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Impact of Foliar-Applied Manganese on Photosynthesis in Pecan in New Mexico

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INTRODUCTION

In the southwestern region of the U.S., pecans [Carya illinoinensis (Wangenh.) K. Koch] have proven to be a major economic resource with a combined in-shell production value of $213 million in 2012 (USDA-NASS, 2013). Adequate levels of plant nutrients within the pecan leaf tissue play a vital role in this production. Within the leaf tissue, macronutrients are those minerals needed in higher concentrations (usually expressed as a percentage of leaf dry weight) compared to the micronutrients (usually expressed as parts per million dry weight, ppm). Nevertheless, micronutrients are just as important as the macronutrients in that the plant cannot complete its life cycle without them and they aid in regulating a plant's physiological processes (Marschner, 1995).

Symptoms of micronutrient deficient plants are not always visible to the grower even when the plant is indeed deficient and this well documented phenomenon is known as the "hidden hunger" state (Brady & Weil, 1999; Herrera, 1998; Heerema, 2013). Nutrients within the plant tissue need to remain within certain levels in order for the plant to perform its metabolic and physiological processes effectively.

Pecans' ability to take up micronutrients from the soil is dependent on the pH of the soil because as pH of soil increases the nutrients transition into ionic states that are not in the form the plant utilizes (Brady & Weil, 2007). Soils in the Southwest are typically alkaline (pH above 7.0) and calcareous, thus making phosphorus and most micronutrients, including manganese (Mn), poorly available for root uptake (Chang, 1953; Herrera, 1998; Sims, 1986). In these soils with low organic matter Mn mostly resides along the exchange complex as Mn4+ and thus is not in the usable form as Mn2+. Smith & Cheary (2001) published the first report of Mn deficiency in pecan. In their study, they showed that trees on Texas soil with pH 7.2 had suppressed Mn uptake such that leaf concentrations were only 1 to 18 ppm and "shoot growth was short with pale green foliage but no discernible pattern of chlorosis." When the trees were supplied a Mn Sulfate (MnSO4) foliar applied fertilizer, leaf Mn concentrations increased and foliage color returned to more healthy green appearance.

Manganese is essential for the photosynthesis process, specifically in the oxidation side of the photosystem II complex and as a coenzyme for biosynthesis of chlorophyll. Henriques (2003) revealed that with decreasing Mn availability in field grown pecans, total chlorophyll content and photosynthetic rates decline dramatically below 11 ppm Mn leaf tissue concentrations. The NMSU Cooperative Extension Service recommendations for New Mexico (NM) pecans are 100-300 ppm Mn in July sampled leaflet tissue (Heerema, 2013). A published survey of NM pecan orchards showed, on average, only 85 ppm Mn in leaf tissue, but the level of Mn at which photosynthesis is optimum is not yet known (Pond & Walworth et al., 2006).

The objective of our study was to characterize the impacts of foliar applied Mn as amino acid chelate (Manganese Metalosate®, Albion Plant Nutrition, Clearfield, UT) on photosynthesis and chlorophyll content over a broad range of increasing leaf Mn concentrations.

MATERIALS AND METHODS

Study Site, Experimental Design, and Treatments

Two experiments were conducted at the NMSU Linwood Nursery Research Orchards at the Leyendecker Plant Science Research Center in Las Cruces, NM (latitude 32˚ 11' 39" N, longitude 106˚ 44' 18" W; elevation 1174 m; Armijo clay loam and Harkey loam). Experiment 1 lasted the duration of the 2011 and 2012 growing seasons, and Experiment 2 was conducted in the 2013 growing season. Experiment one consisted of 24 'Pawnee' cultivar pecan trees planted in 2010. Trees were irrigated, pruned, and supplied other macro- and micronutrients using standard cultural practices for the New Mexico pecan industry. Four treatments were assigned to trees (six single-tree replications per treatment) according to a complete randomized design. Treatments consisted of foliar applications of varying concentrations of amino acid chelate Mn fertilizer (Manganese Metalosate®, Albion Plant Nutrition, Clearfield, UT) solution. There were three applications in 2011and five applications in 2012 at four different concentrations:

1) High – 2% v/v Mn Metalosate solution
2) Medium – 1% v/v Mn Metalosate solution
3) Low – 0.5% v/v Mn Metalosate solution
4) Control – 0% v/v Mn Metalosate solution - H2O only

In 2011 the Low treatment received one application, Medium treatment received two, and Control and High received three. In 2012 the Low treatment received three applications, Medium received four, and Control and High received five. The reason for increasing applications per increasing concentration rate of Mn was to create greater differences between Mn treatments.

Experiment 2 consisted of 30 'Pawnee' and 30-third leaf 'Western' cultivar pecan trees. Trees were irrigated, pruned, and supplied other macro- and micronutrients using standard cultural practices for the New Mexico pecan industry. Five treatments were assigned to trees (six single-tree replications per treatment) according to a complete randomized design. There were six total applications using the same Mn fertilizer as in Experiment 1at five different concentrations:

1) Ultra High – 4% v/v Mn Metalosate solu tion
2) High – 2% v/v Mn Metalosate solution
3) Medium – 1% v/v Mn Metalosate solution
4) Low – 0.5% v/v Mn Metalosate solution
5) Control – 0% v/v Mn Metalosate solution - H2O only

The Low treatment received three applications, Medium received four, High received five and Control and Ultra High received six applications across the season.

Data Measurements

Gas exchange was measured using the LI-6400XT portable photosynthesis system (LI-COR Biosciences, Lincoln, NE) one week after every application **(Figure 1)**. Three fully sun-exposed leaflets per tree were selected for gas exchange measurements **(Figure 2)**. Soil Plant Analytical Development (SPAD) was measured on the same leaflets measured for gas exchange avoiding major leaf veins using a portable SPAD 502 chlorophyll meter. Midday Stem Water Potential (MDSWP) was measured from one completely shaded fully-expanded leaf (sealed in a reflective plastic bag for ~20-40 minutes) from each experimental tree on the same dates as gas exchange and SPAD were measured to determine plant moisture stress levels since photosynthesis is negatively impacted with decreasing water availability.

Tissue Sampling and Nutrient Analysis

Leaf samples were collected from 12 non-fruiting shoots per experimental tree on July 25, 2011, August 25, 2012, and on August 24, 2013 after all applications were completed according to the method recommended by NMSU Cooperative Extension Service (Herrera, 2000). On May 24, 2012 leaf samples were collected in Experiment 1

early in the season prior to any Mn applications to determine if there were any carryover effects from 2011 Mn applications. The middle leaflet pair of the middle leaf on each shoot was sampled for a total of 24 leaflets per tree. Leaflets were washed briefly in phosphorous free soap bath, deionized water rinse, 0.1M hydrochloric acid wash, followed by two deionized water rinses and the baths were drained and replenished after each treatment group to avoid cross contamination from any Mn Metalosate residue on the leaflets. Samples were then dried in drying oven at 60˚ C for 48 hours. In 2011 and 2012, leaf samples were analyzed by Inductively Coupled Plasma. Leaf analysis for 2013 samples has yet to be conducted.

Statistics

Data were analyzed by year as a randomized complete block design with repeated measures using SAS proc mixed software version 9.3 (SAS Institute, Cary, NC, 2010). In addition to fixed effects for treatment, time and their interaction, the model included random effects for block and block by time, and fitted a heterogeneous compound symmetric variance structure to the repeated measures through time. Sensitivity of findings to extreme data points was examined using the outlier strategy with outliers identified as those observations with studentized residual magnitude greater than 2.5 (Ramsey & Chafer, 2002). Statistical significance was defined as $P \leq 0.05$.

RESULTS AND DISCUSSION

Average leaf Mn concentrations in 2011 were 38, 52, 149, and 302 ppm in the Control, Low, Medium, and High treatments, respectively. Concentrations in 2012 were 53, 84, 147, and 329 ppm in the Control, Low, Medium, and High treatments, respectively (Figure 3). All other nutrients were within normal ranges. Leaf Mn concentrations were not significantly different across treatments on May 2012 (prior to 2012 Mn applications), indicating no carryover of Mn from 2011. The Medium Mn treatment in both years supplied enough Mn to the pecan trees to bring their Mn levels into the recommended range level of 147-149 ppm. The Control and Low treatments supplied levels below the recommendation with 35-53 ppm and 52-84 ppm respectively, and the High treatment above the 300 ppm level of what is currently recommended. Even at the low level of 35 ppm in the Control treatment there were still no visible signs of Mn deficiency.

Analyzed across dates the Medium Mn treatment had significantly higher photosynthesis and stomatal conductance ($\alpha = 0.05$) than the other treatments in both experiments and in both cultivars over three consecutive growing seasons. In 2011 mean photosynthesis rates of the Control, Low, Medium, and High treatments showed 14.2, 14.3, 15.0, and 14.5 μmol CO2 m-2 s-1 and in 2012 were 14.9, 14.8, 16.2, and 15.2 μmol CO2 m-2 s-1, respectively **(Figure 4)**. The percent increase in photosynthesis between the Medium Mn treatment and the Control was 5.8% in 2011 and 8.7% in 2012. Stomatal conductance indicated a similar pattern with the Medium Mn treatment being significantly higher than all other treatments. Stomatal conductance percent increase between the Medium Mn treatment and Control were 8.5% in 2011 and 16.3% in 2012 (data not shown).

In the 2013 growing season average photosynthesis rates across the season were 15.2, 15.3, 16.3, 14.5, and 14.5 μmol CO2 m-2 s-1 for the Control, Low, Medium, High, and Ultra High treatments, respectively, in the 'Pawnee' cultivar **(Figure 5)**. The average photosynthesis rates across the season for the 'Western' cultivar were 14.9, 15.3, 16.5, 14.1, and 14.1 μmol CO2 m-2 s-1 for the Control, Low, Medium, High, and Ultra High treatments, respectively **(Figure 5)**. This indicated a photosynthetic rate increase between the Medium and Control treatments of 7.1% and 10.4% for the 'Pawnee' and 'Western' cultivars, respectively.

A similar pattern was evident for stomatal conductance in both cultivars as well. The 'Pawnee' cultivar was 0.31, 0.33, 0.35, 0.28, and 0.29 mmol m-2 s-1 in the Control, Low, Medium, High, and Ultra High treatments respectively **(Figure 6)**.

The 'Western' cultivar had 0.31, 0.30, 0.35, 0.26, and 0.28 mmol m-2 s-1 for the Control, Low, Medium, High, and Ultra High treatment, respectively **(Figure 6)**. Percent increase in stomatal conductance between the Medium and Control treatments were 12.6% and 14.4% for the 'Pawnee' and 'Western' cultivars, respectively.

Photosynthesis can be negatively impacted in pecan by plant moisture stress. In all three years the average MDSWP of all treatments were above -0.8 megapascals for each measurement date signifying that the photosynthetic rates recorded were not impacted by low moisture stress (St. Hilaire & Othman, 2013).

Manganese is essential to photosynthesis and chlorophyll synthesis and consequently with decreasing amounts of Mn in the leaf tissue these critical processes decline (Kozlowski & Pallardy, 1997). The carbohydrates produced from photosynthesis are utilized by the plant for the production of fruit. Therefore, if the photosynthesis process is not performing at its optimum the question remains if the plant is producing fruit at its optimum.

While these data provide strong evidence of improved tree performance in immature non-bearing trees, we predict a similar response in mature, fruit-bearing trees and the possibility of improvement on flowering, fruit set, nut yield and nut quality with foliar applied Mn. Although the Control treatment in our experiment was within deficient levels of leaf tissue Mn concentrations there were no visible symptoms of Mn deficiency. Nevertheless, photosynthesis was significantly lower for the Control compared to the Medium treatment. This gives evidence that pecan trees in southern NM may have "hidden hunger" for Mn and thus tree performance could benefit from foliar application of Mn. These data confirm a relationship in pecan between photosynthesis and Mn nutrition and, furthermore, indicate that Mn may be a limiting factor on photosynthesis in NM pecan orchards.

SOURCES

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FIGURES

Figure 1.
LI-6400XT (LI-COR Biosciences, Lincoln, NE).

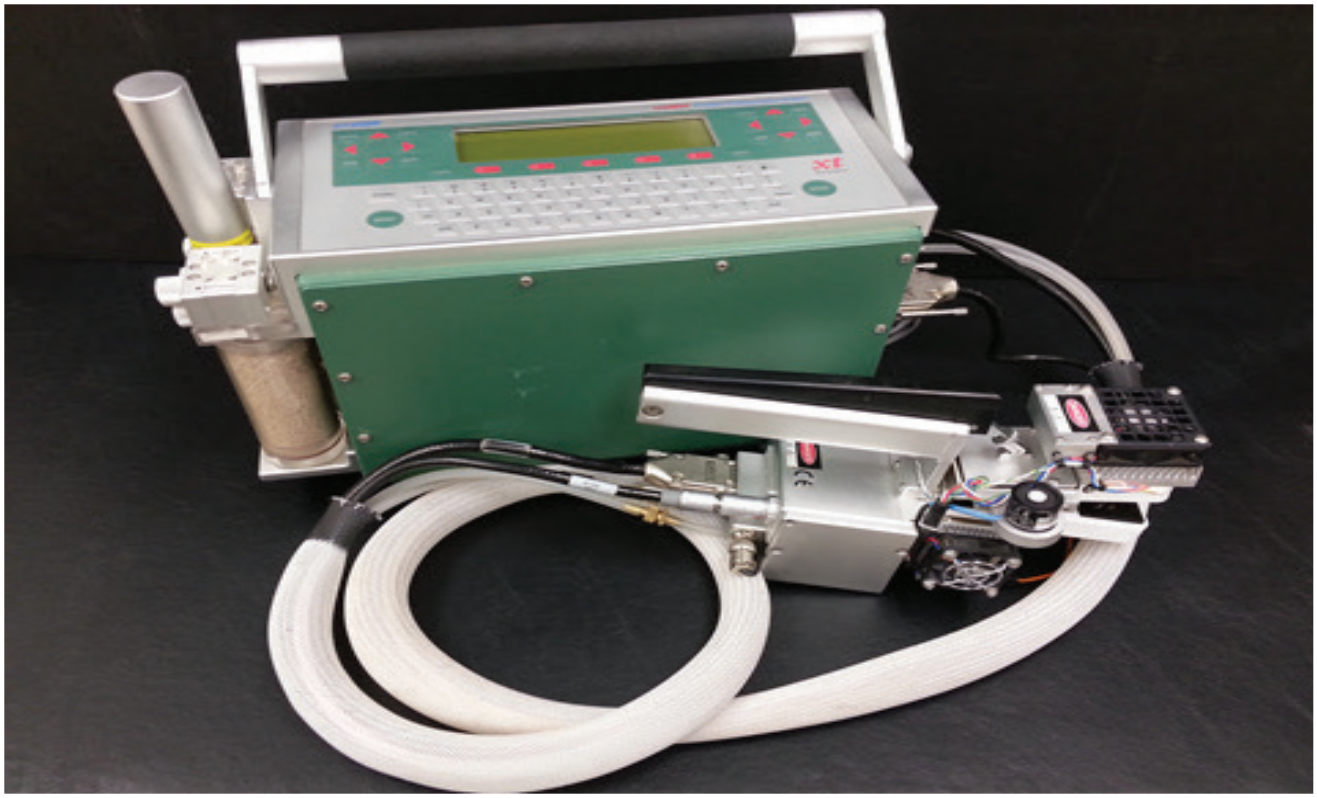


Figure 2.
LI-6400XT leaf chamber clamped on leaflet for gas exchange measurements.

Figure 3.
Manganese Leaf Tissue concentrations for 2011 and 2012. Vertical bars indicate ± standard error of the means.

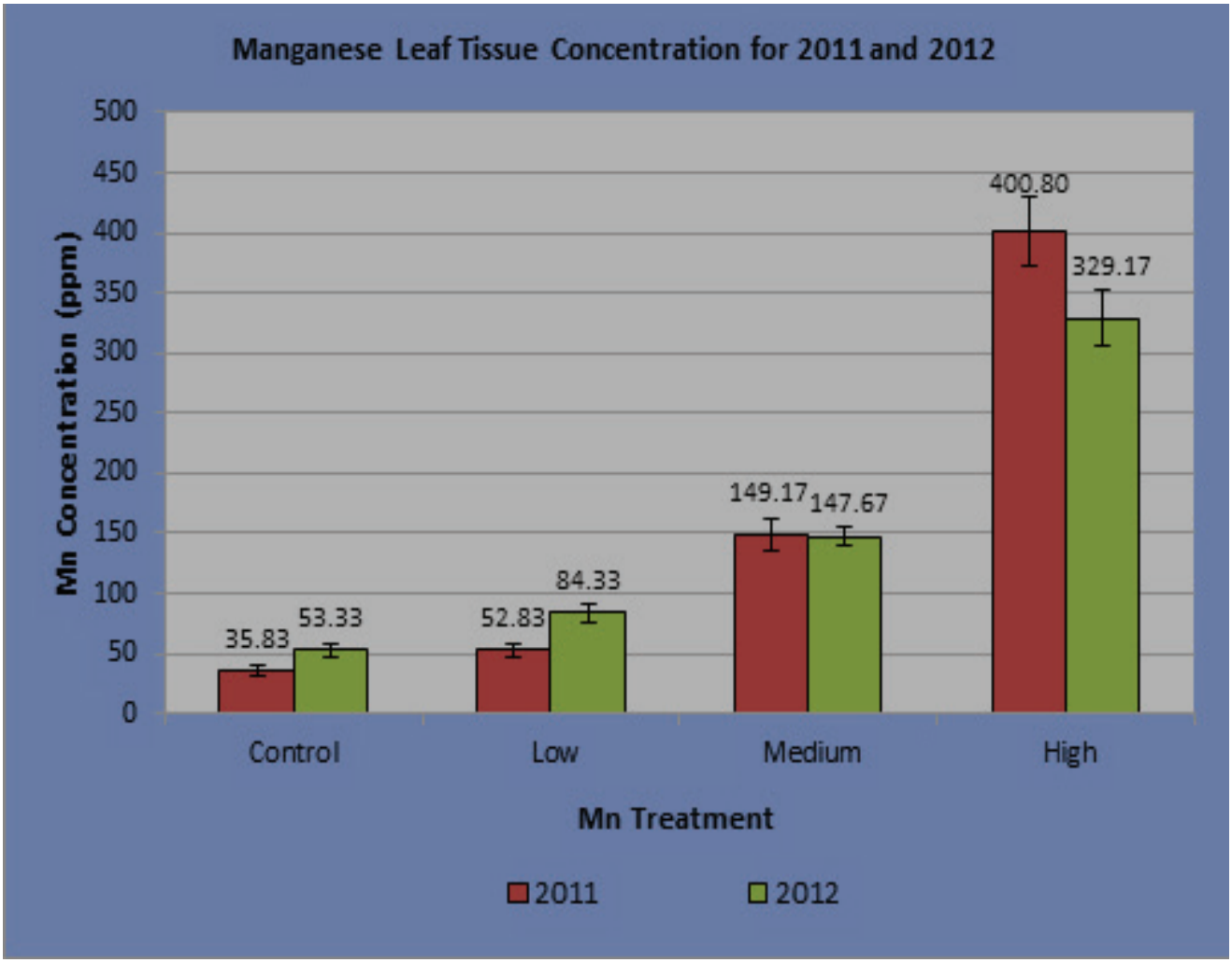


Figure 4.
Average Photosynthesis for 2011 and 2012. Vertical bars indicate ± standard error of the means.

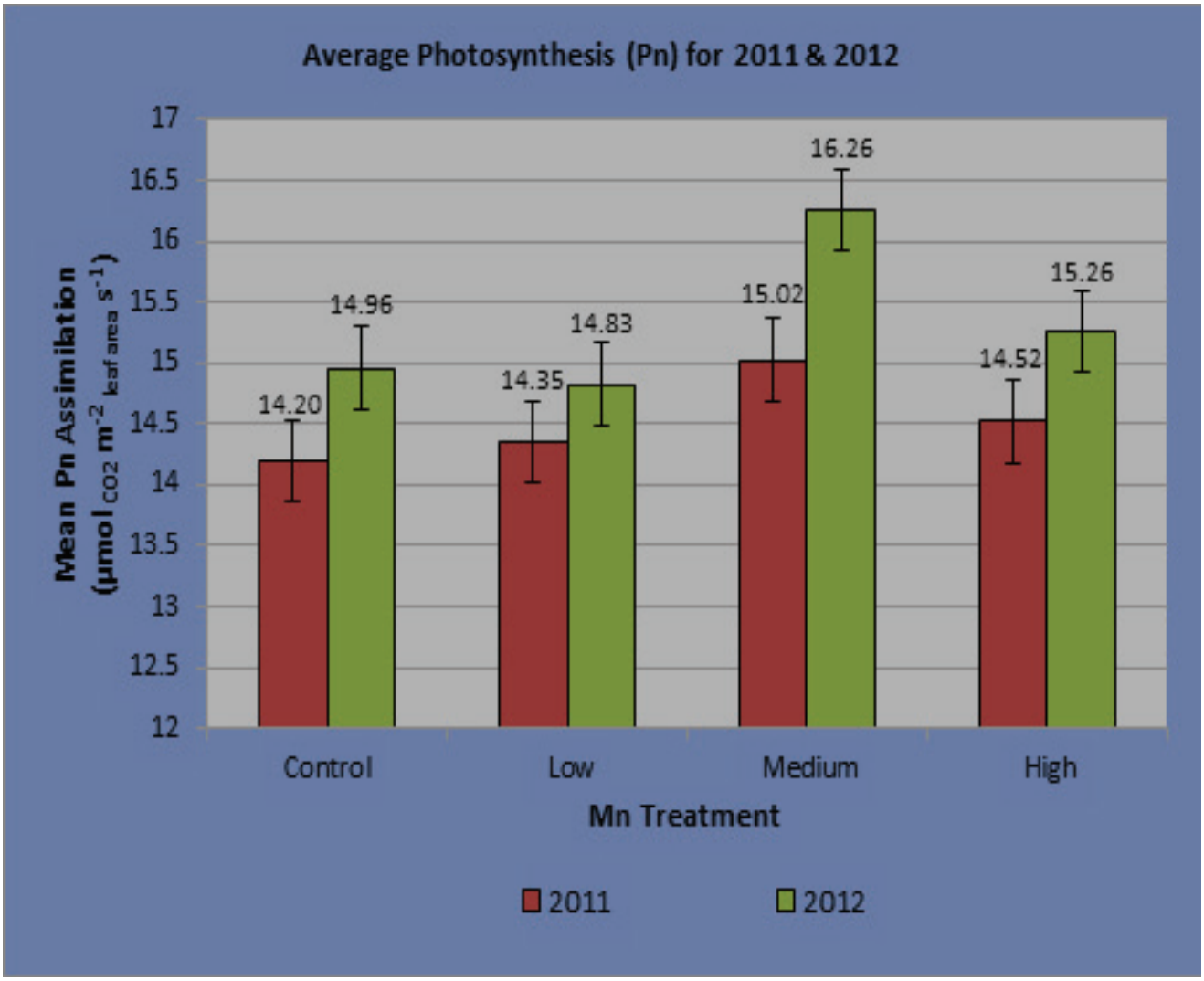


Figure 5.
Average photosynthesis for 2013 'Pawnee' and 'Western' cultivars. Vertical bars indicate ± standard error of the means.

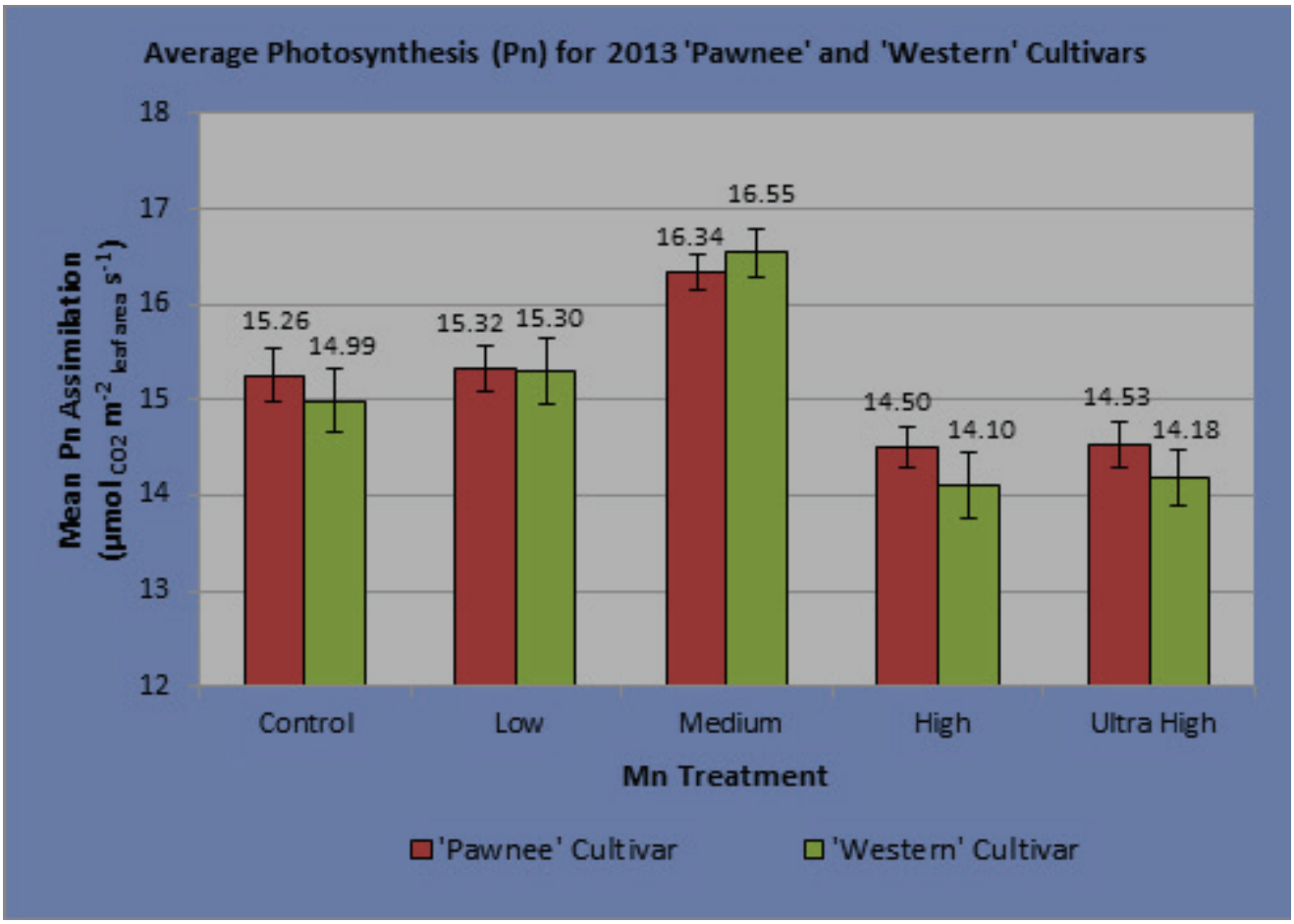
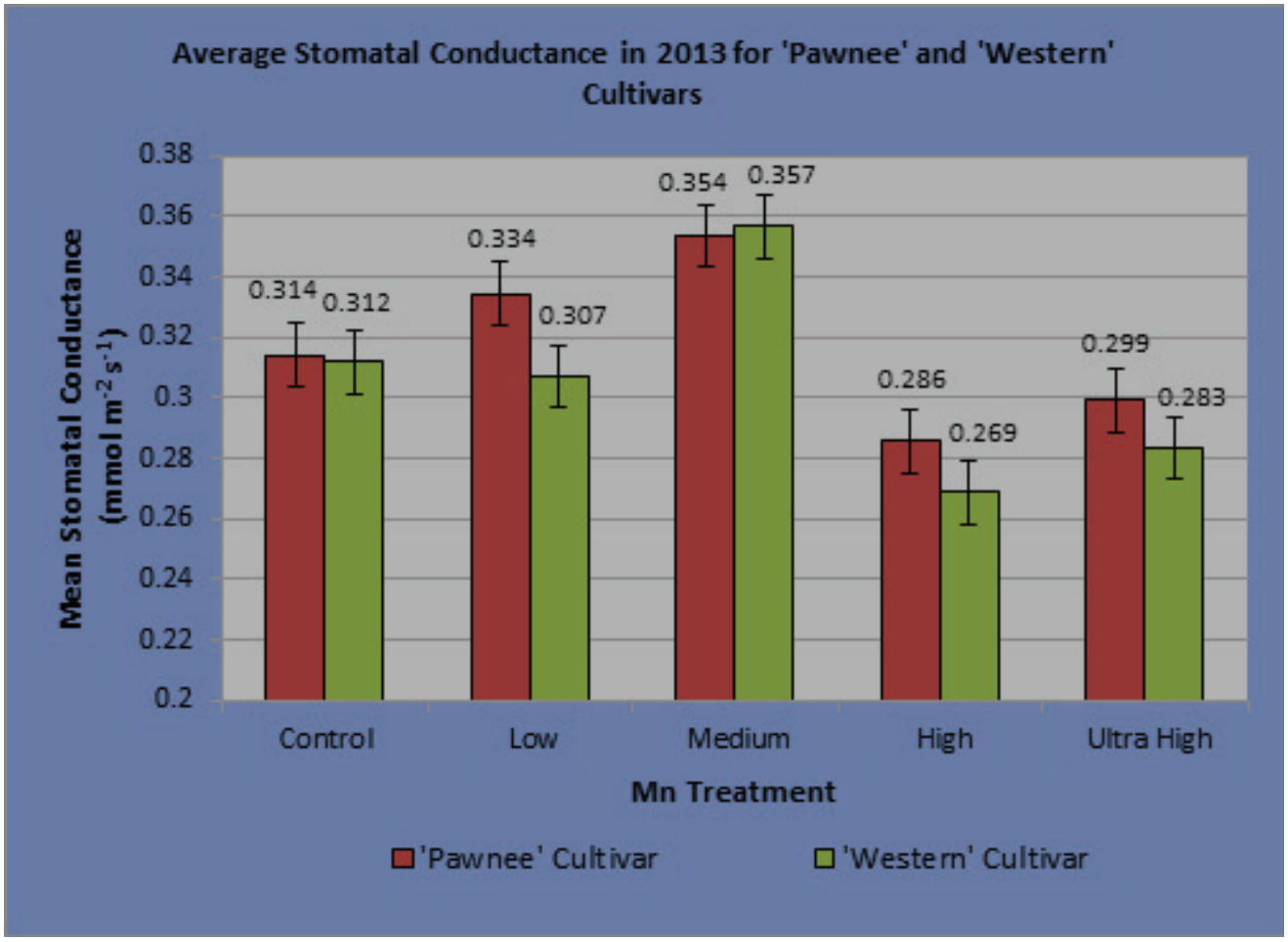


Figure 6.
Average stomatal conductance for 2013 'Pawnee' and 'Western' cultivars. Vertical bars indicate ± standard error of the means.



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