30ab
	18.0	0.45a	2.36a	0.28a	0.98ab	0.45a	2.23a	0.27a	1.20b

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Table 4-2 B. Leaf macronutrient concentrations of field grown NAD-ME C-4 plant, purple amaranth, treated with two sources and different rates of Mn fertilizer. Values are means (n=6).

Mn source	Mn rates	Macro-nutrient elements							
		2006				2007			
		P	K	Mg	Ca	P	K	Mg	Ca
		%							
None	0.0	0.29a[Ŧ]	3.40a	0.72a	3.11a	0.41ab	3.56a	0.59a	2.99a
Manganese beads	4.5	0.30a	3.44a	0.71a	3.11a	0.44a	3.58a	0.58a	2.91a
	9.0	0.29a	3.28a	0.69a	3.29a	0.40b	3.66a	0.57a	2.96a
	18.0	0.26a	3.30a	0.67a	3.04a	0.40b	3.40a	0.56a	2.97a
Manganese chloride	4.5	0.26a	3.42a	0.72a	3.43a	0.42ab	3.71a	0.56a	2.93a
	9.0	0.28a	3.48a	0.70a	3.45a	0.44a	3.65a	0.55a	2.88a
	18.0	0.29a	3.37a	0.71a	3.35a	0.41ab	3.57a	0.58a	2.89a

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

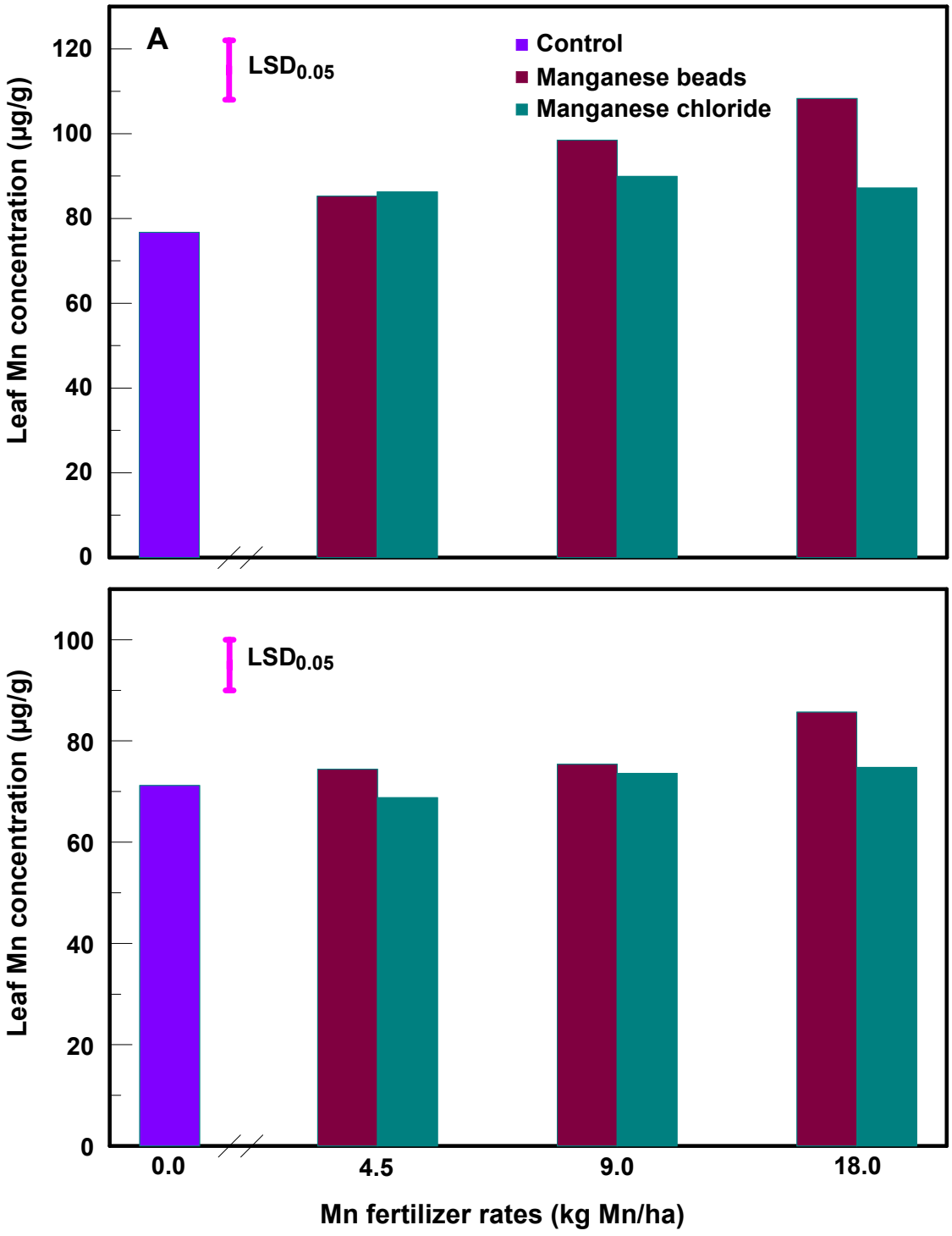


Figure 4-5. Leaf manganese concentration of field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

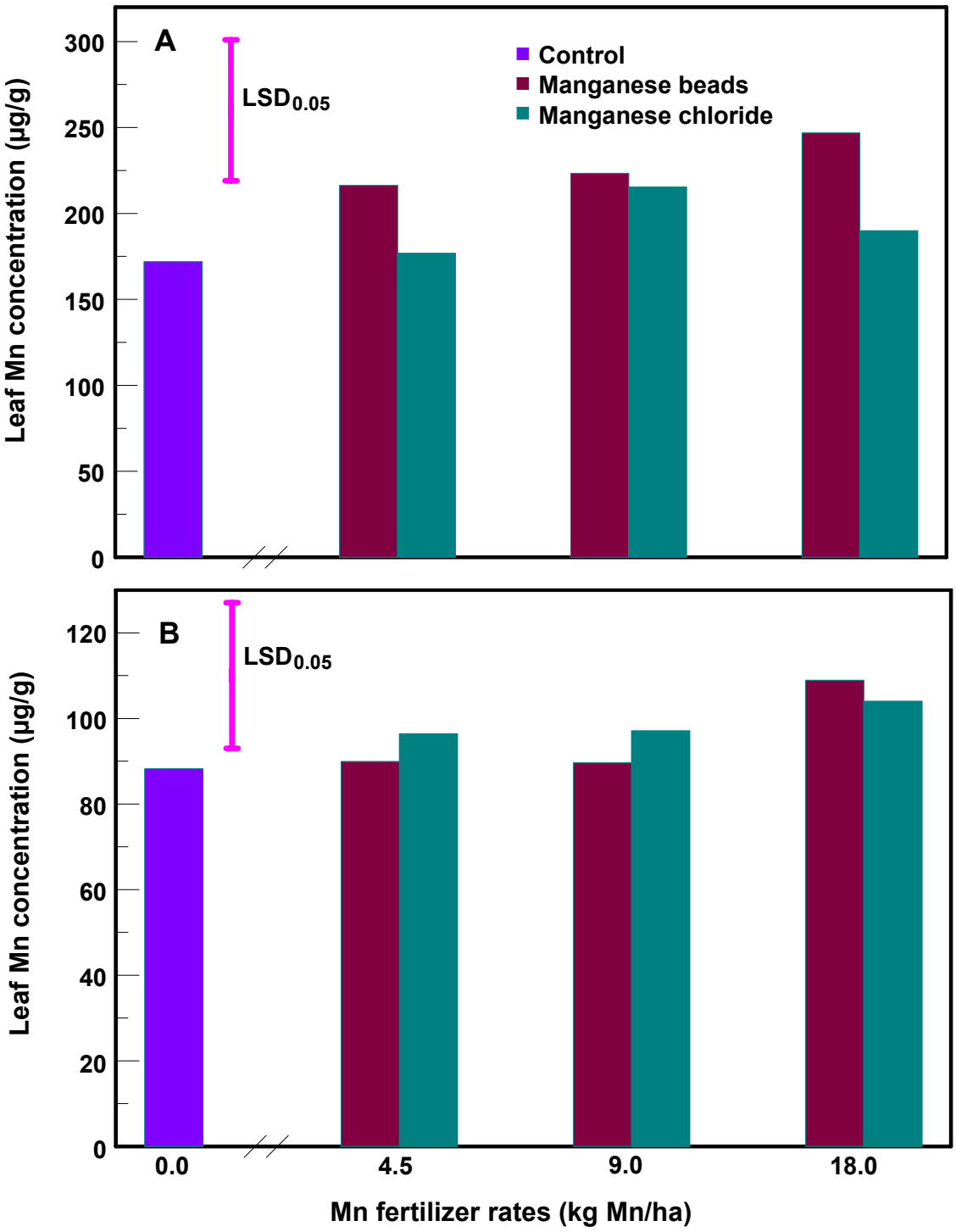


Figure 4-6. Leaf manganese concentration of field grown NAD-ME plant, purple amaranth, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

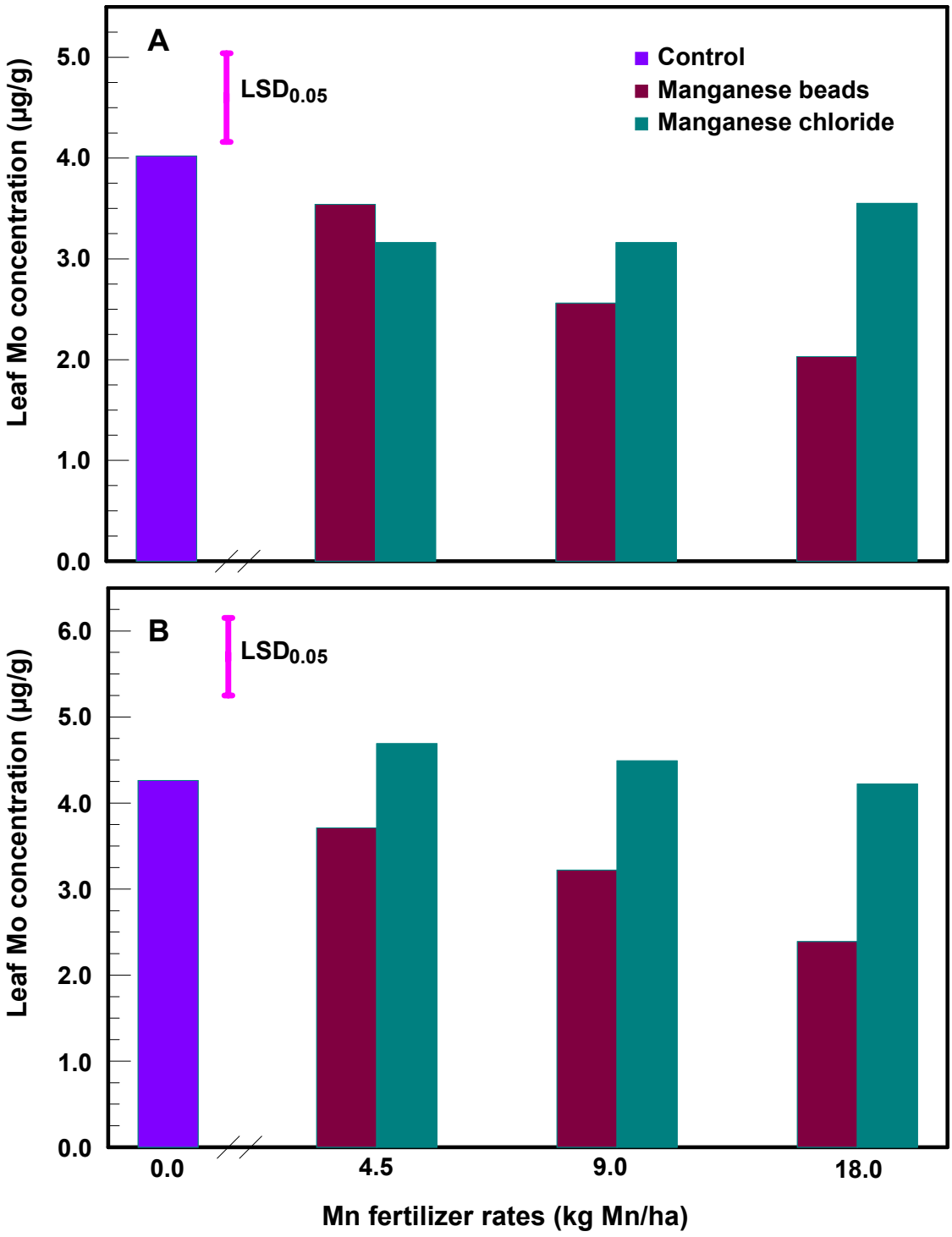


Figure 4-7. Leaf molybdenum concentration of field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

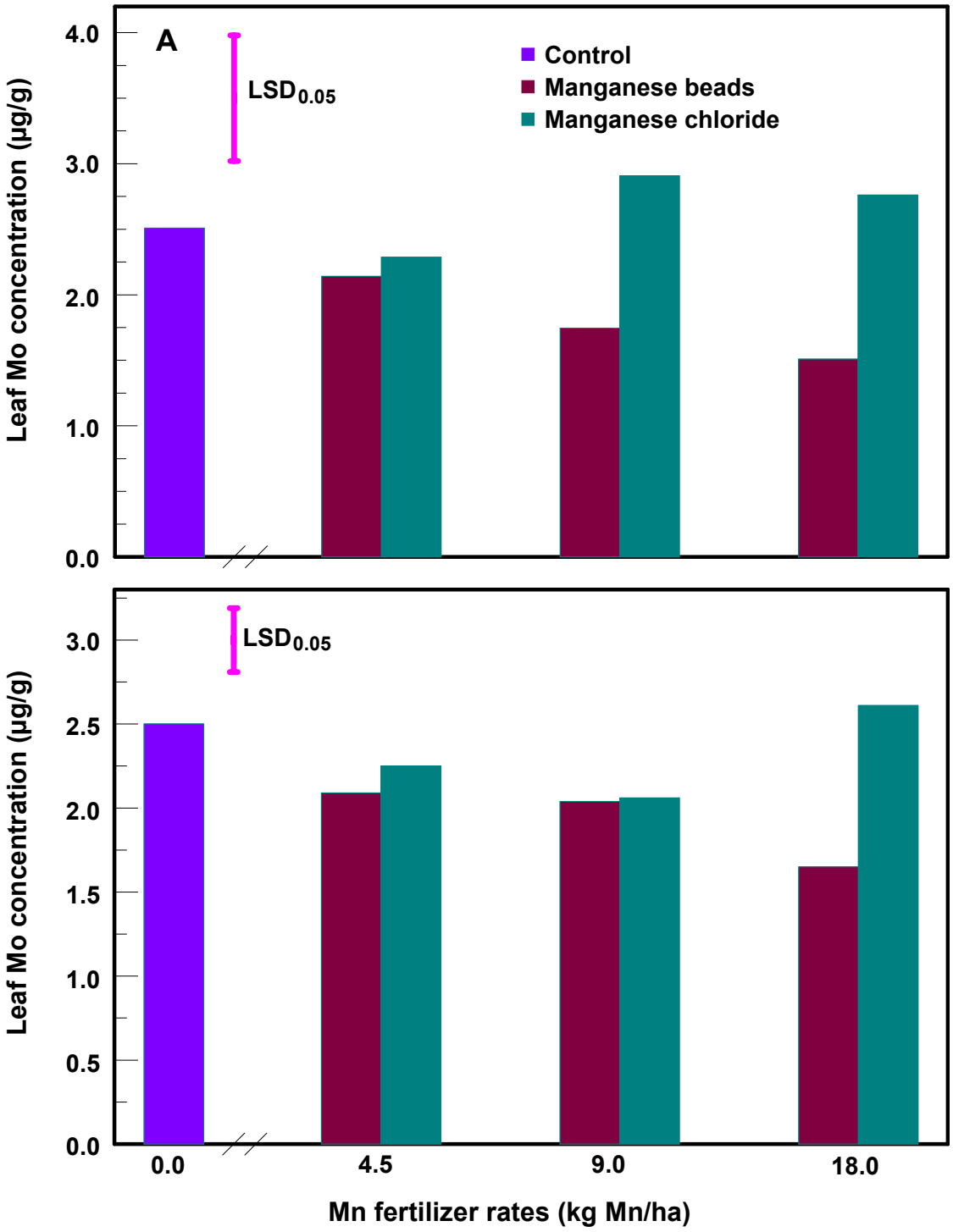


Figure 4-8. Leaf molybdenum concentration of field grown NAD-ME C-4 plant, purple amaranth, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

Other micronutrients

During both years of this study, pearl millet leaf Fe, Cu, and Zn concentrations did not change with Mn fertilization (Table 4-2 B). Similar results were seen for purple amaranth (Table 4-3 B). Among the micronutrients, Zn concentrations seemed comparable between the two species, but amaranth had relatively lower leaf Fe and Cu concentrations.

Seed yield

In the first year, both pearl millet and purple amaranth seed yield from plants receiving 9.0 kg Mn/ha was higher with both Mn sources than that from plants receiving no Mn (Table 4-4). Plants fertilized with 4.5 and 18.0 kg Mn/ha had similar seed yield to those not receiving Mn treatment.

In the second year, all Mn treated pearl millet plants, except for 4.5 kg Mn/ha as Mn beads, produced higher yields than those not receiving Mn (Table 4-4). However, Mn treatment did not show any effect on seed yield of purple amaranth.

Table 4-3 A. Leaf micronutrient concentrations of field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer. Values are means (n=6).

Mn source	Mn rates	Micronutrient elements							
		2006				2007			
		Fe	Zn	Cu	Na	Fe	Zn	Cu	Na
		(µg/g)							
None	**0.0**	165.0a[Ŧ]	54.0b	13.4b	46.4a	121.4a	53.0a	10.4a	46.7a
Manganese beads	**4.5**	169.8a	53.3b	13.9ab	46.8a	124.5a	49.3a	10.1a	44.7a
	9.0	166.7a	50.7b	13.8b	46.7a	117.3a	52.5a	9.9a	46.7a
	18.0	163.3a	62.6a	15.3a	45.7a	125.7a	54.8a	10.0a	44.5a
Manganese chloride	**4.5**	170.2a	49.8b	12.8b	45.6a	117.9a	48.0a	10.4a	45.6a
	9.0	160.8a	48.6b	13.3b	46.4a	122.8a	57.3a	10.4a	44.3a
	18.0	168.3a	48.5b	13.7ab	45.7a	118.2a	55.5a	9.8a	43.8a

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Table 4-3 B. Leaf micronutrient concentrations of field grown NAD-ME C-4 plant, purple amaranth, treated with two sources and different rates of Mn fertilizer. Values are means (n=6).

Mn source	Mn rates	Micronutrient elements							
		2006				2007			
		Fe	Zn	Cu	Na	Fe	Zn	Cu	Na
		(µg/g)							
None	**0.0**	92.4ab[Ŧ]	54.6a	6.4a	142.0a	89.8a	59.1ab	8.8a	48.1ab
Manganese beads	**4.5**	92.6ab	40.3a	6.1ab	140.0a	85.1a	46.2b	7.9b	40.0b
	9.0	86.4ab	48.5a	5.7b	157.0a	82.4a	56.2ab	8.0ab	42.1b
	18.0	78.9b	51.9a	5.8b	149.0a	94.7a	60.4ab	8.8a	52.6a
Manganese chloride	**4.5**	84.4ab	48.6a	5.6b	145.0a	86.6a	57.2ab	8.7a	42.9ab
	9.0	93.3ab	41.7a	6.51ab	144.0a	88.9a	59.9ab	8.4ab	45.6ab
	18.0	98.8a	42.1a	5.7b	151.0a	88.9a	70.0a	8.5ab	49.9ab

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Table 4-4. Effect of Mn fertilization on seed yield of field grown NAD-ME C-4 plants, pearl millet and purple amaranth grown in field plots in 2006 and 2007. Values are means (n=6).

Manganese source	Application rate kg Mn/ha	Seed yield (kg/ha)			
		Pearl Millet		Purple amaranth	
		2006	2007	2006	2007
None	0.0	2487c[Ŧ]	3995c	608d	290ab
Manganese beads	4.5	2787bc	4538bc	720c	207c
	9.0	3008a	5141ab	750bc	279ab
	18.0	2573bc	4400bc	657cd	257bc
Manganese chloride	4.5	2596bc	5151ab	551d	244bc
	9.0	2845ab	5764a	835ab	276ab
	18.0	2568bc	4985ab	895a	339a

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Discussion

The NAD-ME species, pearl millet and purple amaranth grown in a complete nutrient solution with different levels of Mn showed an increase in photosynthetic rate and other photosynthetic characteristics, that was correlated with the increase in Mn supply (Chapter 2 and 3). The results showed that Mn requirement for the optimum performance of these species was up 20-fold higher than that of C-3 and NADP-ME C-4 plants (Chapter 2 and 3). Plant available Mn was between 10-20 ppm during both growing season, a concentration considered low in some soils (Espinoza et al. 2007).

In this part of the study, pearl millet and purple amaranth were grown in the field and treated with increasing levels of Mn fertilizer in the form of Mn beads, or $MnCl_2$. Although the impact of Mn on field-grown plants was much smaller, there was a trend of increasing leaf Mn concentration with higher rate of Mn fertilizer in both pearl millet and purple amaranth for both years (Figure 4-5 and 4-6).

The lowest Mn fertilization level increased the photosynthetic rate by 25% in pearl millet and by 16% in purple amaranth, but no further increase was observed with higher Mn applications. Higher leaf Mn concentrations could contribute to the higher respective photosynthetic rates because of a possible high mitochondrial Mn. High Mitochondrial Mn would increase CO_2 release for Calvin cycle reactions, and increase photosynthesis due to its effects on the activity of NAD-ME.

With the exception of leaf Mo concentration, there was no impact of Mn fertilization on leaf macro- and micronutrient concentrations. A decrease in leaf Mo concentration was observed with Mn beads, but not with $MnCl_2$. The consistent reduction of leaf Mo concentration with Mn bead application during both years and for both species indicates a possible Mo adsorption by the beads. The Mo could even be binding to the sites freed by Mn release.

Only a small effect of Mn fertilization on seed yield was observed. However, in this study, the broadcast method of Mn application could have resulted in Mn oxidation at the soil surface. Poor incorporation of Mn into the soil in combination with Mn oxidation could have significantly reduced Mn availability for plant uptake. It has been reported that the response to Mn fertilization was better with drilled or side-band application than with surface broadcasting (Reuter et al. 1988, Crabtree 1999). Although ~2ppm plant available Mn in soil is considered sufficient in most soils (Buchholz et al. 1983), there are reports that concentration < 40 ppm are low and that in soil with pH >6.5 and plant available Mn < 20 ppm, deficiency symptoms are observed (Espinoza et al. 2007).

In both species, pearl millet and purple amaranth, there was a substantial difference in the seed yield between the two growing seasons. This, most likely, was a result of environmental conditions. While there were no major differences between the years in solar radiation, air temperature, or precipitation, the excessive winds in the first growing season (Appendix Figure 4-1 & 4-2) could have had a significant negative effect on pearl millet production. Furthermore, the pearl millet plants during the second year may have received more N, since the

plots were established in a field previously used for soybean. In purple amaranth, lower seed yield in the second year could be explained by lower plant density due to poor emergence, lodging, and,even more so, due to the seed loss caused by a weather related harvest delay.

Summary

The hypothesis tested in these experiments was whether the Mn response of pearl millet and purple amaranth observed in hydroponic experiments could be observed in field conditions. Plants were grown with varying Mn fertilizer rates. Two sources of Mn fertilizer Mn beads and $MnCl_2$ were compared. The plant available Mn in soil prior to treatments ranged from 10-20 ppm.

Plants receiving Mn fertilizer showed a trend of increasing leaf Mn concentrations with increasing Mn rates. Higher Mn concentrations in the leaves could have led to the observed 16-20% increase in photosynthetic rate with Mn fertilizer increment of 4.5 kg Mn/ha. No further increase in photosynthesis was observed at higher Mn fertilizer rate. Manganese fertilization had minimal effect on leaf macronutrient concentrations and except for Mo, had minimal effect on leaf micronutrient concentrations. Purple amaranth had generally greater leaf macronutrients K, Mg and Ca concentration than pearl millet. Field applied Mn had very small effect on seed yield of both pearl millet and purple amaranth.

Literature cited

Andrews DJ and Bramel-Cox P 1994 Breeding cultivars for sustainable crop production in low-input dryland agriculture in the tropics. In Buxton DA (Ed) International Crop Science. I. CSSA, Madison, WI, pp 211-222.

Andrews DJ, Hanna WW, Rajewski JF, and Collins VP 1996 Advances in grain pearl millet: Utilization and production research. In Janick J (Ed) Progress in New Crops. ASHS Press, Alexandria, VA, pp. 170-177.

Bressani R, Gonzales JM, Zuniga J, Breuner M and Elias LG 1987 Yield, selected chemical composition and nutritive value of 14 selections of amaranth grain representing four species. J. Sci. Food Agric. 38, 347–356.

Buchholz DD, Brown JR, Garrett JD, Hanson RG, and Wheaton HN 1983 Soil test interpretation and recommendations handbook. University of Missouri-college of Agriculture, Department of Agronomy.

Burton GW, Wallace AT and Rachie KO 1972 Chemical composition and nutritive value of Pearl millet [*Pennisetum typhoide* (Burm.) Staph and E. C. Hubbard] grain. Crop Sci. 12,187-188.

Crabtree WL 1999 Deep placement of Mn fertilizer on a sand soil increased grain yield and reduced split seed in *Lupinus angustifolium*. Plant and Soil 214, 9-14.

Ehleringer JR, Cerling TE, and Helliker BR 1997. C_4 photosynthesis, atmospheric CO_2, and climate. Oecologia 112, 285-299.

Ejeta G, Hassen MM and Mertz ET 1987 In vitro digestibility and amino acid composition of pearl millet (*Pennisetum typhoides*) and other cereals. Proc. Nat. Acad. Sci. (USA) 84, 6016-6019.

Espinoza L, Slaton N and Mozaffari M 2007 Understanding the numbers on your soil test report. University of Arkansas, Division of Agriculture, Cooperative Extension Service. FSA 2118.

Fagari 2002 Influence of micronutrients on dry matter yield and interaction with other nutrients in annual crops. Pesq.agropec.bras. 37 no. 12.

Haydon KD and Hobbs SE 1991 Nutrient digestibilities of soft winter wheat, improved triticale cultivars, and pearl millet for finishing pigs. J. Anim. Sci. 69, 719-725.

Hirel B, Le Gouis J, Ney B, and Gallais A 2007 The challenge of improving nitrogen use efficiency in crop plants: towards a more central role for genetic variability and quantitative genetics within integrated approaches. J. Exp. Bot. 58, 2369-2387.

Leakey ADB, Uribelarrea M, Ainsworth EA, Naidu SL, Rogers A, Ort DR, and Long SP 2006 Photosynthesis, productivity, and yield of maize are not affected by open-air elevation of CO_2 concentration in the absence of drought. Plant Physiol.140, 779-790.

Lizaso JI, Batchelor WD, Westgate ME, and Echarte L 2003 Enhancing the ability of CERES-maize to compute light capture. Agric. Sys. 76, 293-311.

Lehmann JW 1990 The potential of grain amaranth in the 1990s and beyond. Proc. 4th National Amaranth Symposium: Perspectives on Production, Processing, and Marketing. Minneapolis, MN. 23–25 Aug. 1990. Univ. Minn., St. Paul, MN, pp. 1–7.

Lloyd J and Farquhar GD 1996 The CO_2 dependence of photosynthesis, plant growth response to elevated atmospheric CO_2 concentration and their interaction with soil nutrient status. 1. General principles and forest ecosystems. Funct. Ecol. 10, 4-32.

Owensby CE, Coyne PI, Ham JH, Auen LM, and Knapp AK 1993 Biomass production in a tall grass prairie ecosystem exposed to ambient and elevated CO_2. Ecol. Appl. 3, 644-653.

Raun WR and Johnson GV 1999 Improving nitrogen use efficiency for cereal production. Agron. J. 91, 357-363.

Reich PB, Hobbie SE, Lee T, Ellsworth DS, West JB, Tilman D, Knops JMH, Naeem S, and Trost J 2006 Nitrogen constrains sustainability of ecosystem response to CO_2. Nature 440, 922-925.

Reuter DJ, Alston AM, and McFarlane JD 1988 Occurrence and correction of manganese deficiency in plants. In Graham RD, Hannam R, and Uren NC (Eds) Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht, Netherlands, pp 205-224.

Sage RF and Pearcy RW 1987 The nitrogen use efficiency of C_3 and C_4 plants. II. Leaf nitrogen effects on the gas exchange characteristics of *Chenopodium Album* (L.) and *Amaranthus retroflexus* (L.). Plant Physiol. 84, 959-963.

SAS Institute, 2004 The SAS system, version 9.1, SAS Institute Inc., Cary, NC, USA.

Senft J 1979 Protein quality of amaranth grain. Proc. 2nd Amaranth Conf., Rodale Research Center, Kutztown, PA. 13–14 Sept. 1979. Rodale Press, Emmaus, PA, pp. 43–47.

Singh DN and Prez-Maldonado R 2003 Pearl millet as an alternative feed grain for pigs and poultry. Asia Pac. J. Clin. Nutr. 12 Suppl S40.

Sleugh BB, Moore KJ, Brummer EC, Knapp AD, Rusells J, Gibson L 2001. Forage nutritive value of various Amaranth species at different harvest dates. Crop Sci. 41, 466-472.

Smith R L, Jensen LS, Hoveland CS and Hanna WW 1989. Use of pearl millet, sorghum, and triticale grain in broiler diets. J. Prod. Agr. 2, 78-82.

Stordahl JL, Sheaffer CC, and DiCostanzo A 1999 Variety and maturity affect amaranth forage yield and quality. J. Prod. Agric. 12, 249–253.

Tollennar M, Dwyer LM, Stewart DW and Ma BL 2000 Physiological parameters associated with differences in kernel set among maize hybrids. In Westergate M and Boote K (Eds) Physiology and Modeling Kernel Set in Maize. South Road Road. Madison Winsconsin, pp 115-130.

Triplett EW, Rolfe BG, Cocking EC Kennedy I, and Vanderleyden J 2007 A nitrogen story. Microbe 2, 372-373.

Yue SX, Sun HL, Chang BY, Chen ZP, and Zuo JW 1987 The nutritional composition of grain amaranth and its potential for utilization. Acta Agron. Sin. 13, 151–156.

CHAPTER 5

PEARL MILLET AND PURPLE AMARANTH SEED PROTEIN, OIL AND MINERAL ELEMENT CONTENT IN RESPONSE TO MANGANESE APPLICATION

Introduction

Pearl millet seed has high, up to 19% protein content (Singh 2004). Compared to corn, pearl millet seed has similar metabolizable energy (Abate and Gomez 1984, Fancher et al. 1987, Amato and Forrester 1995), but contains 40% more lysine and methionine, and 30% more threonine (Burton et al. 1972). It has been reported that chicken layers fed pearl millet had larger-sized and stronger-shelled eggs (Kumar et al. 1991), with higher omega-3-polyunsaturated fatty acids than those fed corn (Collins et al. 1995). Therefore, substituting pearl millet for corn could reduce the need for protein and amino acid supplements, lower the feed cost, and lead to production of healthier eggs. In another study, a weight gain of 8-22% at day 14, and a 50% reduction in mortality rate was observed when pearl millet feed was used for newly hatched bobwhite quail, compared to corn (Savage 1995). It has also been shown that swine given a feed containing 50 to 75% pearl millet reached market weight 10 days earlier than corn fed (Calder 1955). Because of its high nutritional value, pearl millet seed is a significant asset in poultry and non-ruminant production.

Purple amaranth seed also has high protein content, ranging between 13 and 19% (Stordahl et al. 1999, Lehamann 1990, Yue et al. 1987). Amaranth seed amino acid profile shows high lysine, cysteine, and methionine, the three essential amino acids in the diet of monogastric animals that are often deficient in other grains like corn, wheat, and rice (Senft 1979, Bressani et al. 1987). Amaranth seed is also superior because of its high Fe content, and amaranth

derived foods alleviated iron-deficiency (anemia) in young children (Whitteker and Ologunde 1990). Protein from animal protein sources is expensive, therefore amaranth may provide an alternative, lower cost plant protein source for humans.

Management practices, depending on soil and environmental conditions, may have different effects on seed quality and composition. For example, in white lupin, early planting and narrow rows resulted in higher yields of both protein and oil (Faluyi et al. 2000). In another study, N application increased seed protein without sacrificing yield in winter wheat (Subedi et al. 2007). Nitrogen application also increased total yield, total protein, and total oil in soybean (Wesley et al. 1998). Zinc and P applications increased seed yield, seed oil, and seed protein in cotton, and these increases were attributed to greater photosynthetic activity and more photosynthate mobilization to the bolls (Sawan et al 2001). It has been reported that plants under stress, with lower concentrations of photo-assimillates, preferentially store proteins in the seed, while higher photo-assimilate levels favor oil (Campbell and Nable 1988).

Fatty acid biosynthesis in plants occurs predominantly in plastids and it requires carbon in the form of acetyl CoA, ATP, and reducing compounds like NADH (Alonso et al. 2007). Because acetyl CoA cannot cross the plastid membrane, the precursors for acetyl CoA are synthesized inside the plastid or imported from the cytosol (Roughan et al. 1979, Weaire and Kekwick 1975). Malate and PEP, two important organic compounds in the C-4 photosynthetic pathway, were shown to support fatty acid biosynthesis in isolated plastids (Rawthorne 1996), and malate support was the highest of all compared carbon

sources (Pleite, 2005). Acetyl CoA carboxylase, a Mn-activated enzyme, incorporates CO_2 into acetyl CoA to form fatty acids precursors. Lower rates of photosynthesis found in Mn deficient plants could reduce fatty acid synthesis due to limited photosynthate supply (Campbell and Nable 1988).

Proper fertilization management could improve plant photosynthesis, increase photosynthate levels, and therefore influence the seed composition. In this study (Chapter 2 and 3), higher Mn supply increased leaf Mn concentrations in pearl millet and purple amaranth. Higher leaf Mn concentration could increase the activity of the Mn-activated decarboxylating enzyme, NAD-ME, and subsequently the photosynthetic rate and photosynthate production. The resulting higher photo-assimilate levels, as well as the higher Mn supply in the seed for the activation of fatty acid synthesizing enzymes, acetyl CoA carboxylase and phosphatidylinositol synthase, could affect seed protein and oil content.

To test this hypothesis, pearl millet and purple amaranth were grown in soil with pH 7.0 and plant available Mn less than 20 ppm, and supplied with different Mn fertilizer levels. Two sources of Mn fertilizer were utilized. Seeds were analyzed for seed protein and oil content, as well as for the concentration of mineral elements.

Materials and Methods

Experimental site, plant material and planting rate, crop management, and irrigation schedules were the same as in Chapter 4.

Experimental Site

The experiments were carried out at the Rollins Bottom Experiment Site located near the University Golf Course on Hinkson Creek on the University of Missouri-Columbia campus. The soil, a Moniteau silt loam, has 15% sand, 65% silt, and 20% clay. The chemical composition of the soil was analyzed by the University of Missouri Soil and Plant Testing Laboratory (Table 4-1). Soil samples were collected each year before the start of the experiment. The experiments were randomized complete block design with six replicates and seven Mn treatments. Each experimental unit was 0.0023 hectares (10 x 25 ft).

Planting

Pearl millet (*Pennisetum glaucum* (L.) R. Br.) hybrid cv. HGM 686 seed was purchased from Crosbyton Seed Company (Crosbyton, TX), and purple amaranth (*Amaranthus hypochondriacus* (L.) cv. plainsman) seed was purchased from Albert Lea Seed House (Albert Lea, MN). Seeds were planted in a prepared seed bed at seeding rates of 10 kg/ha (9 lbs/acre) for pearl millet and 4.5 kg/ha (4 lbs/acre) for purple amaranth. Seeding depth was approximately 1-2 cm and each experimental plot consisted of seven rows spaced 42 cm apart. Data were collected from the middle five rows in each plot. Initial planting was

done on June 6th and June 1st in 2006 and 2007, respectively. However, due to poor plant emergence, replanting of purple amaranth was done ~ 2 weeks later in both years. After planting, Mn from two different sources was applied to appropriate plots at rates of 0, 4.5, 9.0 and 18 kg Mn/ha (0, 4.0, 8.0 and 16 lb Mn/acre). The sources were conventional fertilizer, $MnCl_2$, and Mn fertilizer pellets supplied by Dr. Larry Sanders (Specialty Fertilizer Products, Kansas City, MO). Nitrogen fertilizer was applied each season at rates of 110 kg/ha (100 lb/acre) for both crops, three weeks after crop emergence.

Crop management

Weed and pest control

In both species, mechanical and chemical methods were used for weed control 2 weeks after plant emergence. In pearl millet, 2, 4 D Lo-V Ester (2,4 - dichlophenoxyacetic acid, ai 65.1%) was applied to kill broadleaved weed species. The application rate was 1.2 L /ha (½ pint/ acre) with a spray rate of 140 L/ha (15 gallons/acre). In amaranth, Select® herbicide was applied to kill grass weeds. Application rate was 1.2 L /ha (½ pint/ acre) with a spray rate of 140 L/ha. Tilling was done following chemical control to remove any weeds tolerant to the chemicals used. Subsequent weeds were controlled by either manual uprooting or with a tilling implement as the need arose. There were no major insect infestations, therefore, no insect control measures were undertaken. However, there was a late season bird infestation of pearl millet each year.

Irrigation

Prolonged dry periods with no or very little precipitation were experienced during both years. During the period of extreme moisture stress, plants were irrigated. In all cases, a single irrigation operation would supply water equivalent to approximately 25 mm (1 inch) of rainfall.

Seed harvesting

At maturity, when seed moisture content was approximately 12%, seeds were harvested by a combine. Seed were harvested from the middle five rows. A representative sub-sample was obtained, cleaned by removal of broken seed, seed husks, and other non-seed debris. Samples were ground and stored at 5^{o}C prior to macro- and micronutrient analysis, and protein and oil content determination.

Seed preparation for macro- and micronutrient analysis

Dried seeds were ground to a fine powder with a modified coffee grinder and the ground material was stored at 5 ^{0}C. For nutrient element analysis, 0.25 g sample of the ground material was placed in a 50 ml microwave digestion tube. Samples were digested in 10 ml of nitric acid solution (5 ml of double de-ionized water and 5 ml of concentrated nitric acid (6 N HNO_3)). Digestion was done with an accelerated microwave digestion system (MARSXPress by CEM, Matthews, NC). The digested material was brought to a volume with DDI H_2O, filtered, and stored at 5 ^{0}C, pending mineral element analysis by Inductively Couple Plasma

Absorption Emission Spectroscopy (ICP-AES, Varian, Inc. Palo Alto, Ca). Mineral elements analyzed were macronutrients P, K, Mg, and Ca and micronutrients Fe, Mn, Zn, Cu, Na, and Mo.

Protein and oil determination

0.5 g sample of ground seed material was used for crude oil and protein determination. Total crude fat was determined by acid hydrolysis (Method 954.02; AOAC, 2006). Total crude protein was determined by combustion analysis (Method 990.03; AOAC, 2006). Crude oil and protein analysis were done at Agricultural Experiment Station Chemical Laboratories, University of Missouri-Columbia.

Statistical analysis

Statistical analysis was conducted using PROC MIX program in SAS version 9.2 (SAS 2004). The effect of Mn treatment was tested in both species, separately for each year of study. Means with significant differences ($P<0.05$) were separated using Fisher's Protected Least Significant Difference.

RESULTS

Seed macro- and micronutrient concentrations

Macronutrients

Manganese fertilization had little effect on pearl millet seed macronutrient concentration. Only the highest Mn rate from the Mn bead source during the first year of experiment showed a significant difference in seed P, K, Mg, and Ca concentrations, as compared to unfertilized plots (Appendix Table 5-1). There was no response to Mn in the second year. In purple amaranth, Mn fertilization had no effect on seed macronutrients, except for K that was higher at 4.5 and 18 kg Mn/ha of Mn beads (Appendix Table 5-2).

Micronutrients

Manganese fertilization also had very little effect on seed micronutrients concentrations. There was no change in seed Mn even with the highest Mn applications (Table 5-1). However, in purple amaranth seed Mn concentration was much higher in the first than in the second year. In the first year of the experiment, there was, however, a significant increase in seed Fe concentrations, noted for both sources of Mn fertilizer (Appendix Table 5-3). Small increases in seed Na concentration were also found in pearl millet during the first growing season.

Seed protein

In the first year, Mn fertilization produced little effect change in pearl millet and purple amaranth seed protein content (Figure 5-1 and 5-2). The only significant increases were found with 9.0 kg Mn/ha as Mn beads for pearl millet, and with 4.5 kg Mn/ha of Mn beads in purple amaranth. In the second year, Mn fertilization of pearl millet had no effect on seed protein concentrations. However, the seed protein of pearl millet varied between the first and the second year of the experiment, and averaged ~9% and ~11%, respectively.

Seed oil

There was little effect of Mn fertilization on pearl millet or purple amaranth seed oil content (Figure 5-3 and 5-4). There was a trend for increased oil concentration with increased Mn application in the first year of Mn bead treatment, although the only significant difference was found between 0 and 18 kg Mn/ha application.

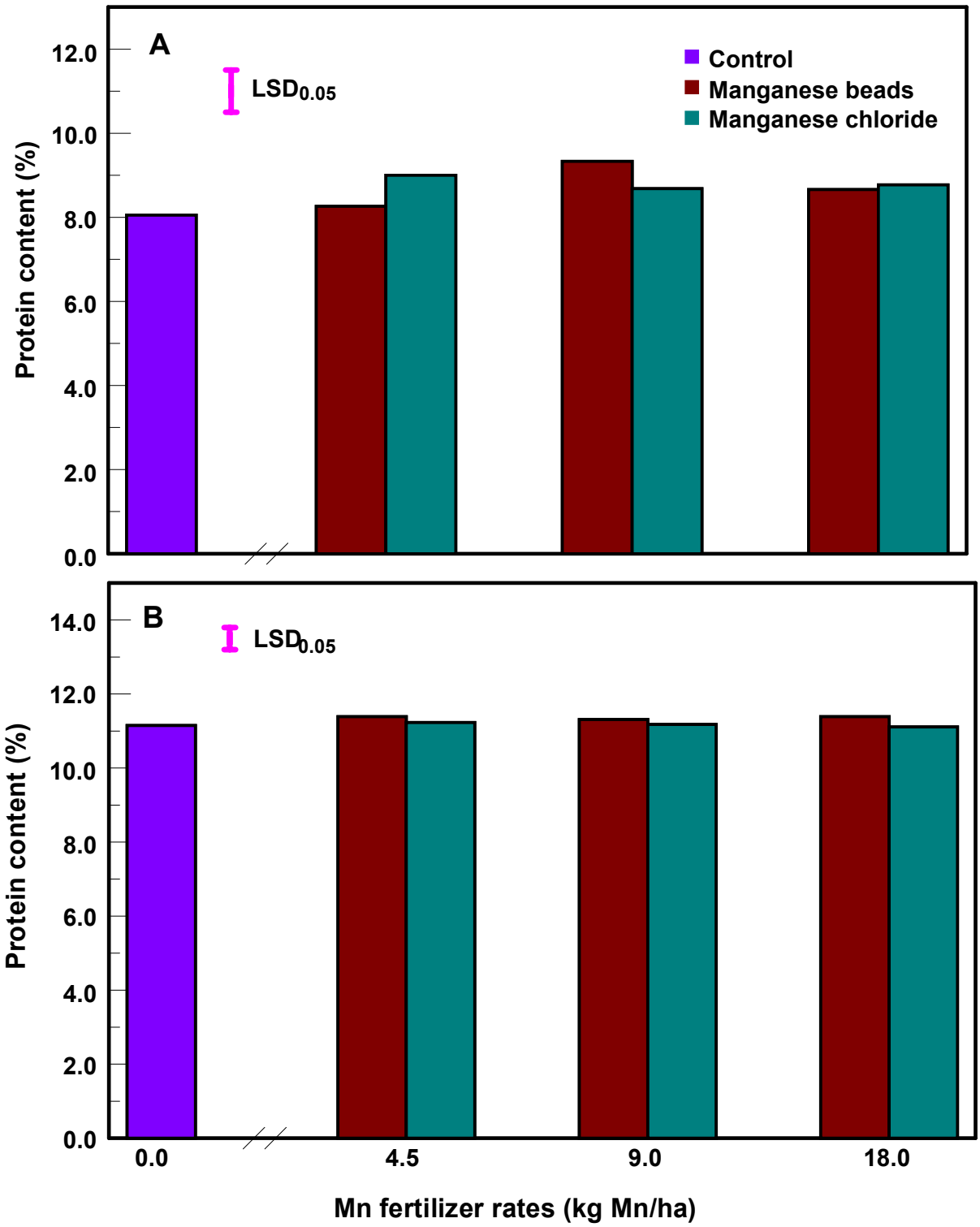


Figure 5-1. Seed protein content of field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

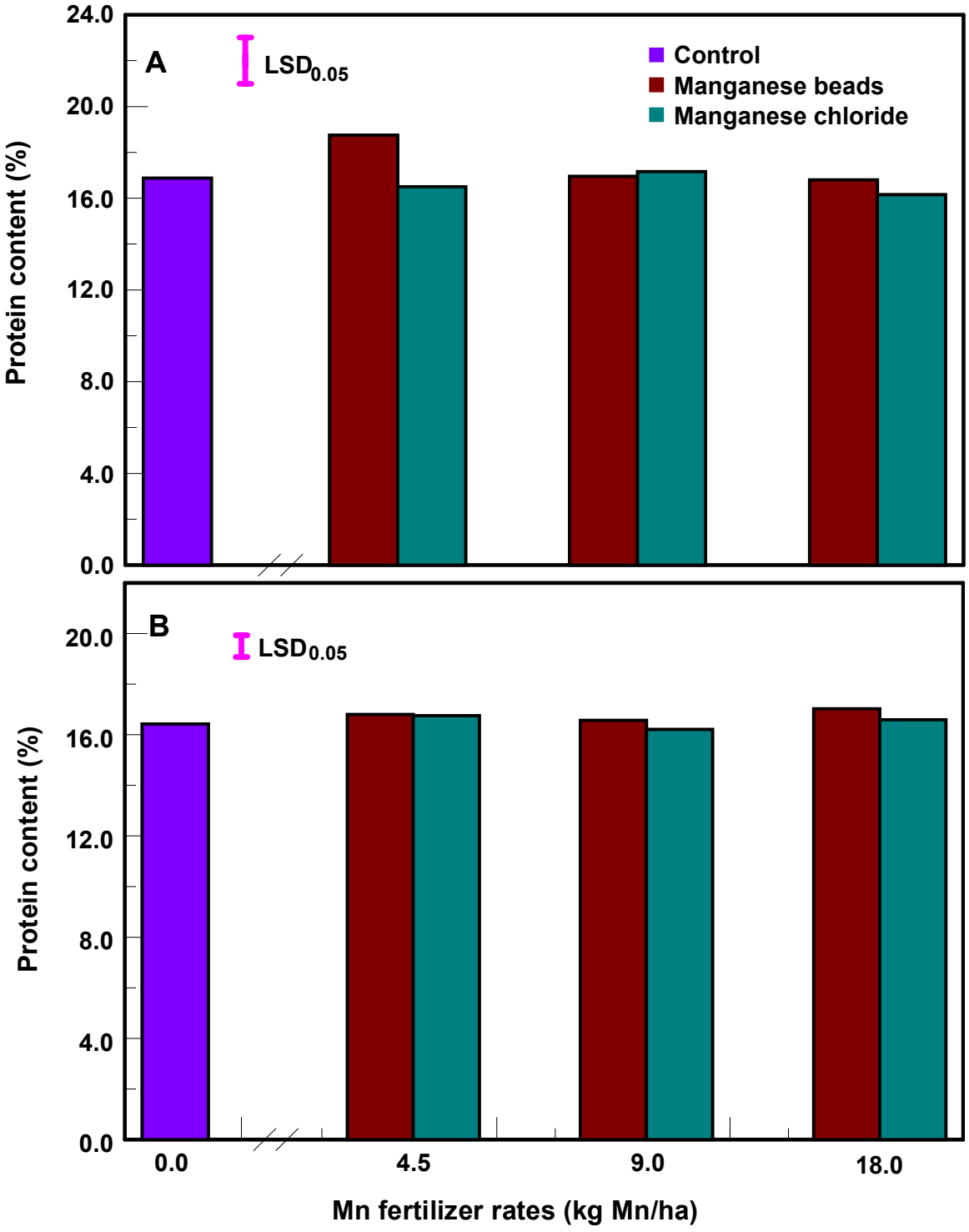


Figure 5-2. Seed protein content of field grown NAD-ME C-4 plant, purple amaranth, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

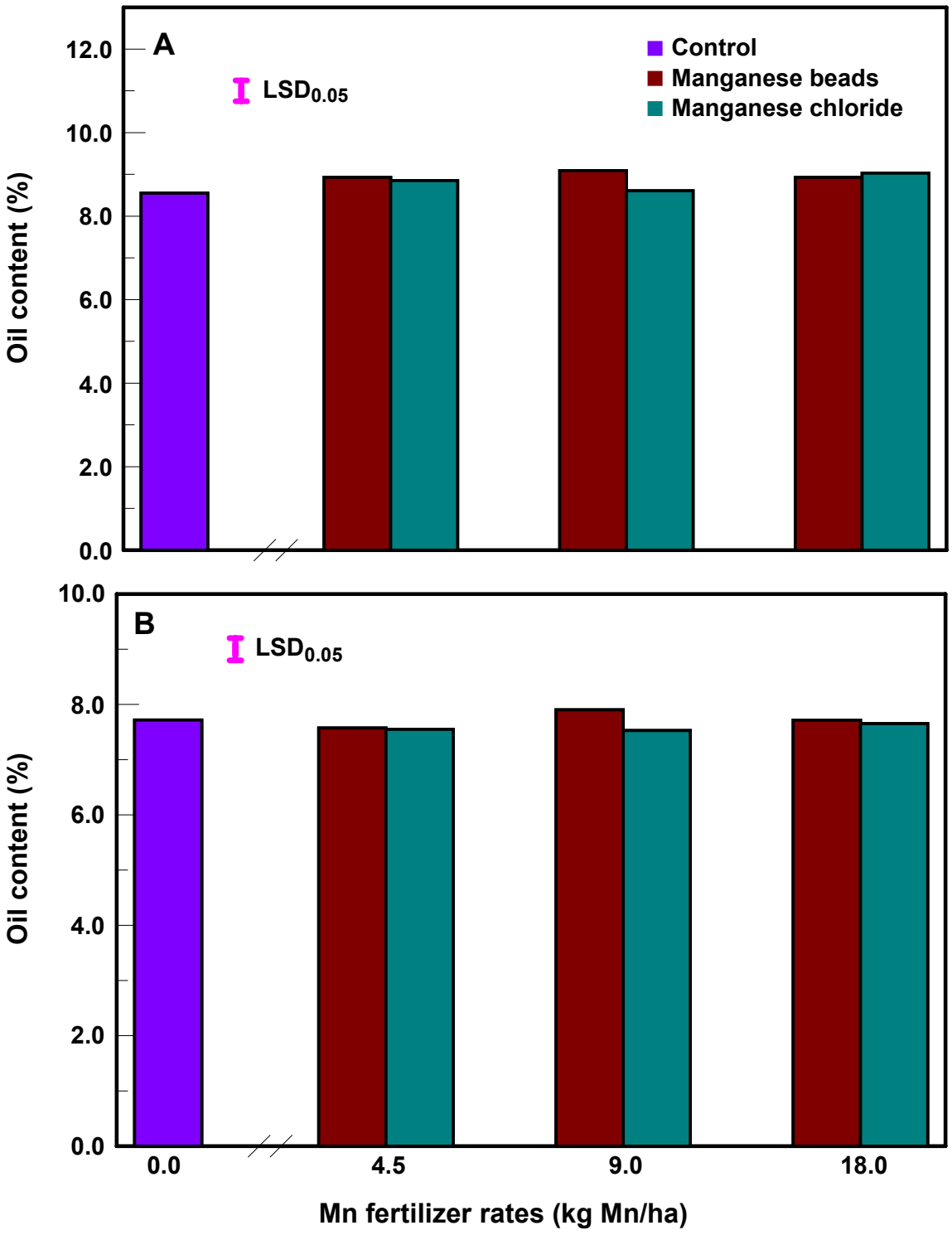


Figure 5-3. Seed oil content of field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

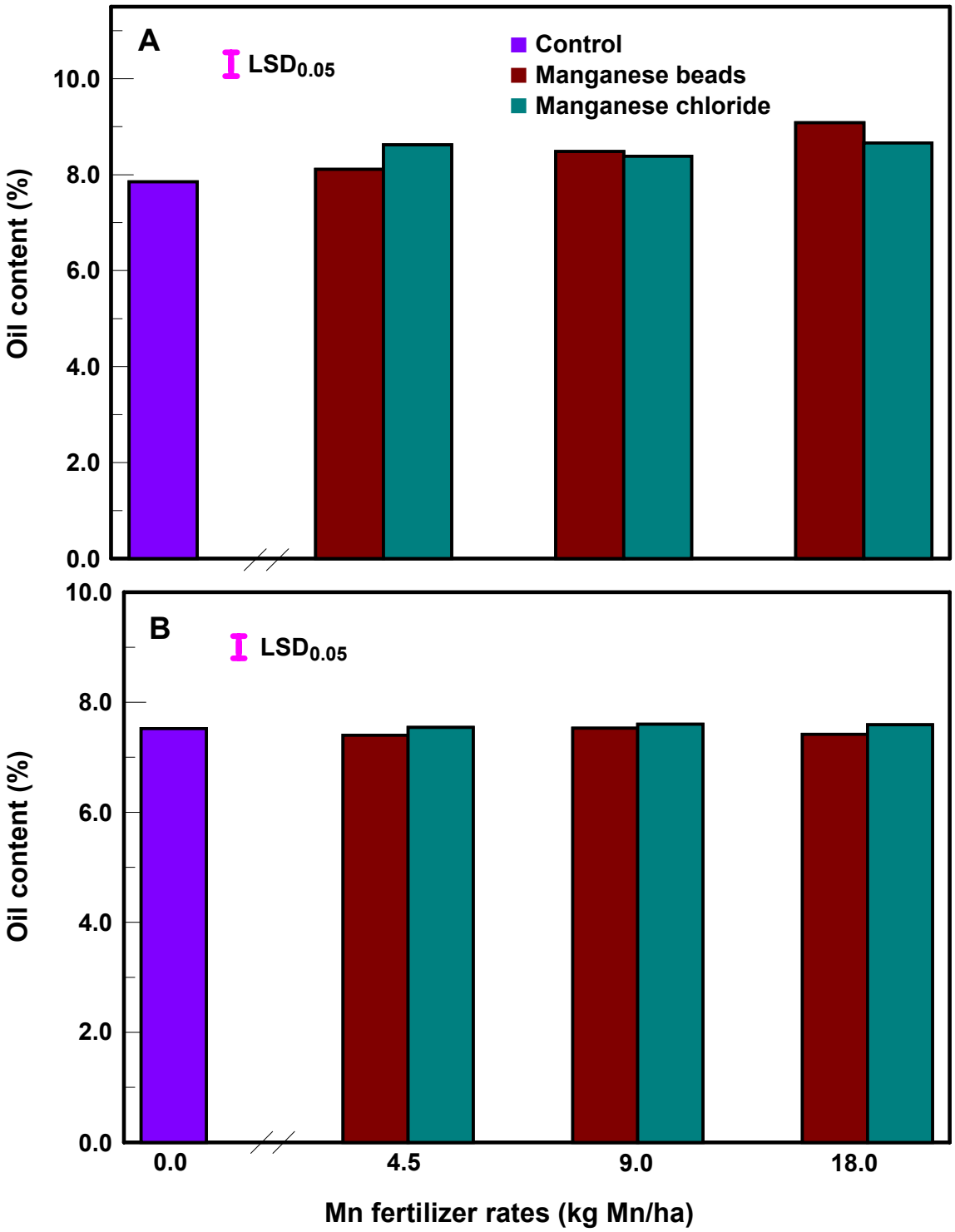


Figure 5-4. Seed oil content of field grown NAD-ME C-4 plant, purple amaranth, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are means (n=6).

Table 5-1. Seed Mn concentration of field grown NAD-ME C-4 plants, pearl millet, and purple amaranth, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are mean (n=6).

Mn source	Mn rates (kg/ha)	Seed Mn			
		Pearl millet		Purple amaranth	
		2006	2007	2006	2007
		(µg/g)			
None	0.0	11.1a	10.8a	56.2a	33.3a
Manganese beads	4.5	11.2a	10.9a	50.8a	34.2a
	9.0	10.8a	10.9a	58.1a	32.8a
	18.0	10.9a	11.1a	66.2a	38.2a
Manganese chloride	4.5	10.3a	11.1a	66.5a	34.1a
	9.0	11.1a	11.7a	58.3a	39.8a
	18.0	11.6a	11.5a	50.1a	36.2a

† Values in same column followed by a different letter are statistically different at $p < 0.05$.

Discussion

As reported in Chapters 2 and 3, increasing Mn rates in nutrient solutions resulted in progressively higher leaf Mn concentrations in hydroponically grown pearl millet and purple amaranth. The higher leaf Mn concentrations corresponded with higher photosynthetic rates and higher shoot dry weight (Chapters 2 and 3). The effect of Mn on the performance of these species was likely related to Mn-activation of NAD-ME, followed by a higher CO_2 release and assimilation.

Manganese fertilization of field-grown pearl millet and purple amaranth had smaller effect than did hydroponic experiments on leaf Mn concentrations, as well as on photosynthetic characteristics. Although there were no significant differences, a trend of increasing leaf Mn concentrations with increasing Mn application was observed (Chapter 4).

Different rates and forms of Mn fertilizer in the field had even less effect on pearl millet and purple amaranth seed composition. Seed levels of P, K, Mg, and Ca were comparable with the respective leaf concentrations and showed no effect from Mn fertilizer in either species. There was no change in seed Mn or other micronutrient concentrations, even with the highest Mn application. However, a significant increase in seed Fe concentrations was recorded in the first year for both sources of Mn fertilizer. However, purple amaranth seed Mn concentration was higher in the first than the second year of study.

The lack of change in seed Mn concentrations in pearl millet and purple amaranth with increased Mn fertilization could be related to different factors.

Based on many reports, Mn is not very mobile in phloem, a tissue that feeds developing seed (Pearson and Rengel 1994, Pearson et al. 1995, Rengel 2002, Page et al. 2006). In fact, it has been suggested that xylem could be the principal source of seed Mn (White et al. 1981, Grusak et al. 1999, Rengel 2002). Therefore, despite higher leaf Mn concentration in pearl millet and purple amaranth, seed Mn levels may remain unaffected due to low Mn remolization from the leaf.

It has also been reported that in some wheat cultivars, seed Mn concentration was positively correlated with plant height. A cross made between a short low seed Mn plant and a tall high seed Mn plant produced plants intermediate in both height and seed Mn content, indicating genetical control of Mn deposition in the seed (Singh and Bhartis 1985).

On the other hand, an 80-100-fold increase in seed Mn was found in white lupin grown in solution containing 9 mM Mn compared to 2.0 μM Mn (Hockings et al. 1977). Therefore, it is possible that seed Mn deposition is controlled by different mechanisms in monocots and dicots. This could be in agreement with the fact that seed Mn concentrations were the same in both growing seasons for the monocot, pearl millet, while they were different in the dicot, purple amaranth.

Manganese fertilization had very little effect on pearl millet and purple amaranth seed protein and oil content (Figure 5-1 and 5-2). However, the seed protein content in pearl millet varied between the first and second year of experiment and averaged 9 and 11%, respectively. This ~2% difference could have been a result of a higher plant N status, because in the second year pearl

millet was planted in an area previously used for soybean. Higher residual soil N, in addition to the 110 kg N/ha supplied, could have increased plant tissue N concentrations. At seed fill, leaf N could have been remobilized and used for seed protein synthesis.

Summary

The objective of this study was to determine the effect of Mn fertilization on seed composition of pearl millet and purple amaranth grown in the field.

In both species Mn application had little effect on seed macronutrient and micronutrient concentrations. Even with the highest Mn fertilizer rate, no change in seed Mn was observed. A difference in seed Mn concentrations was observed for purple amaranth between the two growing seasons. In general, purple amaranth had higher seed macronutrient concentration, especially for Ca which was 10-fold higher than for pearl millet.

Manganese fertilization had little effect on seed protein and oil contents of pearl millet and purple amaranth. The ~2% difference in seed protein content in pearl millet between the first and second year of experiment was attributed to the environmental effect.

Literature cited

Abate AN and Gomez M 1984. Substitution of finger millet (*Eleusine coracana*) and bulrush millet (*Pennisetum typhoides*) for maize in broiler feeds. Anim. Feed Sci. Tech. 10, 291-299.

Amato SV and Forrester RR 1995. Evaluation of pearl millet as a feed ingredient for broiler rations. In Teare ID (Ed.), Proc. 1st Nat. Grain Pearl Millet Symp. Univ. Georgia, Tifton, pp 125-128.

AOAC 2006 Official Methods of Analysis. 18th ed. Assoc. Offic. Anal. Chem., Washington, DC.

Alonso AP, Goffman FD, Ohlrogge JB, and Sachar-Hill Y 2007 Carbon conversion efficiency and central metabolic fluxes in developing sunflower (*Helianthus annuus* L.) embryos. Plant J. 52, 296-308.

Bressani R, Gonzales JM, Zuniga J, Breuner M and Elias LG 1987 Yield, selected chemical composition and nutritive value of 14 selections of amaranth grain representing four species. J. Sci. Food Agric. 38, 347–356.

Calder A 1955. Value of munga (pearl millet) for pig feeding. Rhod. Agr. J. 52, 161-170.

Campbell LC and Nable RO 1988 Physiological functions of manganese in plants. In Graham RD, Hannam R, and Uren NC (Eds) Manganese in Soils and Plants. Kluwer Academic Publishers, Dordrecht, Netherlands, USA, pp 139-154.

Collins VP, Cantor AH, Pescatore AJ, Straw MI and Ford MJ 1995 Effect of pearl millet in laying hen diets on egg fatty acids. Poultry Sci. 74 (supp. 1) 4

Faluyi MA. Zhou XM, Zhang F, Leibovitch S, Migner P and Smith DL 2000. Seed quality of sweet white lupin (*Lupinus albus*) and management practice in eastern Canada. Euro. J. Agron. 13, 27-37.

Fancher BI, Jensen LS, Smith RL, and Hanna WW 1987. Metabolizable energy content of pearl millet. Poultry Sci. 66:1693-1696.

Grusak M, Pearson JN, and Eduardo M 1999 Physiology of micronutrient homeostasis in field crops. Field Crop Res. 60, 41-56.

Hocking PJ, Pate JS, Wee SC, and McComb AJ 1977 Manganese nutrition of Lupinus spp. Especially in relation to developing seeds. Annals of Bot. 41, 677-688.

Kumar AM, Reddy VR, Reddy PV, and Reddy RS 1991. Utilization of pearl millet (*Pennisetum typhoides*) for egg production. Poultry Sci. 32, 463-469.

Lehmann JW 1990 The potential of grain amaranth in the 1990s and beyond. Proc. 4th National Amaranth Symposium: Perspectives on Production, Processing, and Marketing. Minneapolis, MN. 23–25 Aug. 1990. Univ. Minn., St. Paul, MN, pp. 1–7.

Page V, Le Bayon Renée-Chlairen, and Feller U 2006 Partitioning of zinc, Cadmium, manganese and cobalt in wheat (*Triticum aestivum*) and lupin (*Lupinus albus*) and further release into the soil. Environ. and Exp. Bot. 589, 269-278.

Pearson JN and Rengel Z 1994 Distribution and remobilization of Zn and Mn during grain development in wheat. J. Exp. Bot. 45, 1829-1835.

Pearson JN, Rengel Z, Jenner CF, Graham RD 1995 Transport of zinc and manganese to developing wheat grains. Physiologia Plantarum 95, 449–455.

Pleite R, Pike MJ, Garces R, Martinez-Forces E, and Rawsthorne S 2005 The sources of carbon and reducing power for fatty acid synthesis in the heterothropic plastids of developing sunflower (*Helianthus annuus* L.) embryos. J. Exp. Bot. 56, 1297-1303.

Savage S 1995 Quail performance on pearl millet grain. In Teare ID (Ed.), Proc. 1st Nat. Grain Pearl Millet Symp., Univ. Georgia, Tifton, pp 121-123.

Sawan ZM, Hafez SA, and Basyony AE 2001 Effect of phosphorus fertilization and foliar application of chelated zinc and calcium on seed, protein and oil yields and oil properties of cotton. J. Agri. Sci. 136, 191-198.

Senft J 1979 Protein quality of amaranth grain Proc. 2nd Amaranth Conf., Rodale Research Center, Kutztown, PA. 13–14 Sept. 1979. Rodale Press, Emmaus, PA, pp. 43–47.

Singh DK and Bhartis S 1985 Seed manganese content and its relationship with the growth characteristics of wheat cultivars. New Phytol. 101, 307-391.

Subedi KD, Ma BL and Xue AG 2007 Planting date and nitrogen effects on grain yield and protein content of spring wheat. Crop Sci. 47, 36-44.

Stordahl JL, Sheaffer CC, and DiCostanzo A 1999 Variety and maturity affect amaranth forage yield and quality. J. Prod. Agric. 12, 249–253.

Rengel Z 2002 Xylem and phloem transport of micronutrients. In Horst W, Schenk M, Bürkert A, Claassen N, Flessa H, Frommer W, Goldbach H, Olfs H, Römheld V, Sattelmacher B, Schmidhalter U, Schubert S, Wirén NV, and Wittenmayer L (Eds) Plant Nutrition. Food Security and Sustainability of Agro-ecosystems through Basic and Applied Research. Springer Publisher, Netherlands, pp 628-629.

Roughan PG, Holland R, and Slack CR 1979 Control of long-chain fatty acid synthesis in isolated intact spinach (*Spinacia oleracea* L.) chloroplasts. Biochem. J. 184, 193-202.

WesleyTL, Lamond RM, Martin VL, and Duncan RS 1998 Effects of late-season nitrogen fertilizer on irrigated soybean yield and composition. J. Prod. Agric. 11, 331–336.

Weaire PJ and Kekwick RGO 1975 Fractionation of fatty-acid synthetase activities of avocado mesocarp plastids. Biochem. J. 146, 439-445.

White M, Decker AM, and Chaney RL 1981. Metal complexation in the xylem fluid. I. Chemical composition of tomato and soybean stem exudates. Plant Physiol. 67, 292–300.

Whitteker P and Ologunde O 1990 Study of iron bioavailability in native nitrogen grain amaranth cereal for young children using a rat model. Cereal Chem. 67, 505-508.

Yue SX, Sun HL, Chang BY, Chen ZP, and Zuo JW 1987 The nutritional composition of grain amaranth and its potential for utilization. Acta Agron. Sin. 13, 151–156.

CHAPTER 6

SUMMARY AND PERSPECTIVE

Despite the wealth of evidence for the critical role of Mn in photosynthesis of C-4 plants, there is a lack of information on their Mn requirements, as well as an absence of specific Mn recommendations for their cultivation. The objective of this study was to establish and compare the Mn requirements for optimum performance of NAD-ME C-4 species pearl millet and purple amaranth, C-3 species wheat and squash, and NADP-ME C-4 species corn and sorghum.

The results demonstrate that optimal Mn concentrations required for maximum growth of NAD-ME C-4 species were up to 20-fold higher than those supporting maximum growth of C-3 and NADP-ME C-4 plants, and range between 50 and 100 μM. Although shoot tissue Mn concentrations increased in parallel with Mn concentration in nutrient solution, the higher leaf Mn presence correlated with a large, up to 100% increase in photosynthetic rate only in NAD-ME C-4 pearl millet and purple amaranth, while it had no effect on C-3 and NADP-ME C-4 species. The highest photosynthetic rate across a 0-1000 umol quanta m^{-2} s^{-1} PPFD range was recorded for pearl millet supplied with 50 μM Mn and purple amaranth supplied with 100 μM Mn, while in C-3 and NADP-ME C-4 species it was recorded at plants supplied with 2-5 μM Mn. The photosynthetic rate at saturated PPFD was higher in NAD-ME C-4 plants receiving high Mn, while in C-3 and NADP-ME C-4 species it maximized with 2-5 μM Mn. In contrast to growth and photosynthetic characteristics, Mn had no effect on NAD-ME C-4 plants leaf stomatal conductance.

I proposed that the higher photosynthetic rates and dry matter production with higher Mn treatment of NAD-ME C-4 plants could be a result of increased

activation of the Mn-dependent NAD-ME in mitochondria of BSC resulting in a higher CO_2 supply for Calvin cycle reactions. This explanation is supported by the finding that the higher photosynthetic rate with higher Mn nutrition occurred without a corresponding increase in stomatal conductance, indicating that the higher CO_2 supply was most likely due to fast malate decarboxylation that maintained a steep CO_2 concentration gradient between the atmosphere and the intercellular space.

The impact of Mn fertilization on field grown pearl millet and purple amaranth was smaller than that in hydroponic culture. There was only a trend of increasing leaf Mn concentration with higher rate of Mn fertilizer, and this trend was observed in both species for both growing seasons. The lowest field Mn application increased the photosynthetic rate by 25% in pearl millet and by 16% in purple amaranth, but no further increase was observed with higher Mn fertilization. Only a small effect of Mn was observed on seed yield and seed composition.

The smaller impact of Mn fertilizer in the field could be, at least in part, due to the broadcast method of Mn application, which could result in Mn oxidation on the soil surface. A poor incorporation of Mn into the soil in combination with Mn oxidation could significantly reduce Mn availability for plant up take.

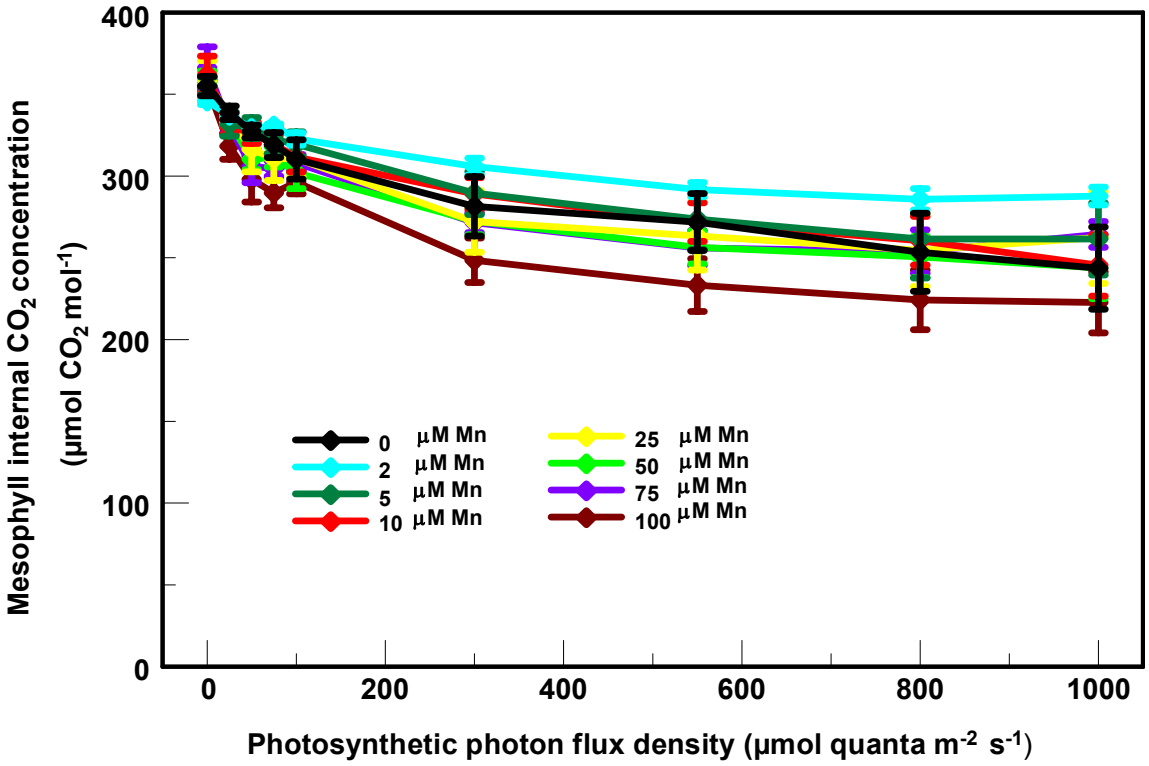
This is, to my knowledge, the first information on the substantial, 20-fold higher Mn requirements for optimum performance of NAD-ME C-4 plants, compared to other plant species. This finding should be considered in future research, as well as in future cultivation of NAD-ME C-4 crops. This dissertation

is just the beginning. As more information is collected about the NAD-ME C-4 plants biochemistry, physiology, and structure, the guidance it can provide should lead us to improve recommendations for specific nutrient requirements and specific Mn management strategies.

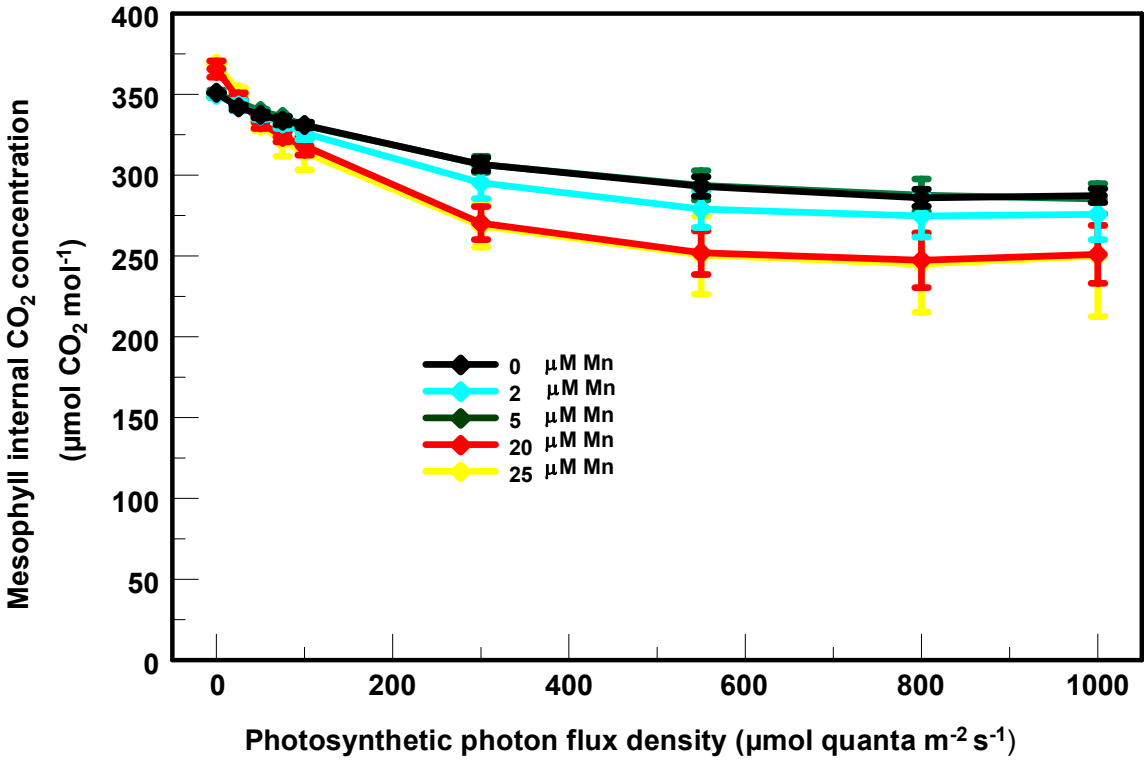
APPENDICES

Appendix A

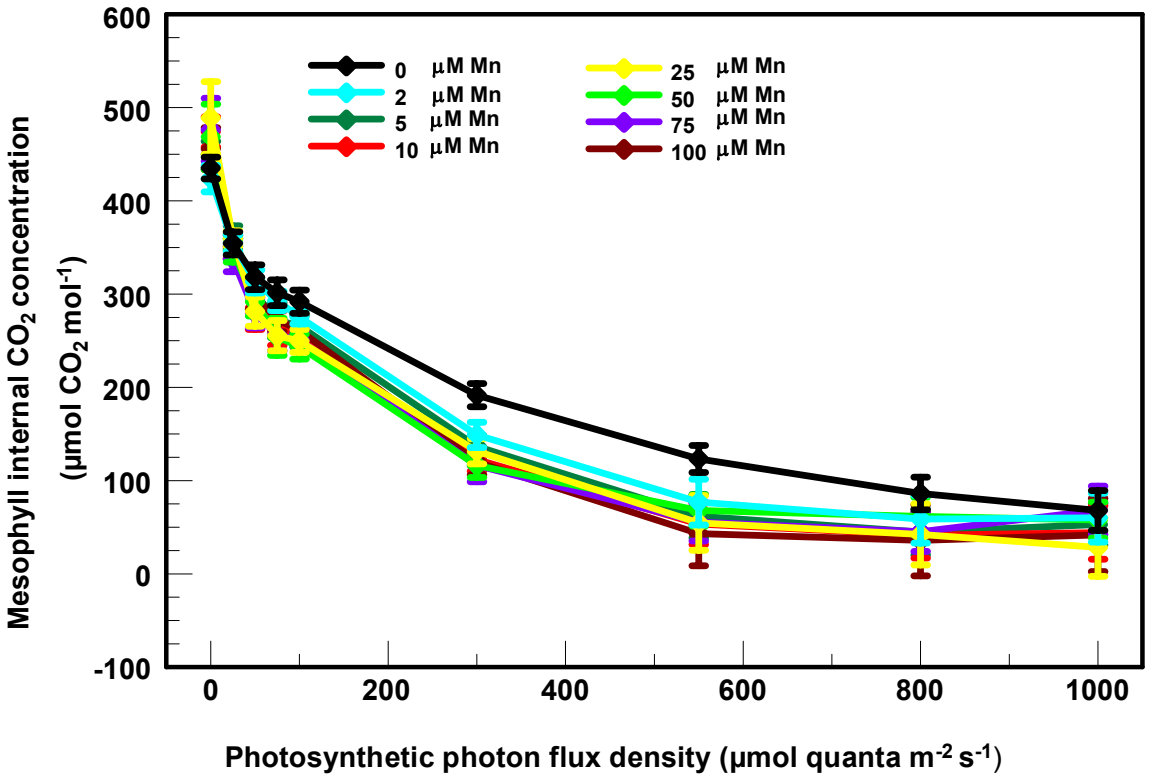
Figures



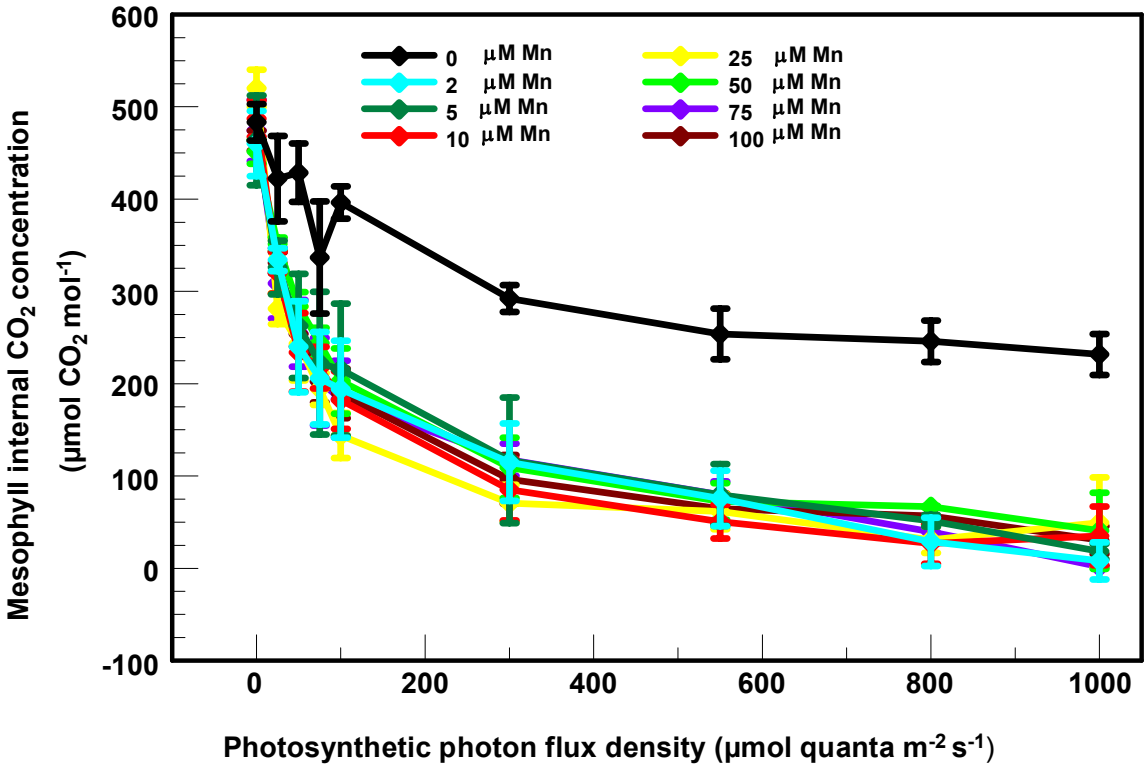
Appendix Figure 3-1. Response of mesophyll internal CO_2 concentration of C-3 wheat, to changing PPFD when grown hydroponically with different Mn concentrations. Values are means ± SE (n=6).



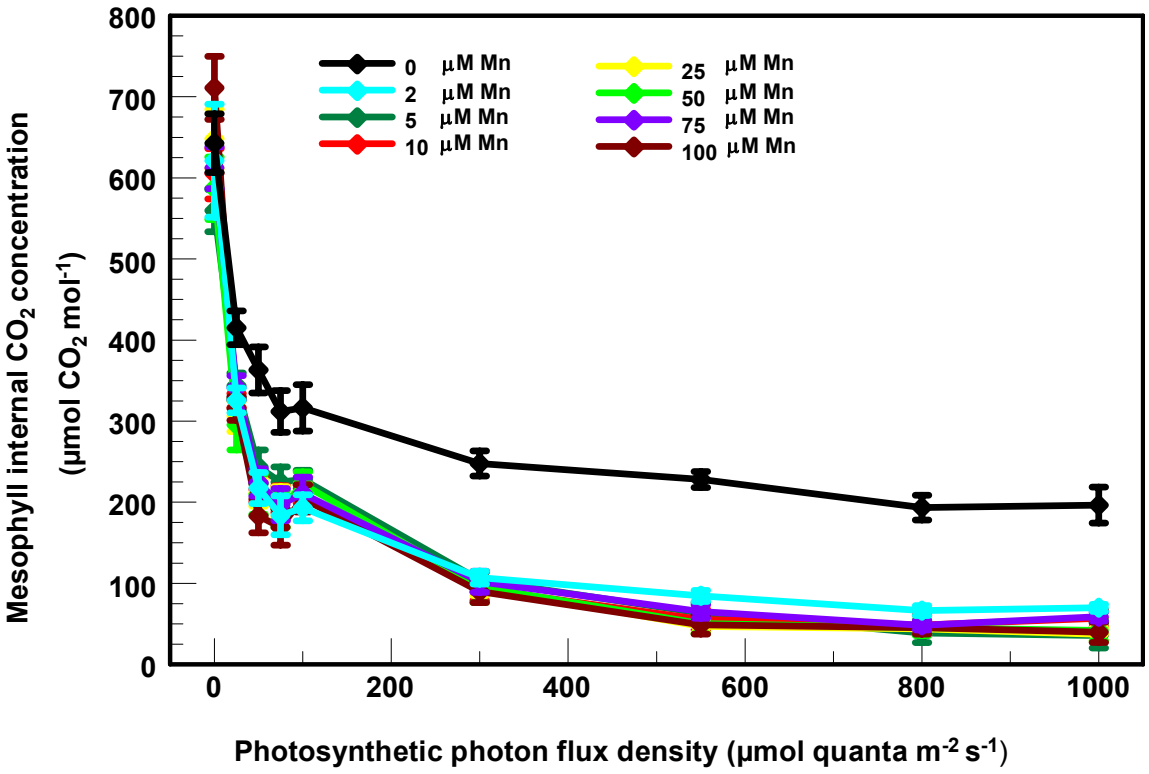
Appendix Figure 3-2. Response of mesophyll internal CO_2 concentration of C-3 squash, to changing PPFD when grown hydroponically with different Mn concentrations. Values are means ± SE (n=6).



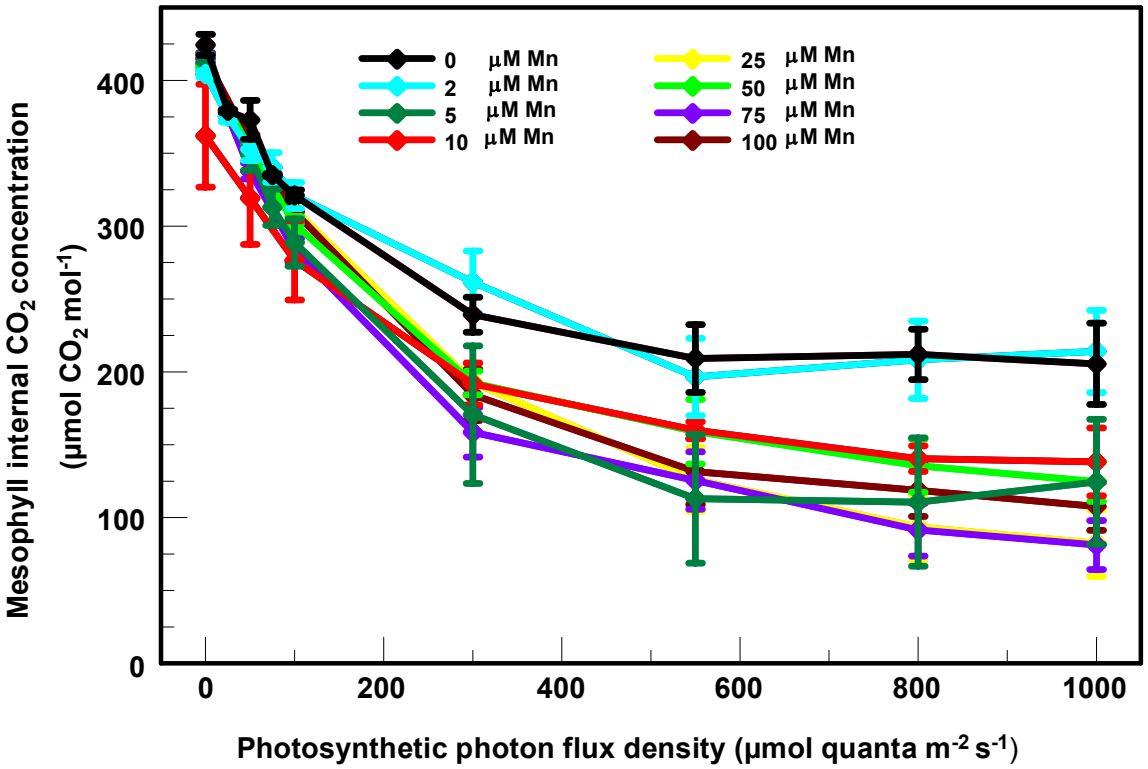
Appendix Figure 3-3. Response of mesophyll internal CO_2 concentration of NADP-ME C-4 corn, to changing PPFD when grown hydroponically with different Mn concentrations. Values are means ± SE (n=6).



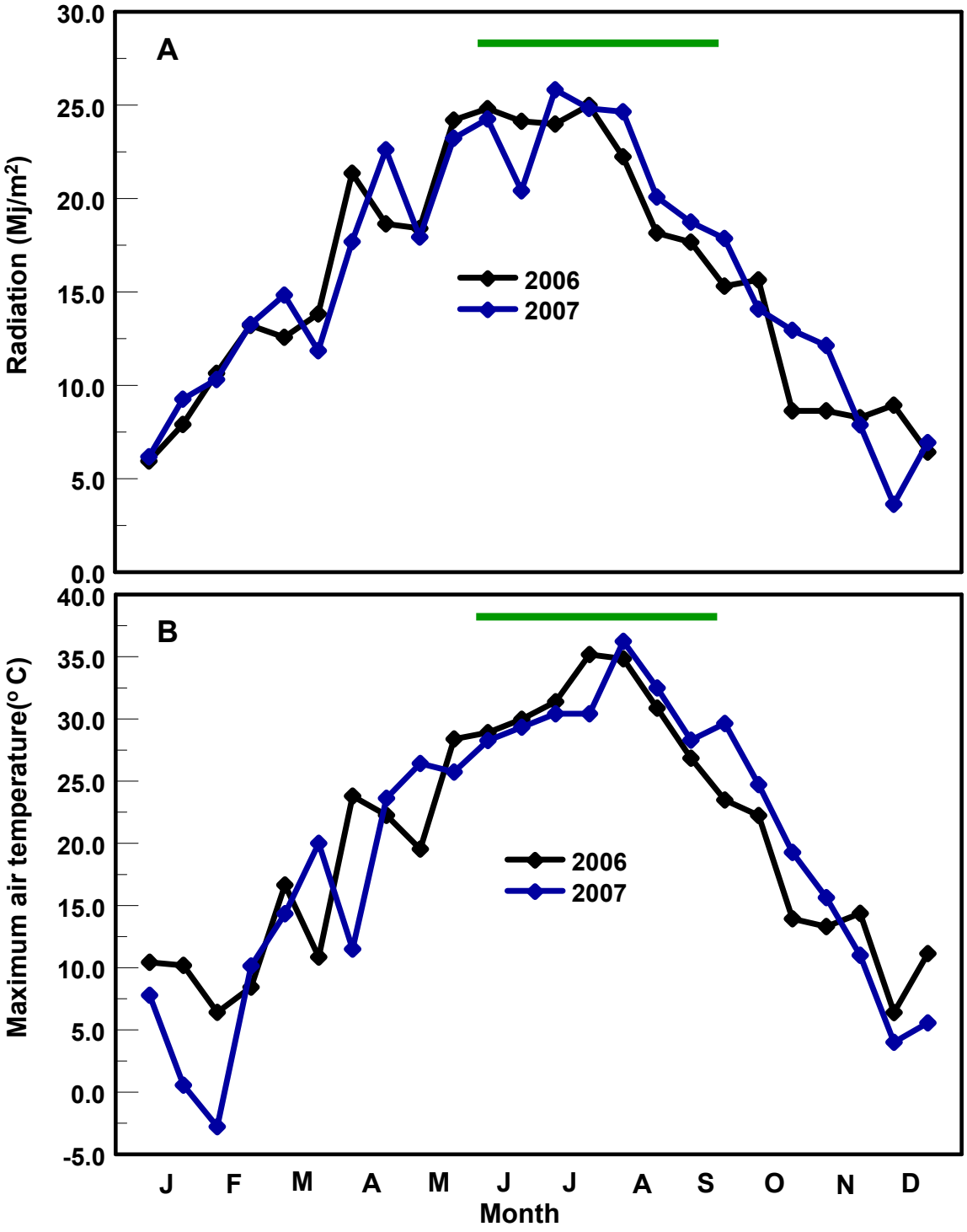
Appendix Figure 3-4. Response of mesophyll internal CO_2 concentration of NADP-ME C-4 sorghum, to changing PPFD when g grown hydroponically with different Mn concentrations. Values are means ± SE (n=6).



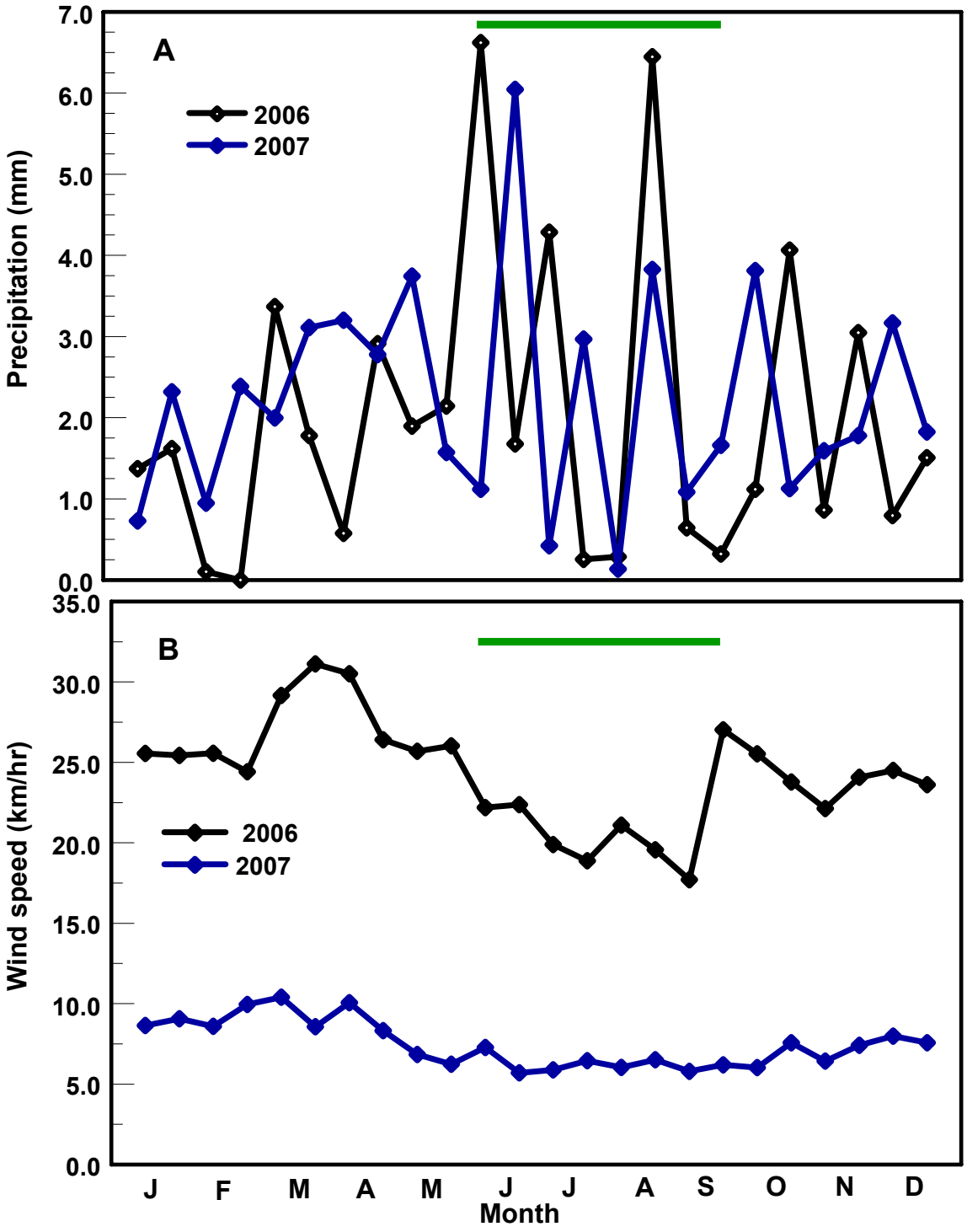
Appendix Figure 3-1. Response of mesophyll internal CO_2 concentration of NAD-ME C-4 pearl millet, to changing PPFD when grown with a range of solution Mn concentrations. Values are means ± SE (n=9).



Appendix Figure 3-2. Response of mesophyll internal CO_2 concentration of NAD-ME C-4 purple amaranth, to changing PPFD when grown hydroponically with different Mn concentrations. Values are means ± SE (n=9).



Appendix Figure 4-1. Solar radiation (A) and maximum air temperature (B) average for the first and last half of each month in 2006 and 2007. Green horizontal line represents approximate plant growth period. Data collected at Sanborn field weather station, University of Missouri-Columbia.



Appendix Figure 4-2. Mean precipitation (A) and wind speeds (B) averaged for the first and last half of each month in 2006 and 2007. Green horizontal line represents approximate plant growth period. Data collected at Sanborn field weather station, University of Missouri-Columbia.

Appendix B

Tables

Appendix Table 2-1. Tissue nutrient element concentration of the NAD-ME C-4 plants, pearl millet and purple amaranth grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means (n=9).

Manganese (µM)	NAD-ME C-4 plants											
	Pearl millet						Purple amaranth					
	Macronutrient (%)				Micronutrient (µg/g)		Macronutrient (%)				Micronutrient (µg/g)	
	Shoot						Shoot					
	P	K	Mg	Ca	Fe	Zn	P	K	Mg	Ca	Fe	Zn
0	1.15a[Ŧ]	9.41a	0.23a	0.57a	363a	38.4a	1.26a	6.60a	0.87a	2.49a	541a	75.8a
2	0.87b	8.92a	0.23a	0.57a	252b	48.3a	0.83b	6.79a	0.63a	1.93a	207b	51.3ab
5	0.87b	9.09a	0.24a	0.60a	235b	38.0a	0.82b	6.65a	0.64a	1.97a	152b	61.3ab
10	0.87b	9.11a	0.25a	0.61a	243b	38.4a	0.74b	6.60a	0.78a	2.70a	208b	52.0ab
25	0.81b	9.35a	0.23a	0.60a	235b	37.8a	0.67b	6.56a	0.77a	2.16a	241b	45.9b
50	0.86b	9.45a	0.24a	0.62a	230b	40.0a	0.61b	6.06a	0.66a	2.15a	186b	49.9ab
75	0.82b	9.16a	0.22a	0.58a	230b	34.1a	0.65b	6.23a	0.63a	1.85a	181b	55.2ab
100	0.79b	8.84a	0.20a	0.58a	226b	39.2a	0.59b	6.94a	0.61a	2.30a	207b	51.2ab
	Root						Root					
0	1.84a	5.20a	0.59a	0.13a	10700a	167.6a	3.43a	4.11a	0.46a	0.33a	9144a	49.7a
2	1.31b	5.25a	0.90a	0.17a	5053a	96.4b	1.23b	4.76a	0.35a	0.37a	10533a	45.3a
5	1.20b	5.50a	0.96a	0.16a	4498a	69.5b	1.24b	5.49a	0.36a	0.45a	10091a	52.3a
10	1.34b	5.97a	0.88a	0.15a	4628a	76.3b	2.28b	4.84a	0.59a	0.46a	2313b	75.3a
25	1.14b	5.46a	0.95a	0.16a	4559a	70.3b	1.98b	5.79a	0.53a	0.48a	1749b	75.4a
50	1.28b	5.77a	0.91a	0.17a	4834a	55.4b	1.29b	5.01a	0.50a	0.39a	1147b	51.1a
75	1.23b	5.40a	0.87a	0.16a	4299a	73.5b	1.46b	5.13a	0.47a	0.36a	1206b	50.0a
100	1.24b	5.76a	0.88a	0.16a	4468a	67.4b	1.83b	5.40a	0.48a	0.45a	1630b	73.8a

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Appendix Table 2-2. Tissue nutrient element concentration of the NADP-ME C-4 plants, corn and sorghum, grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means (n=6).

Manganese (µM)	NADP-ME C-4 plants											
	Corn						Sorghum					
	Macronutrient (%)				Micronutrient (µg/g)		Macronutrient (%)				Micronutrient (µg/g)	
	Shoot						Shoot					
	P	K	Mg	Ca	Fe	Zn	P	K	Mg	Ca	Fe	Zn
0	1.18a[Ŧ]	8.39a	0.19a	0.56a	151.8a	78.0a	1.38a	4.18a	0.37a	0.82a	144.6a	62.1a
2	0.95a	7.71a	0.15a	0.40a	100.6a	41.2a	0.98a	6.48a	0.28a	0.59a	61.1b	34.9a
5	1.07a	8.01a	0.16a	0.45a	59.9a	83.1a	0.98a	6.32a	0.28a	0.57a	57.5b	36.9a
10	1.19a	8.51a	0.19a	0.63a	134.2a	81.6a	1.03a	5.60a	0.29a	0.61a	94.8b	57.3a
25	1.14a	8.32a	0.20a	0.57a	141.5a	73.8a	0.95a	5.82a	0.28a	0.57a	89.8b	54.5a
50	1.17a	8.65a	0.20a	0.62a	119.5a	78.1a	0.93a	6.18a	0.27a	0.54a	86.8b	58.5a
75	1.14a	8.15a	0.19a	0.58a	113.5a	79.6a	0.88a	5.95a	0.28a	0.56a	99.5b	61.4a
100	1.11a	8.19a	0.21a	0.55a	100.9a	82.2a	0.92a	6.11a	0.29a	0.57a	80.5b	64.5a
	Root						Root					
0	1.09a	5.95a	.58a	1.51a	451a	181.2a	1.56a	5.11a	0.27a	0.22a	1550a	61.3a
2	1.17a	5.30a	0.33a	0.76a	12957a	85.6a	1.73a	6.29a	0.62a	0.65a	1220a	131a
5	1.40a	5.51a	0.36a	0.85a	12850a	109.3a	1.69a	6.08a	0.57a	0.61a	1450a	103a
10	0.81a	5.23a	0.56a	1.72a	3633a	114.8a	1.13a	5.84a	0.49a	0.38a	981a	70.8a
25	0.87a	5.53a	0.59a	1.71a	4318a	133.8a	1.12a	6.01a	0.49a	0.49a	1135a	74.7a
50	0.91a	5.47a	0.56a	1.81a	4288a	118.9a	1.12a	6.01a	0.47a	0.55a	1236a	68.7a
75	0.92a	5.37a	0.58a	1.81a	4169a	139.9a	1.19a	5.45a	0.46a	0.52a	1185a	73.5a
100	1.01a	5.29a	0.57a	1.65a	4666a	127.1a	1.23a	5.94a	0.47a	0.64a	1427a	76.4a

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Appendix Table 2-3. Tissue nutrient element concentrations of the C-3 plants, wheat and squash grown hydroponically in complete nutrient solution with different Mn concentrations. Values are means (n=6).

Manganese (µM)	C-3 plants											
	Wheat						Squash					
	Macronutrient (%)				Micronutrient (µg/g)		Macronutrient (%)				Micronutrient (µg/g)	
	Shoot						Shoot					
	P	K	Mg	Ca	Fe	Zn	P	K	Mg	Ca	Fe	Zn
0	1.23a[Ŧ]	4.55a	0.15a	0.37a	162a	26.0ab	1.27a	3.92a	0.59a	3.48a	88.2b	3.71a
2	1.01b	3.92bc	0.13b	0.31bc	129b	19.6abc	0.96c	3.12bc	0.57a	2.99a	79.0b	3.91a
5	0.99b	3.93bc	0.13b	0.33abc	125bc	29.2a	1.01c	3.07bc	0.58a	3.62a	91.5b	3.54ab
10	0.99b	3.88bc	0.13b	0.35ab	117bc	16.8bc	1.04bc	3.21bc	0.59a	3.62a	87.0b	3.44abc
25	1.09ab	4.02b	0.13b	0.34abc	122bc	18.2bc	1.16ab	3.54ab	0.56a	3.17a	114.7ab	3.19abc
50	1.07ab	4.02b	0.13b	0.35ab	109cd	16.6bc	1.09bc	3.16bc	0.55a	3.10a	107.9ab	2.70c
75	0.86b	3.55c	0.11c	0.28c	99d	15.5c	1.14b	2.96c	0.58a	3.51a	123.5a	2.84bc
100	0.85b	3.60c	0.11c	0.31abc	92d	16.3bc	1.03c	2,95c	0.57a	3.49a	11.9ab	3.47abc
	Root						Root					
0	0.95a	3.52a	0.11b	0.16c	7676a	81.7a	1.32a	6.69a	0.16a	0.44a	6199a	73.0a
2	0.91a	3.30a	0.11a	0.18ab	7502a	75.2ab	1.26a	7.01a	0.16a	0.43a	5590a	61.5ab
5	0.84b	3.35a	0.12ab	0.19a	6481a	67.0ab	1.23a	6.46a	0.16a	0.44a	5474a	63.5ab
10	0.85b	3.26a	0.12ab	0.19a	6422a	69.8ab	1.26a	7.04a	0.16a	0.42a	4945a	55.3bc
25	0.87ab	3.32a	0.12ab	0.18ab	6465a	65.3ab	1.28a	7.02a	0.16a	0.43a	6193a	55.6bc
50	0.87ab	3.26a	0.12ab	0.18ab	6725a	61.8b	1.25a	6.93a	0.16a	0.44a	6193a	57.5bc
75	0.85a	3.33a	0.13ab	0.17bc	6052a	66.0ab	1.24a	6.69a	0.15a	0.44a	6404a	48.3c
100	0.95a	3.54a	0.14a	0.19a	6856a	69.5ab	1.29a	7.10a	0.16a	0.47a	5945a	53.5bc

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Appendix Table 5-1. Seed macronutrient concentrations of field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are mean (n=6).

Mn source	Mn rates (kg/ha)	Macronutrients							
		2006				2007			
		P	K	Mg	Ca	P	K	Mg	Ca
		%							
None	**0.0**	0.29b[Ŧ]	0.38b	0.13b	0.022b	0.36ab	0.34a	0.14a	0.028a
Manganese beads	**4.5**	0.31ab	0.40ab	0.13b	0.022b	0.37a	0.33a	0.14a	0.027ab
	9.0	0.31ab	0.39b	0.14ab	0.023ab	0.35ab	0.32a	0.14a	0.030a
	18.0	0.33a	0.41a	0.15a	0.028a	0.35ab	0.31a	0.14a	0.027ab
Manganese chloride	**4.5**	0.32a	0.40ab	0.14ab	0.022b	0.34b	0.32a	0.13a	0.023b
	9.0	0.31ab	0.39b	0.14ab	0.023ab	0.35ab	0.32a	0.14a	0.025b
	18.0	0.32a	0.40ab	0.14ab	0.025ab	0.37a	0.33a	0.14a	0.026ab

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Appendix Table 5-2. Seed macronutrient concentrations of field grown NAD-ME C-4 plant, purple amaranth, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are mean (n=6).

Mn source	Mn rates (kg/ha)	Macronutrients							
		2006				2007			
		P	K	Mg	Ca	P	K	Mg	Ca
		%							
None	**0.0**	0.59ab[Ŧ]	0.47ab	0.32ab	0.22ab	0.58a	0.455b	0.29a	0.20a
Manganese beads	**4.5**	0.63a	0.50a	0.34a	0.23a	0.63a	0.494a	0.31a	0.20a
	9.0	0.57abc	0.46ab	0.31ab	0.21b	0.62a	0.478ab	0.31a	0.20a
	18.0	0.62a	0.51a	0.34a	0.23a	0.62a	0.486a	0.31a	0.21a
Manganese chloride	**4.5**	0.51c	0.42b	0.28b	0.20b	0.61a	0.467ab	0.30a	0.20a
	9.0	0.54bc	0.44b	0.29b	0.20b	0.61a	0.484ab	0.30a	0.20a
	18.0	0.55bc	0.45ab	0.30b	0.21b	0.60a	0.471ab	0.30a	0.20a

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Appendix Table 5-3. Seed micronutrient concentrations of field grown NAD-ME C-4 plant, pearl millet, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are mean (n=6).

Mn source	Mn rates (kg/ha)	Micronutrients							
		2006				2007			
		Fe	Zn	Cu	Na	Fe	Zn	Cu	Na
		(µg/g)							
None	0.0	46.7c[Ŧ]	34.0a	3.7b	11.2b	61.6a	47.5ab	5.1a	16.3bc
Manganese beads	4.5	46.3c	36.4a	4.2ab	13.6ab	61.5a	43.9b	4.6b	17.6b
	9.0	50.7ab	35.6a	4.2ab	13.3ab	61.8a	50.3ab	5.3a	21.8a
	18.0	50.9ab	37.6a	4.6a	14.8a	62.2a	50.5ab	5.1a	22.4a
Manganese chloride	4.5	48.0ab	34.9a	4.3ab	12.5ab	57.2a	43.8b	4.8a	13.3c
	9.0	48.5ab	34.7a	4.3ab	13.2ab	59.8a	43.4b	4.8a	15.5bc
	18.0	51.7a	36.7a	4.1ab	14.3a	60.9a	56.1a	5.2a	17.8b

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Appendix Table 5-4. Seed micronutrient concentrations of field grown NAD-ME C-4 plant, purple amaranth, treated with two sources and different rates of Mn fertilizer in 2006 (A) and 2007 (B). Values are mean (n=6).

Mn source	Mn rates (kg/ha)	Micronutrients							
		2006				2007			
		Fe	Zn	Cu	Na	Fe	Zn	Cu	Na
		(µg/g)							
None	**0.0**	184a[Ŧ]	37.2ab	6.8ab	4.0b	207a	36.5b	7.10b	10.1b
Manganese beads	**4.5**	194a	36.2ab	7.1a	6.4ab	220a	37.9a	8.09a	15.1a
	9.0	228a	36.3ab	6.6ab	7.7ab	205a	36.3ab	7.53ab	9.3b
	18.0	242a	38.7a	6.6ab	5.8ab	259a	38.3a	8.00a	11.5ab
Manganese chloride	**4.5**	167a	35.3b	6.5ab	9.2a	204a	35.2ab	7.91a	10.9ab
	9.0	182a	35.3b	5.8b	5.6b	235a	36.5ab	7.89a	13.4a
	18.0	121a	34.8b	5.9b	5.9ab	211a	36.9ab	8.01a	11.3ab

[Ŧ] Values in same column followed by a different letter are statistically different at $p < 0.05$.

Vita

Maru Kipleting Kering was born and grew up in Uasin Gishu plateau, in the western highland of the Great Rift Valley, Kenya. Kipleting, has was then known, completed his pre-college education in schools near Eldoret, the Uasin Gishu district's commercial and administrative headquaters. During the same period and in line with the Nandi community traditions, he received the name Maru, a first derivative of his Dad's name.

He graduated with a Bachelor degree in Agriculture from the University of Nairobi, and a Master of Philosophy in Soil Science from Moi University before moving to the United States in the fall of 2001. In December 2003, he received his M.S. degree in Plant Science from Missouri State University in Springfield. In May 2008, he received his Ph.D. degree in Agronomy from the University of Missouri with an emphasis in plants nutrition and physiology. Prior to graduation he received a post-doctoral fellowship at The Samuel Roberts Noble Foundation Inc., Ardmore, Oklahoma.