Spring 2017

Effects of Phosphorus Availability on Growth and Leaf Nutrient Concentrations in Wheat, Oat, and Cereal Rye

Jerri Lynn Dodson

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EFFECTS OF PHOSPHORUS AVAILABILITY ON GROWTH AND LEAF

NUTRIENT CONCENTRATIONS IN WHEAT, OAT, AND CEREAL RYE

A Masters Thesis

Presented to

The Graduate College of

Missouri State University

In Partial Fulfillment

Of the Requirements for the Degree

Masters of Science, Plant Science

By

Jerri Lynn Dodson

May 2017

EFFECTS OF PHOSPHORUS AVAILABILITY ON GROWTH AND LEAF NUTRIENT CONCENTRATIONS IN WHEAT, OAT, AND CEREAL RYE

Agriculture

Missouri State University, May 2017

Masters of Science

Jerri Lynn Dodson

ABSTRACT

Adequate soil phosphorus (P) is critical for the growth and nutrient content of forages for grazing animals. On low fertility soils, tall fescue responds to P fertilization with increased yields and improved leaf nutrient contents of P, magnesium (Mg), calcium (Ca), and potassium (K) in winter months. My objective was to examine the effect of P availability on growth and leaf nutrients in annual cereal grains commonly grown for winter forage. Soft red winter wheat (*Triticum aestivum*), oat (*Avena sativa*), and cereal rye (*Secale cereale*) were grown hydroponically in greenhouse conditions in complete nutrient solutions with varying P concentrations of 0, 200, 400, and 800 µM P (3 blocks, 3 replicates per block). After 32 days, plants were harvested, dried, weighed, and analyzed for P, Mg, Ca, and K content. Shoot growth of all three species increased from 0 to 200 µM P, however only wheat shoots increased incrementally with P treatment concentrations. Leaf P also responded to P treatments incrementally in all three species. Leaf Ca increased from 200 and 800 µM P in cereal rye, while Mg increased from 400 and 800 µM P (using 1:4 Na_2HPO_4 and NaH_2PO_4) in wheat and cereal rye. No changes in leaf K were found in any of the grain species in 200 µM P or greater. These findings support the hypothesis that increased P availability can influence nutrient concentrations in leaf tissue of winter annual forage species.

KEYWORDS: phosphorus, grazing, winter annual forages, grass tetany ratio, hydroponics, wheat, oat, cereal rye

This abstract is approved as to form and content

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Melissa Remley, PhD
Chairperson, Advisory Committee
Missouri State University

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Lastly, I thank the Lord Jesus Christ for allowing me opportunities greater than I have ever imagined on my own. Without His sacrifice and love, I would forever truly be lost.

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INTRODUCTION

Forages in Missouri Agriculture

Missouri agriculture is a $33 billion industry, making up 9.3% of Missouri revenue in products and inventory alone (USDA, 2016). Value added beef cattle ranch sales account for $1.678 billion and $276 million are from dairy cattle and products. While Missouri is the 3rd state in number of beef cattle (USDA, 2016) and 24th in number of dairy cattle nationwide (USDA, 2015), it is 2nd in forage land acreage (USDA, 2016). Forages are the primary commodity used in feeding beef and dairy cattle. While 3,040,000 acres (USDA, 2016) were mechanically in 2012 as hay or haylage, valued at more than $593,902,000, forages used in cattle grazing are typically not accounted for economically.

Missouri is ideal for forage production due to land classification, temperate climate, and diverse plant communities. Common forages for grazing include tall fescue (*Schedonorus arundinaceus*), Kentucky blue grass (*Poa pratensis*), bermudagrass (*Cynodon dactylon*), orchardgrass (*Dactylis glomerata*), and annual ryegrass (*Lolium multiflorum*) (Roberts and Gerrish, 2001).

It is typical to have a mixture of cool season and warm season forages for grazing. Forage mixtures allow for biomass growth and accumulation spanning multiple growth periods. In Missouri, cool season forages typically grow mostly in March through May, decline in growth rate May through September, and experience a second period of growth September through November before becoming dormant November through March. Warm season forages increase in growth during April and decline in September before

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becoming dormant September through April (Ball et al., 2015). Mixtures of cool and warm season forages are common because they allow for forage production in the spring through the fall; however growth is still sparse during winter months resulting in a period of time where alternative feeding practices are needed.

Practices for Winter Feeding

Common practices for winter feeding include stockpiling forages (Hancock and Josey, 2017), haying (Meteer, 2013), and planting winter annuals (Ball et al., 2008). Stockpiled forages are grown during the seasons of rapid growth, predominately fall, and set aside until forage is needed for grazing. Some species, like tall fescue, are able grow during the winter months when conditions are more favorable and add the ability to winter graze (Hancock and Josey, 2017).

Haying requires mechanically harvesting forages at peak quality by cutting, drying, and then baling it for storage until needed. Forages lose part of their nutritional value when hayed, including total digestible nutrient and protein values (Henning and Wheaton, 1993). Storage can lead to up to 5% loss of dry matter and losses can increase if not properly stored or it comes in contact with moisture.

Lastly, winter annuals can be planted to add another option for winter feeding. Winter annuals such as winter wheat, oat, and cereal rye have seasonal growth November through December then again February through June, varying depending on climate conditions (Ball et al., 2015). Winter annuals are typically planted late fall and growth through late winter (November through December) or are planted early spring (February

through June). This growth pattern allows forage to be grown, grazed, or cut and stored when other warm and cool season grasses are not accumulating growth.

While all of these practices are viable options for consideration for biomass, forage quality is also a concern. Stockpiled fescue is subject to freezing and nutrient leaching, thus decreasing forage quality (Blevins et al., 2011). Haying can leach nutrients due to storage, moisture, or weather (Henning and Wheaton, 1993). Additionally, winter annuals are subject to mineral imbalances within new plant tissue during periods of rapid growth and exposure to weather.

Mineral imbalances within the forage tissue can create mineral imbalances within cattle consuming that forage. One imbalance of particular consideration in Missouri is grass tetany.

Grass Tetany

Grass tetany is the most critical and fatal nutritional disease in grazing cattle, causing annual death losses estimated at $50-150 million in the United States (Mayland and Sleper, 1993). Symptoms of grass tetany typically become visible once the disease is untreatable. The first symptoms of grass tetany include muscle twitching, stiff gait, and staggering (Underwood, 1966). It is typically linked to low levels of magnesium (Mg) in the blood serum of cattle (Stewart et al., 1981). The lack of Mg in blood serum is directly associated with nutrient concentrations of the plant tissues consumed by the animal.

Grass tetany is not solely due to the lack of Mg in the blood serum, but rather a ratio of potassium (K), Mg, and calcium (Ca) in the blood: milliequivalents K / (milliequivalents Mg + milliequivalents Ca). Cattle become susceptible to grass tetany

when the ratio is greater than 2.2 (Kemp and t'Hart, 1957). In both plants and animals these three macronutrients are interlinked in their absorption and translocation.

Mineral Nutrition in Plants. Potassium poses an antagonistic relationship with Mg in plant shoot tissue (Ohno and Grunes, 1984). High levels of soil K depressed Mg uptake in the plant (Wilkinson, 1983) and Ragab (1979) found that K depressed leaf Mg and Ca concentrations in oat. When studying winter wheat grown in perlite with nutrient solutions varying in K and Mg solution concentrations, Ohno and Grunes (1984) found that Mg fertilization had no effect on Ca or K total uptake. They also found that Mg tissue concentrations will increase with Mg fertilizer without added K fertilizer. K fertilization showed no effect on total Mg or Ca root influx, but decreased Mg concentrations in shoots. Increased K concentrations led to decreased shoot Mg concentrations, thus creating greater grass tetany ratio values.

The effect of phosphorus (P) on Ca and Mg movement in plants has also been studied. Reinbott and Blevins (1999) found that an increase in P availability in hydroponically grown squash increased the Ca and Mg translocation from roots to shoots. Increased concentrations of P from 50 μM P to 400μM P caused a 180% increase in the xylem exudate volume and a slight increase in the macronutrient concentrations of xylem exudate. Increases of P had no effect on K exudate concentrations, but increased Mg by 119% and Ca by 141%. While nutrient concentrations of Mg, Ca, and K all increased due to higher levels of P availability, greater increases were observed of Mg and Ca concentrations as compared to K concentrations. Greater increases in Mg and Ca versus K decrease the grass tetany ratio, thus reducing the risk of cattle falling susceptible to the disease.

Mineral Nutrition in Animals. Proper Ca, Mg, and K concentrations are important in the forages, but imbalances have negative implications within the ruminant as well. High concentrations of K depress the absorption of Mg through the rumen wall into the blood stream (Reinhardt et al., 1988). Of the two mechanisms of Mg transport across the rumen wall (Schonewille et al., 1999), Bhanugopan et al. (2010) suggests it is most likely that high K concentrations increase the differences in potential between the inside and outside of the rumen wall and reduce the rate of diffusion of Mg into the bloodstream.

When both forage mineral concentrations and the serum concentrations were measured within the ruminant, Chelliah et al. (2008) found that forage mineral nutrition was adequate for the ruminant (McDowell, 2003), but excessively high (above 4-5%) concentrations of K will double the Mg dietary requirements.

Adequate dietary balances of Ca, Mg, and K ratios are especially critical during parturition and during the first few weeks of calves' lives. The early prepartum period poses the highest risk of periparturient hypocalcemia, or insufficient serum calcium (Bhanugopan et al., 2010). Potassium supplementation before parturition could carry over high K concentrations from prepartum to post parturition and create a short lived hypomagnesemia in calves.

These macronutrients may also affect calves when feeding primarily off their mothers. Calves fed by mothers with low milk Mg maintained insufficient balances of Mg and Ca (Naik et al., 2010). Hypomagnesemia in calves is thought to be caused by reduced absorption in the intestine up to three months in age.

Prevention of Grass Tetany

Prevention methods have been established to decrease the susceptibility to grass tetany. Recognized methods include supplemental mineral feeding, soil liming, and P fertilization.

Mineral supplementation is commonly used to compensate for forage mineral nutrition deficits. Minerals can be mixed into a complete ration, or all missing mineral may be mixed together. Trace minerals may be fed separately in complete rations situations. Minerals can be also be added to grain to meet sufficient levels for cattle dietary needs. Minerals can be fed by completely free choice, where cattle come to minerals when needed or desired (Hale and Olsen, 2001). Free choice minerals may not completely meet dietary needs in some cattle due to lower consumption levels. Mineral supplementation can be convenient, but can be costly and time consuming for the producer.

Applications of calcium carbonate (lime) to soil can increase soil pH and change the availability of nutrients for plant uptake. Annual applications of lime increase plant available nutrients as soil pH reach optimum levels (5.5-7.0) Brady and Weil, 2017). For example, in permanent grasslands, P previously adsorbed on the soil colloid becomes more available, and allows for greater uptake by the resident grasses; subsequently increasing Mg and Ca translocation from root to shoot (Higgins et al., 2012). Soil liming does not increase the overall soil P, but does make more soil P available for plant utilization (Hamilton et al., 2012).

Phosphorus is the second most critical soil absorbed nutrient to plants, but is the most dilute and least mobile in soil (Poirier and Bucher, 2002). It is essential for plant

genetic composition, ATP energy synthases, photophosphorylation, and the transport of carbon from the chloroplast during photosynthesis. Phosphorus availability in soil affects root fineness, root length, and overall root fitness (Poirier and Bucher, 2002; Hong Liao and Yan, 2003). Plants will allocate more root growth to pockets of high soil P concentrations, than into pockets of lower P (Hong Liao and Yan, 2003).

Linkohr et al. (2002) found that increases in soil P increased root dry weight and lateral root density, while suppressed lateral root elongations in rice. These findings are consistent with previous studies on root responses to P availability; as roots elongate to obtain P in times of scarcity to locate higher concentrations of P instead of lateral root growth (Poirier and Bucher, 2002).

P increases osmotically driven xylem exudate and translocation of Mg and Ca (Reinbott and Blevins, 1991; 1999). In hydroponically grown winter wheat, increased availability of P increased concentrations of Mg and Ca in the shoots, but decreased K concentrations in the shoot.

From the studies of Reinbott and Blevins (1991; 1999) came field applications of P, particularly in tall fescue. Additional studies examined P applications in the field. Many of these studies focused on tall fescue, a commonly grown cool season perennial grass forage known to cause grass tetany in cattle (Sleper, 1979).

Reinbott and Blevins (1994) used Kentucky 31 tall fescue, established in low P soils, at the Southwest Research Center in Mt. Vernon, MO to evaluate the effects of increased P availability. Various P rates (0, 28, 56, and 112 kg ha^{-1}) were broadcasted with and without Mg (102 kgha^{-1}) .Compared to 0 P treatments, all P treatments resulted in increased leaf P, Ca, and Mg, and decreased leaf K concentrations. Mg fertilization

alone had no effect on P or Ca leaf concentrations, but when Mg was applied with P increased leaf P, Ca, and Mg were observed.

In another tall fescue study, Lock et al. (2002) compared fescue grown on low available P soils (6 lbs/acre Bray I) with and without 30 lbs/acre P fertilizer treatments. The 0 P fertilizer plots exhibited low leaf Mg in new spring growth. The fertilized plots exhibited greater Ca, Mg, and K leaf concentrations than the control. Both treated and untreated plots remained under the 2.2 grass tetany ratio level, but P treated plots maintained higher Mg and Ca concentrations throughout than the control (Lock et al., 2002).

Continued studies of P availability in fall fescue suggest that increased and repeated applications of P fertilizer can aid in decreasing the variation of grass tetany ratio due to increased soil available P (McClain and Blevins, 2007).

Cattle producers have tried for many years to prevent grass tetany, but several of the common practices have recently been discredited. One particular practice is applying animals manure to fields as fertilizer. One common application in Missouri is poultry litter. However, applications of poultry litter have been found to increase leaf K concentrations, while decreasing leaf Mg and Ca concentrations over time (McClain and Blevins, 2009). Even though poultry litter does contain P, high inputs of K found in poultry litter have a negative influence on Mg and Ca concentrations in the shoot tissue and accelerate the loss of these cations from the pasture (Kayser and Isselstein, 2005).

Similarly, dairy manure applications have been discredited as preventative measures for grass tetany. Although it is an inexpensive fertilizer, it often causes an accumulation of excessive soil K (Schonewille, 2013). In fact, the cation ratio in grass

pastures can remain above 2.2 in dairy manured pastures, even after three years of no additional applications (Cherney et al., 2002).

Annual Cereal Crops as Forages

More pasture managers are transitioning to use winter annual forage crops, such as winter wheat (*Triticum astevum*), oat (*Avena sativa*), and cereal rye (*Secale cereale*) as forages. Although winter annual forage crops provide biomass during winter months, Han and McCormick (2010) observed that winter annual forage crops are the most prone to mineral imbalances such as grass tetany. Chelliah et al. (2008) found when grown separately and mixed in Florida; the grass tetany ratio remained above the critical 2.2 level. Villalobos and Brummer (2017) examined cool season annual forages (monoculture and mixes) for forage quality. However, neither study included the effects of applications of P fertilizers on the species studies. To my knowledge it has not been documented how P availability affects leaf Ca, Mg, and K in winter annual forage crops.

This study addresses the need for further investigation of the effects of P availability on macronutrient concentrations within plant tissues by using hydroponic systems. I hypothesize that if I measure leaf nutrient concentrations of winter annual forage species grown hydroponically with various concentrations of P availability then I will find a decrease in the grass tetany ratio, K/ (Mg+Ca), in responses to higher levels of P availability. This study will use three species of annual cereal grains; winter wheat, oat, and cereal rye, grown in four different solutions varying in P: 0 μM, 200 μM, 400 μM, and 800 μM P.

MATERIALS AND METHODS

The hydroponic system was setup using clear polypropylene plastic tubs (35.375 inch x 16.75 inch x 5.875 inch) were used as base support for single pot replications of plants. A foam insert was placed inside the base with 12 (2 inch x 2 inch) square holes cut out to prevent tipping of solution containers (Figure 1A). High density polypropylene plastic containers (400 mL) were painted black to prevent light penetration then metallic gray to reflect light and prevent heat absorption (Figure 1B). In the lid, a two inch diameter hole was removed to place a two inch net pot and a 0.1875 inch diameter hole was removed (Figure 1B), to insert an aeration tube. The aeration tube consisted of natural latex tubing (0.25 inch ID, 0.375 inch OD, 0.0625 inch) attached to a high pressure, high output aquatic air pump (Figure 1C) (112 Watts, 110 liter per minute) (ActiveAqua, HydroFarm, Grand Prairie TX , USA). For oat and cereal rye, latex tubing (0.15625 inch OD, 0.046875 inch ID) (Figure 1A) with a 1 ml plastic pipette tip was attached to ActiveAqua 6-outlet metal air manifold (Figure 1C) (ActiveAqua, HydroFarm, Grand Prairie TX, USA). For wheat, the tubing was connected by a hypodermic needle (0.8mm x 25mm) to a main line (latex tubing (0.25 inch ID, 0.375 inch OD, 0.0625 inch natural latex tubing) coming from the pump.

Seeds were laid on top of cheese cloth that covered rockwool mineral fiber cubes (0.5 inch x 0.375 inch x 0.375 inch) (Gro|dan Grow Cubes, Roermond, The Netherlands) that filled 2 inch net pots (Figure 2). Approximately 22 seeds were placed in each pot. Winter wheat (Kingrazer Wheat, MO), oat (Bob Oats, AR), and cereal rye (Winter King Rye, KS) were started on September 6, 2016, July 11, 2016, and August 8, 2016,

respectively. Pots were placed in deionized (DI) water and under artificial fluorescent

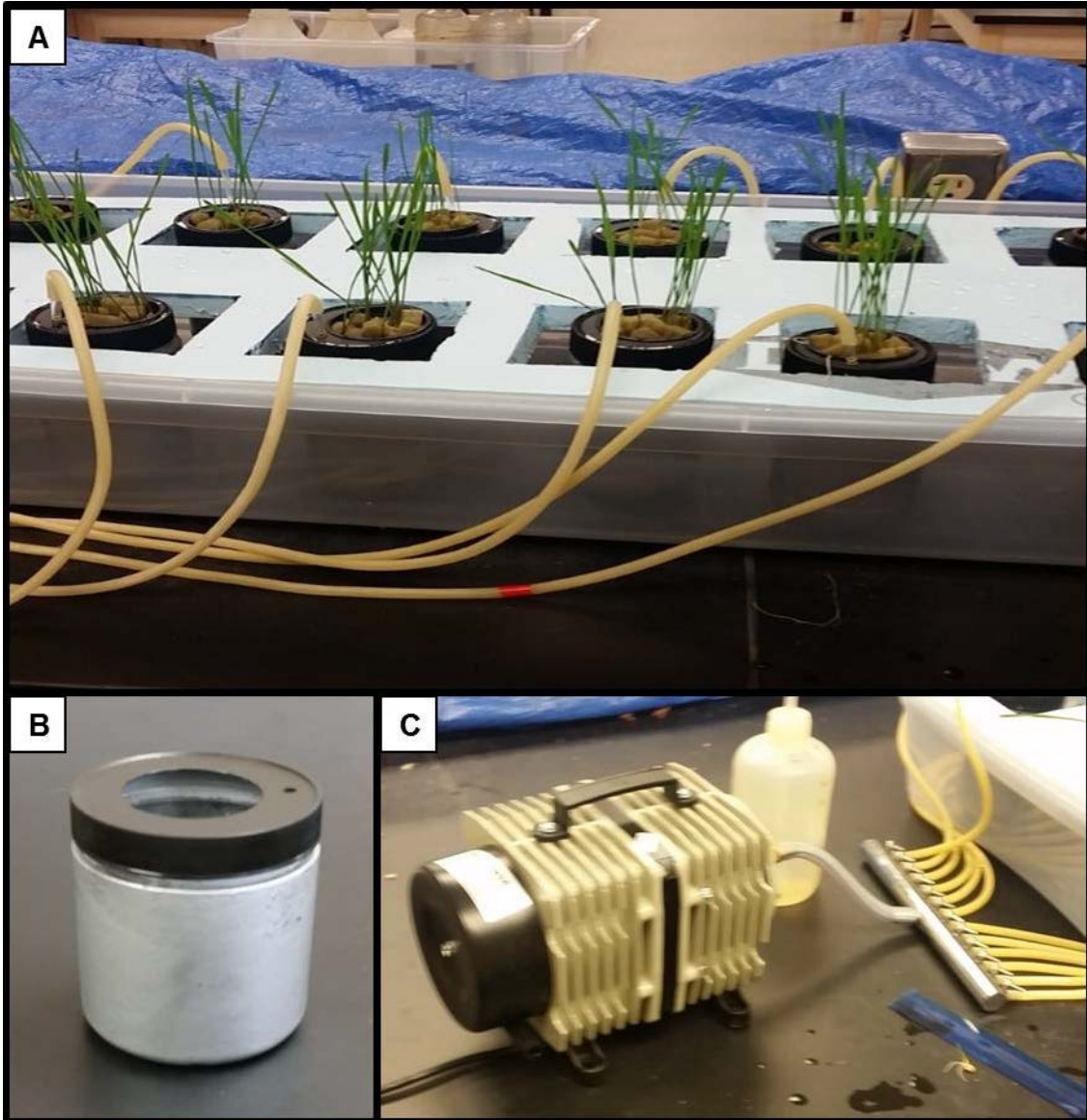light to germinate for seven days.

Figure 1. Hydroponic setup with (A) the clear polypropylene base support tub, foam insert, and latex aeration tubing, (B) the 400 ml containers and lids, and (C) the ActiveAqua air pump and manifold for aeration.

Figure 2. Two inch diameter net pots filled with rockwool mineral fiber cubes and topped with cheese cloth and seeds. Net pots are sitting in DI water.

On the seventh day, seedlings were culled to 15 plants per pot and the net pots were placed in the 400 mL hydroponic containers and moved to the greenhouse of Karls Hall, College of Agriculture building on the Missouri State University campus, Springfield, MO. Plants were grown in aerated DI waters for three days after being moved to the greenhouse. On the fourth day, the DI water was replaced by nutrient solution treatments. Four treatments were assigned randomly with three replicates of each treatment per block, and three replicate solutions. Solutions were constantly aerated. Three replicate blocks were randomly placed in the greenhouse. Solutions were a complete nutrient solution: (2 mM $CaCl_2$, 0.5 mM $MgSO_4$, 2.4 mM KCl, 2.5 mM NH_4NO_3, 12.5 μM FeSeq.330, 0.6 μM $ZnSO_4$, 0.1 μM $NaMoO_4$, 0.11 μM $NiCl_2$, 0.01 μM $CoCl_2$, 0.15 μM $CuSO_4$, 2.3 μM H_3BO_3, 0.9 μM $MnSO_4$) with P treatments of 0, 200, 400, and 800 μM P (using 1:4 Na_2HPO_4 and NaH_2PO_4). Sodium chloride was added to the solutions to balance the amount of sodium across all P treatments. Solutions were pH adjusted between 6.2 and 6.6 using HCl. After every three days of growth, the

nutrient solutions were discarded and replaced with new solutions. Daily maximum and daily minimum temperature and humidity at plant height were recorded daily and recorded using a Fisher Scientific Hygro-thermometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA).

After 32 days of growth from planting date, instantaneous photosynthesis was measured with 400 μM $CO_2m^{-2}s^{-1}$ and 1500 $\mu molm^{-2}s^{-1}$ light intensity using Li-6400XT (Li-Cor Inc., Lincoln, Nebraska, USA). Measurements were taken between 10 AM and 2 PM. Temperature at the time of measurement were 71, 89, and 89 °F in wheat, oat, and cereal rye, respectively.

Plants were harvested, directly after photosynthesis measurements (after 32 days of growth). From each pot, number of plants and tillers were counted, shoots were combined into one sample, and roots were combined into one sample. Roots were washed in DI water three times and weighed, placed in paper bags, and, dried in a temperature control forced air (Cacsade Tek, Cornelius, Oregon, USA) oven to dry at 50°C, and analyzed (1 pot = 1 sample). Dry weights of each sample were measured and recorded; dry weight of sample was divided by the total number of plants in that sample to report biomass as a dry weight per plant. Winter wheat and oat samples were ground using a Cyclone Mill Sample Mill (UDY Corporation, Fort Collins, Colorado, USA). Cereal rye was ground using a modified coffee grinder (Jarden Customer Solutions, Boca Raton, Florida, USA). Ground samples were placed in two ounce Whirl-Pack® write-on bags for storage (Nasco, Fort Atkinson, Wisconsin, USA).

Near infrared spectroscopy (SpectraStar, Unity Scientific, Milford, Massachusetts, USA) was used to analyze sample quality measurements of percent crude protein (CP),

acid detergent fiber (ADF), and neutral detergent fiber (aNDF) in the samples using the Unity Scientific forage analyzer package "Grass Hay".

Samples were digested to evaluate mineral concentrations. Dried tissue samples weighing 0.2495- 0.2505 g from each sample were digested in 5 ml of trace grade nitric acid (ThermoFisher Scientific, Waltham, Massachusetts, USA) using a MARS 6 Microwave Accelerated Reaction System (CEM Corp., Matthews, North Carolina, USA). The MARS 6 Plant Material method was used in order to completely digest samples. This method included a 20 minute ramp to 200 °C where it was held at a constant for 10 minutes. Samples were allowed to cool to 70 °C before ventilation. Digested samples were transferred to 20 ml clear polypropylene vials and diluted with DI water to a final volume of 25 ml. Samples were filtered using Q8 course, fast flowing, 11cm filter paper (ThermoFisher Scientific, Waltham, Massachusetts, USA) and stored in 25 ml polypropylene scintillation vials.

Phosphorus concentrations (% by weight) in digested samples were determined using a colorimetric assay (Murphy and Riley, 1962). Samples were diluted 1:20 (50 μL sample: 950 μL DI H_2O) or 1:40 (25 μL: 975 μL DI H_2O). Diluted samples (1ml) were pipetted into cuvette, followed by an ascorbic acid working solution (4 mL), vortexed, and allowed to develop for 30 minutes. Standard solutions of 0.00, 0.50, 1.00, 2.50, and 5.00 ppm P in DI H_2O were used to determine the absorbance curve using a GENESYS™ 20 visible spectrophotometer (Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 660 nm.

Atomic Absorption/ Flame Emission Spectrophotometry (Agilent Technology, 200 Series AA, Santa Clara, California, USA) was used to determine concentrations (%

by weight) of Ca, Mg, and K in the digested samples. The analytical wavelengths were set at 766.5 nm (K), 285.2 nm (Mg), and 422.7 nm (Ca). All samples were diluted 1:20 using 0.105% lanthanum (La) (from lanthanum oxide). Standard solutions were made using a background 1% HNO_3 and 0.105% La. Standards were 1.00, 2.00, 3.00 and 4.00 ppm for Ca, 0.25, 0.50, 1.00, 1.50, and 2.00 ppm for Mg, and 0.25, 0.50, 1.00, and 2.50 ppm for K.

This experiment was a randomized complete block design. P treatments and block were fixed factors. This model was used to test for statistical significance of P treatments effects as well as interaction with block and treatment using a general linear model (PROC GLM) in SAS version 9.4 (SAS Institute, 2017). All effects and interactions were considered significant when means differed at $P<0.05$. Means were separated by Tukey's pairwise comparison.

Greenhouse Temperature and Humidity

For the wheat experiment, daily temperatures ranged from 66.6 to 108.2 °F and daily humidity ranged from 25 to 97% (Figure 3). For oat, daily temperatures ranged from 70.2 to 101.8°F and daily humidity ranged from 45 to 98% (Figure 4). And for cereal rye, daily temperatures ranged from 63.2 to 107.8°F and daily humidity ranged from 21 to 98% (Figure 5).
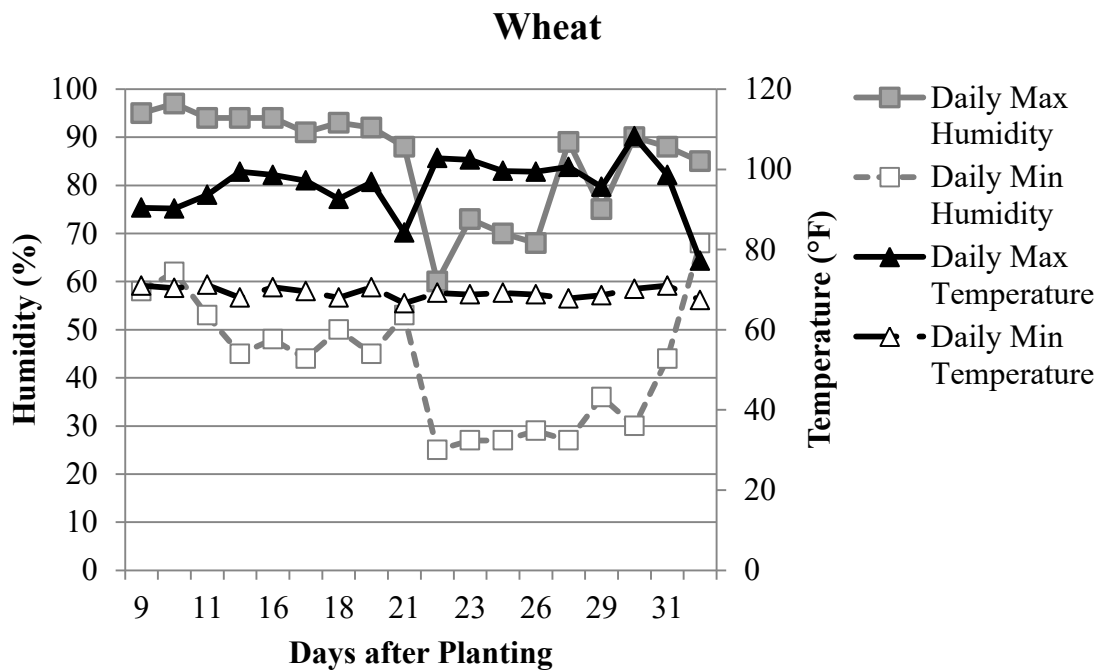
Wheat

Figure 3. Daily minimum (Min) and maximum (Max) temperature and humidity of wheat over 32 days of growth in greenhouse conditions.
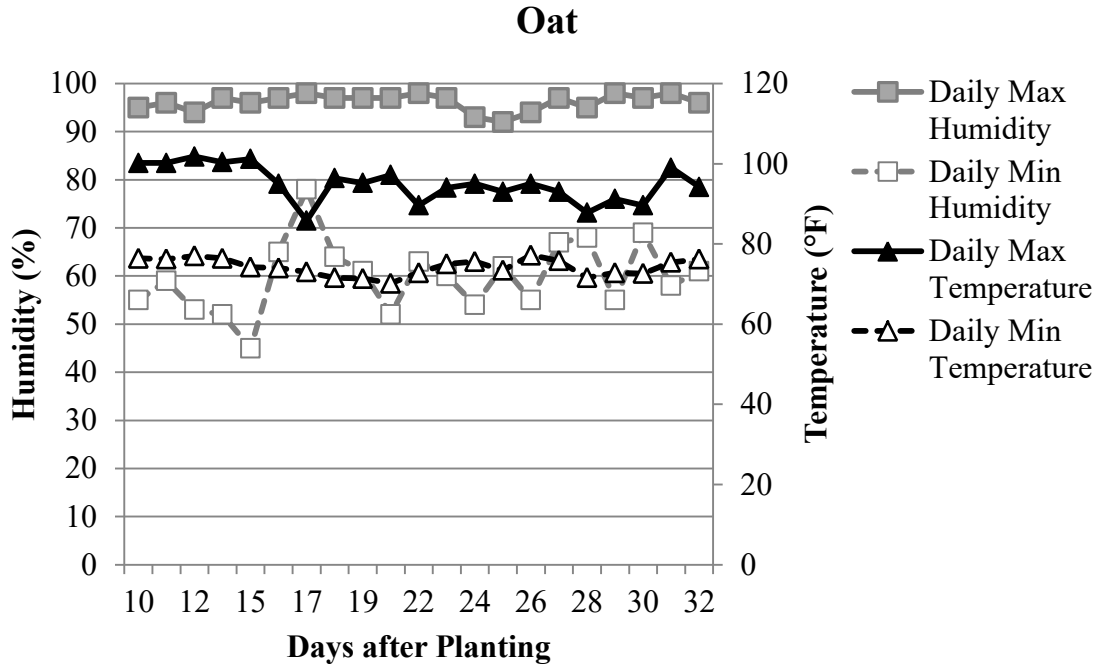
Oat

Figure 4. Daily minimum (Min) and maximum (Max) temperature and humidity of oat over 32 days of growth in greenhouse conditions.
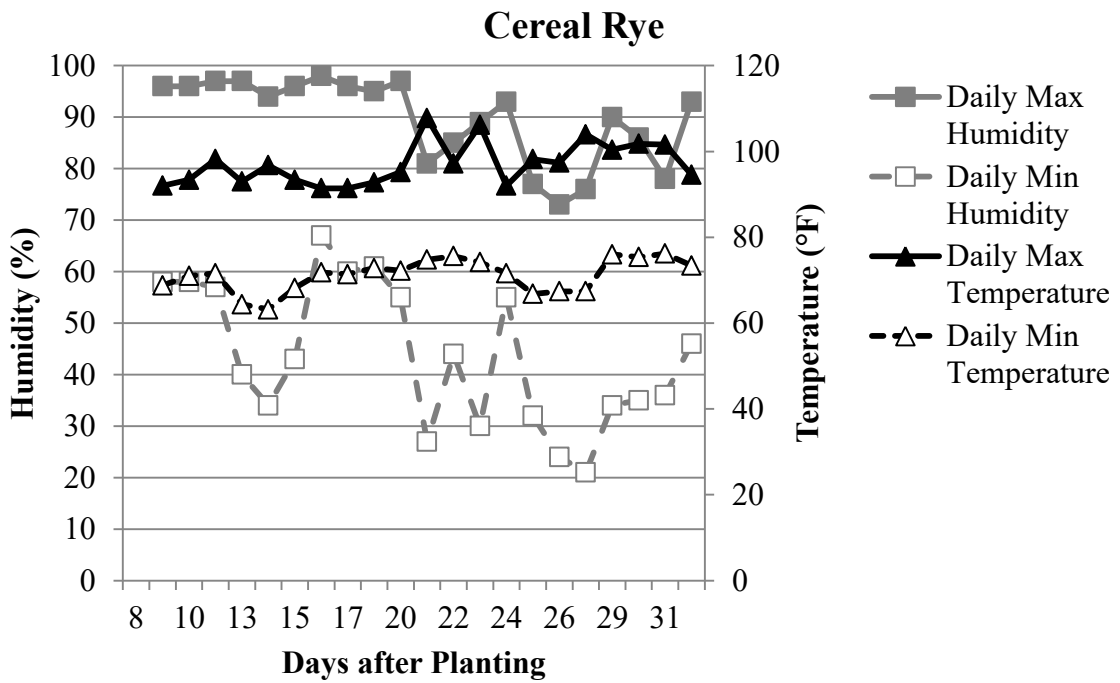
Cereal Rye

Figure 5. Daily minimum (Min) and maximum (Max) temperature and humidity of cereal rye over 32 days of growth in greenhouse conditions.

Biomass

Shoot biomass increased with increasing P treatments in all species studied (Figure 6). Differences were incremental in wheat leaves from 0 to 800 µM P, but in oat and cereal rye leaves only the 0 µM P growth was different. Wheat shoot biomass increased 1.7- fold from 0 and 200 µM P, with an additional 14% increase from 200 and 400 µM P, and 26% increase from 400 and 800 µM P. In oat and cereal rye shoot biomass increased 1.5- and 3.8- increase, respectively, from 0 to 200 µM P. Wheat root growth was suppressed with increasing P treatments. However oat roots exhibited no differences in growth, and cereal rye was only suppressed in 0 µM P (Figure 6).
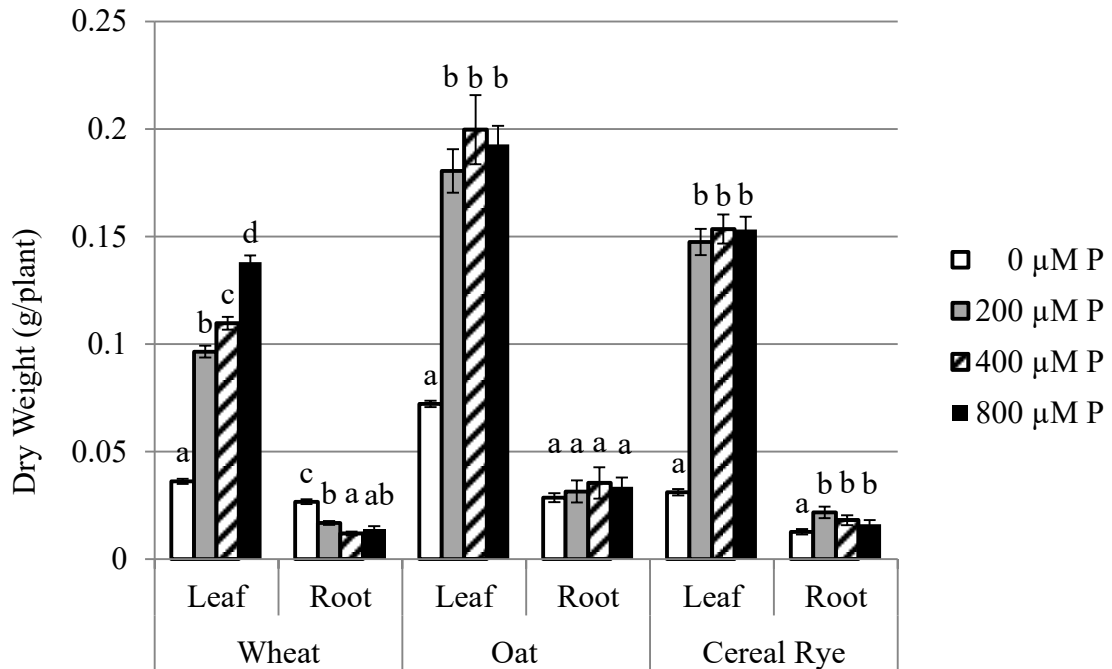

Figure 6. Dry weight of shoots and roots after 32 days of growth of wheat, oat, and cereal rye grown in various P treatments. Values are treatment means ± SE, n=9. Within species and tissue type, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).

Photosynthesis

Photosynthetic rate increased with greater P treatments; wheat showing incremental differences from 0 and 800 µM P, and oat and cereal rye with differences from 0 and 200 µM P (Figure 7). Wheat photosynthesis increased 28% from 0 and 200 µM P, 15% from 200 and 400 µM P, and an additional 18% from 400 and 800 µM P (Figure 7). Photosynthetic rates in oat and cereal rye exhibited a 1.5- and 0.95-fold increase, respectively, from 0 and 200 µM P (Figure 7).
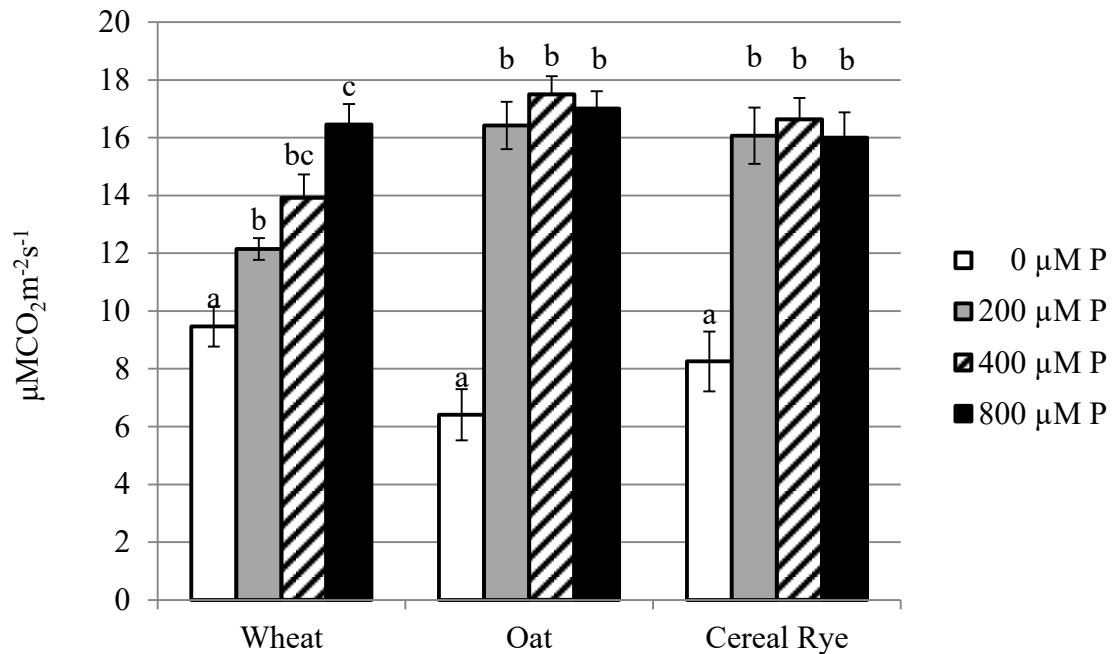

Figure 7. Photosynthetic rates of shoots and roots after 32 days of growth of wheat, oat, and cereal rye grown in various P treatments. Instantaneous rates measured at 400 µM $CO_2 m^{-2} s^{-1}$ and 1500 $\mu mol m^{-2} s^{-1}$. Treatment means $\pm$ SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).

Mineral Concentrations

All species exhibited incremental increases in leaf and root P with increased P treatments. From 0 to 800 µM P there was a 5.5-, 6.7-, and 4.3- fold in wheat, oat, and cereal rye, respectively in leaf P (Figure 8). Root P increased from 0 to 800 µM P a 5.2-, 10.5-, and 5.3- fold in wheat, oat, and cereal rye (Figure 9), respectively.

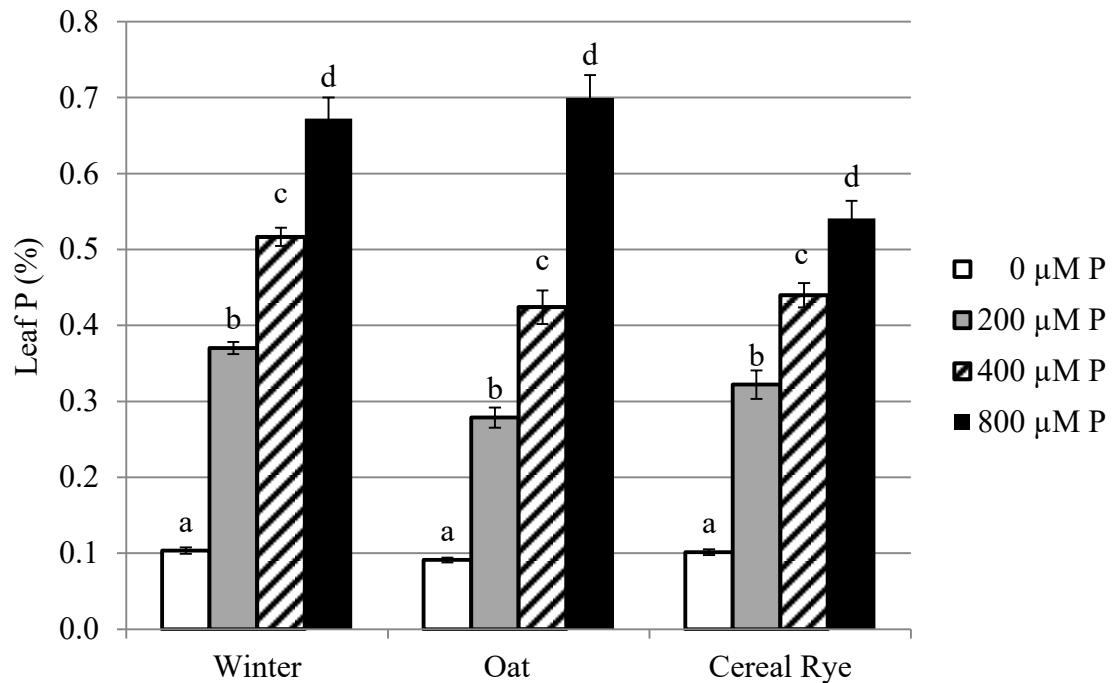Figure 8. Leaf P content of wheat, oat, and cereal rye after 32 days of growth in various P treatments. Treatment means ± SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).
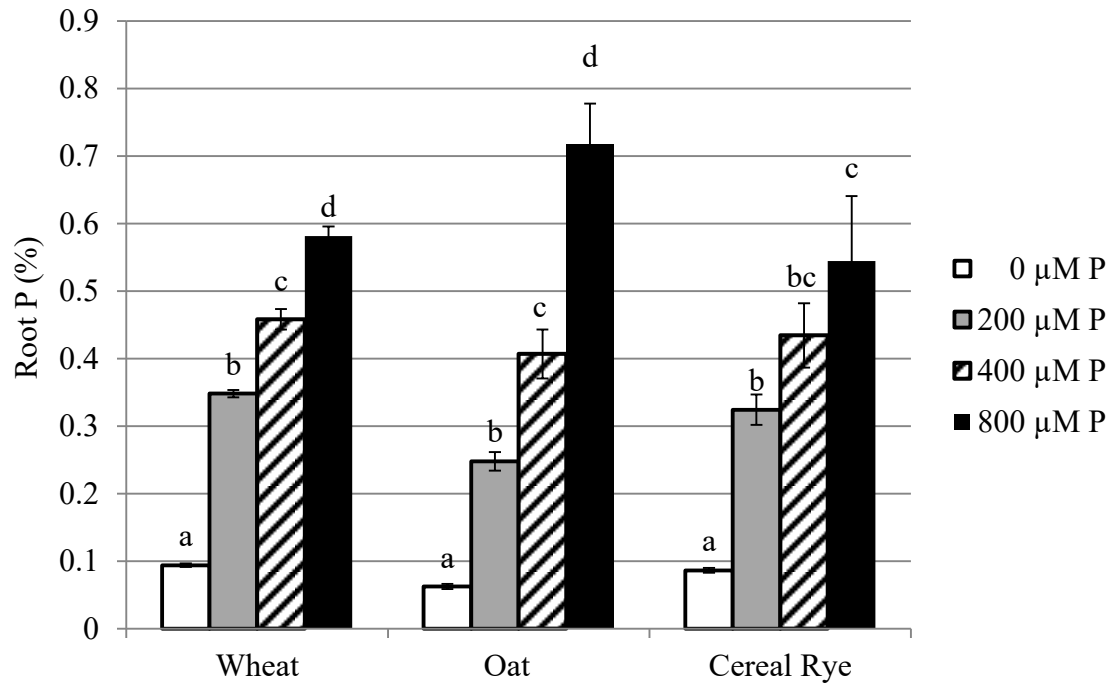
Figure 9. Root P content of wheat, oat, and cereal rye after 32 days of growth in various P treatments. Treatment means ± SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).

In wheat and cereal rye, leaf concentrations of Ca (Figure 11) and Mg (Figure 12) were greater in 0 µM P treatments than 200 µM P. The severe stunting (<0.05 g/plant) and necrosis of the shoots in these species (Figure 10 A and C) suggest the increased nutrients were the result of stunted development and not a factor of greater nutrient uptake and accumulation in healthy tissue. Therefore, when examining the effects of P treatments on nutrient concentration of the shoots of wheat and cereal rye with 0 µM P treatment will not be compared to the treatments with P (200, 400, and 800 µM P).

Figure 10. Shoots and roots of (A) wheat, (B) oat, and (C) cereal rye after 32 days of growth grown in increasing P treatment left to right; 0, 200, 400, and 800 µM P.

Wheat showed incremental increases in leaf Mg from 200 to 800 µM P, resulting in a 17% total increase in leaf Mg (Figure 12). However, leaf Ca (Figure 11) and K (Figure 13) did not change with increased P treatments. Wheat roots increased in Ca (Figure 14) and Mg (Figure 15) from 200 to 400 µM P, but decreased in K incrementally from 200 to 800 µM P (Figure 16).

Oat did not show differences in leaf Mg (Figure 12) or Ca (Figure 11) from 200 and 800 µM P treatments, but did demonstrate upward trends in concentrations. Unlike wheat and cereal rye that exhibited severe stunted growth and necrosis (Figure 10 A and C), oat maintained healthy tissue and growth (>0.05 g/plant) at 0 µM P (Figure 10 B). It should be noted that from 0 and 200 µM P there were significant increases in leaf Ca (Figure 11) and Mg (Figure 12), yet leaf K (Figure 13) did not change with increased P treatments. Oat roots increased in concentrations of Ca (Figure 14) and Mg (Figure 15) from 200 to 800 µM P, and K from 0 to 200 µM P (Figure 16).

Cereal rye showed increases in leaf Ca (27%) (Figure 11) and Mg (15%) (Figure 12) from 200 and 800 µM P. No differences were found in leaf K from 200 and 800 µM P (Figure 13). Additionally, no differences were found in root Ca (Figure 14), Mg (Figure 15), or K in cereal rye (Figure 16).

Figure 11. Leaf Ca content of wheat, oat, and cereal rye after 32 days of growth in various P treatments. Treatment means ± SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).

Figure 12. Leaf Mg content of wheat, oat, and cereal rye after 32 days of growth in various P treatments. Treatment means ± SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).
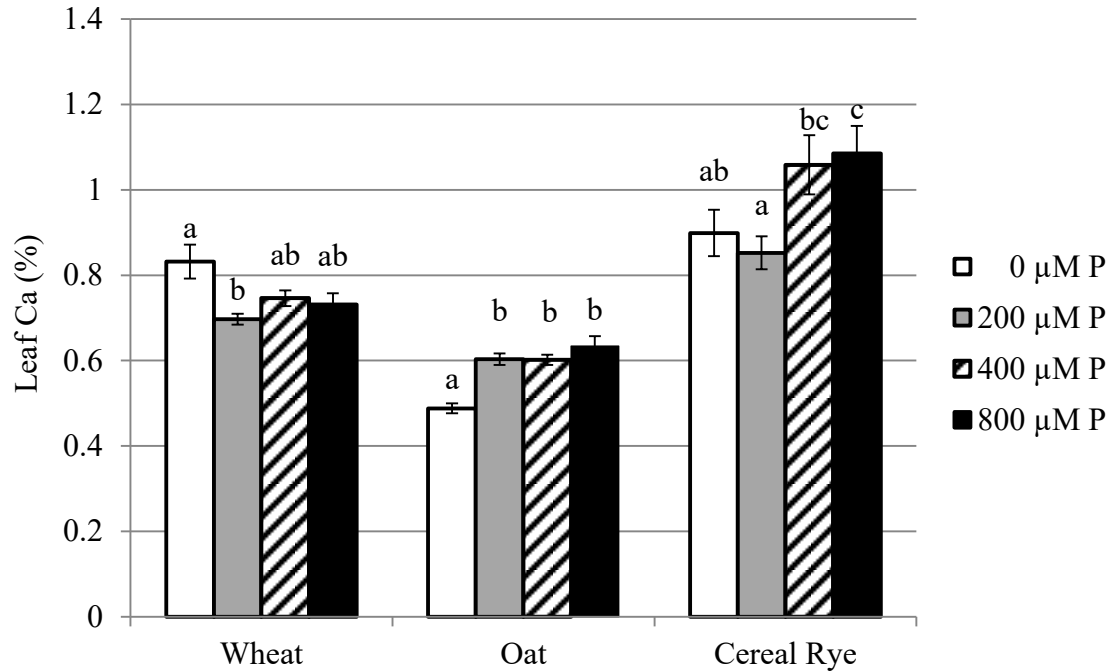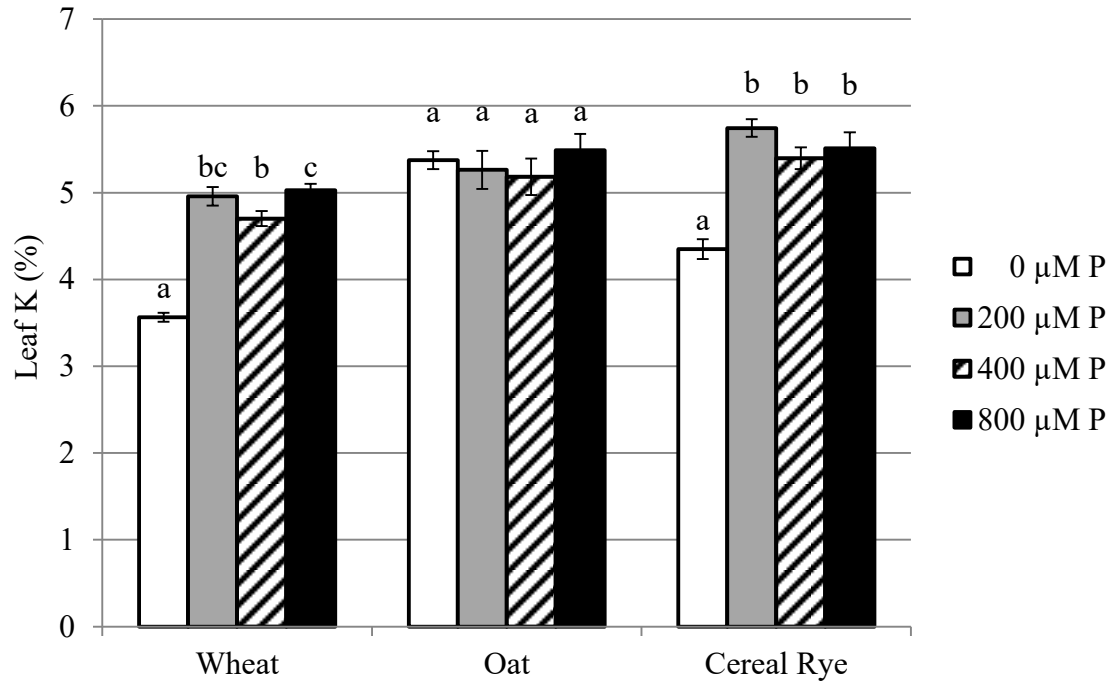
Figure 13. Leaf K content of wheat, oat, and cereal rye after 32 days of growth in various P treatments. Treatment means ± SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).
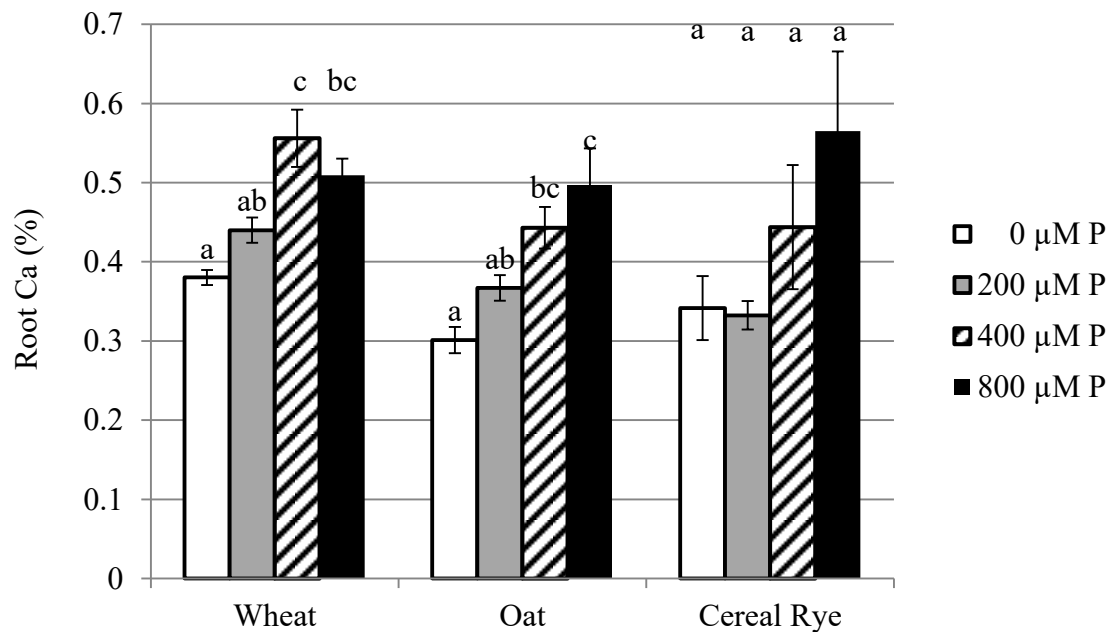
Figure 14. Root Ca content after 32 days of growth of wheat, oat, and cereal rye grown in various P treatments. Treatment means ± SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).

Figure 15. Root Mg content after 32 days of growth of wheat, oat, and cereal rye grown in various P treatments. Treatment means ± SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).
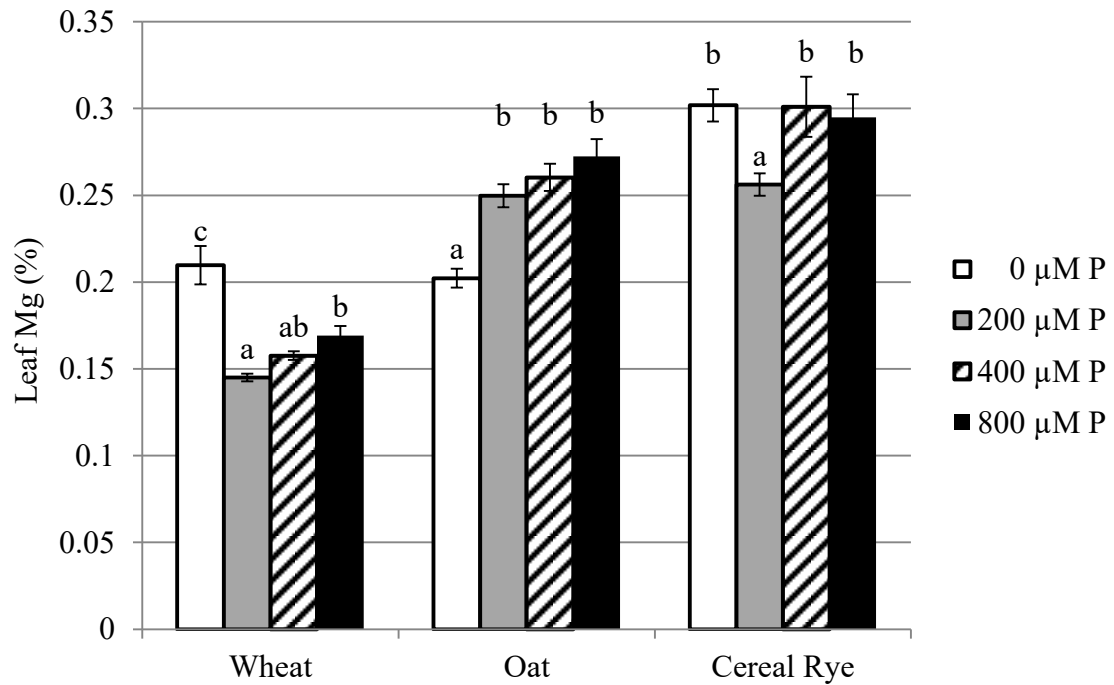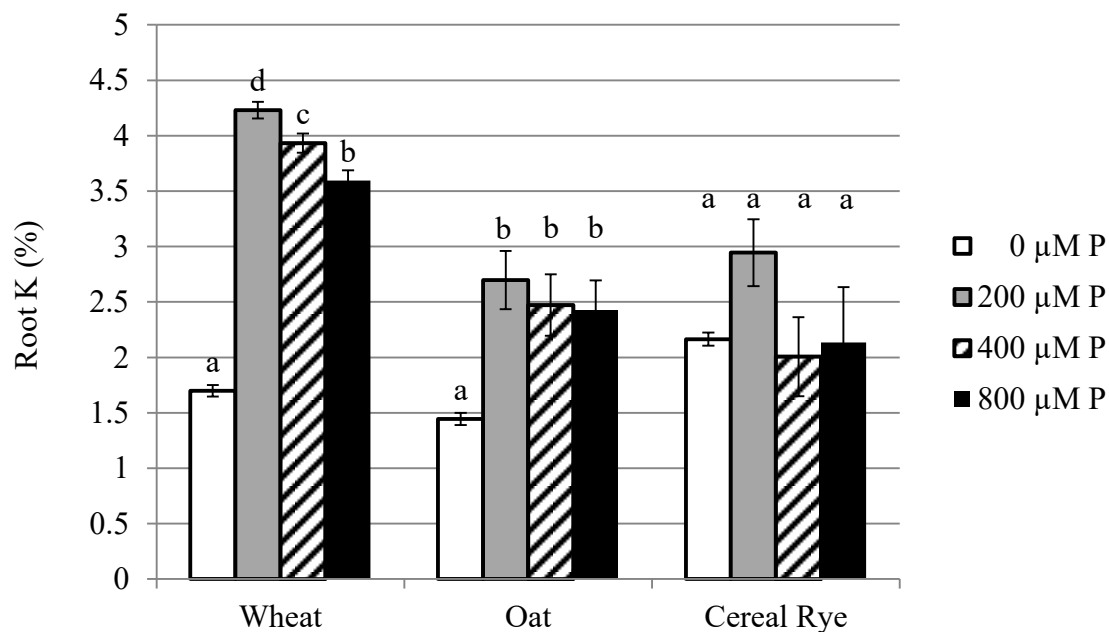

Figure 16. Root K content after 32 days of growth of wheat, oat, and cereal rye grown in various P treatments. Treatment means ± SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).
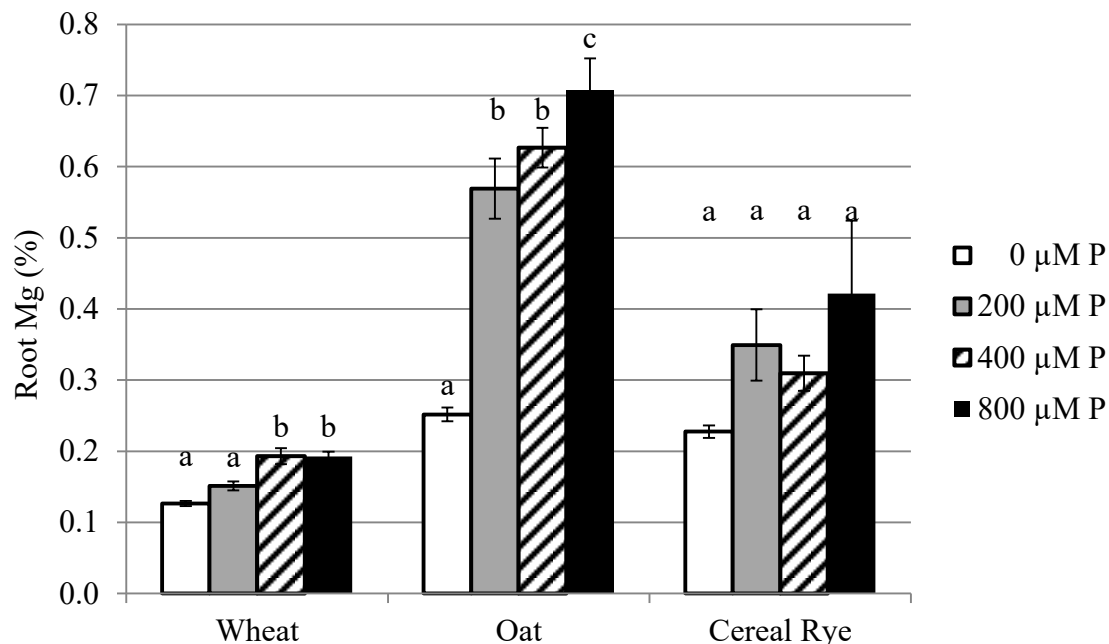
Grass Tetany Ratio

The grass tetany ratio was decreased due to P treatments in all species studied. Discounting 0 µM P due to stunting and necrotic tissue in wheat, decreases in the grass tetany ratio were observed from 200 and 400 µM P (Figure 17). Decreases were observed from 0 and 200 µM P in oat and from 200 and 400 µM P in cereal rye (Figure 17).
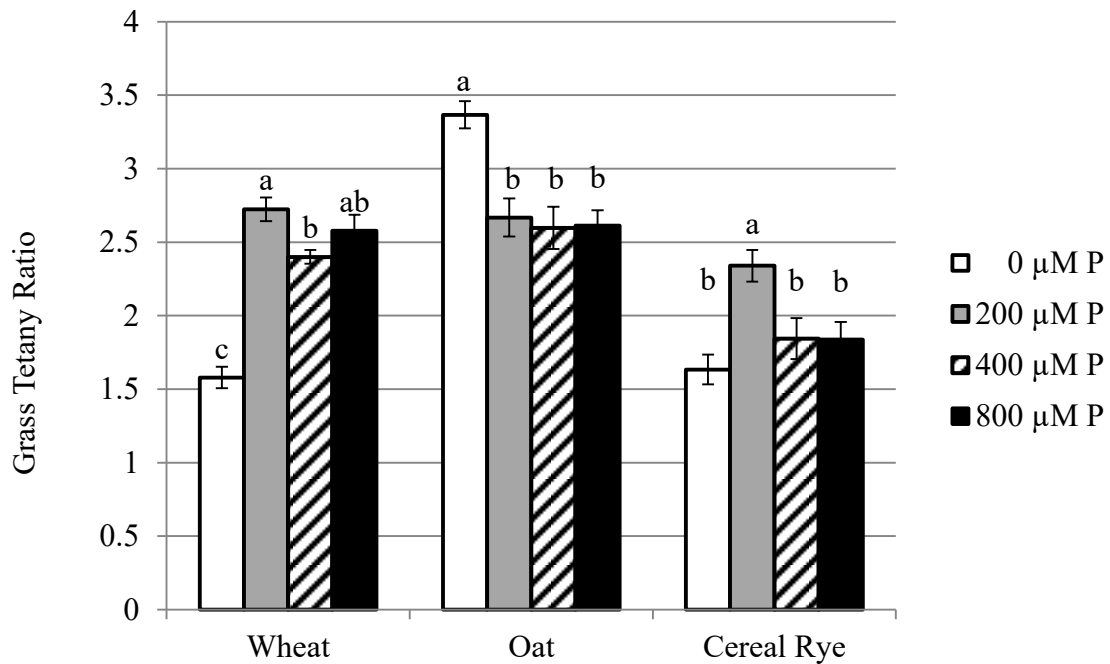
Figure 17. Grass Tetany Ratio of leaf tissue after 32 days of growth. Treatment means ± SE, n=9. Within species, values not followed by the same letter are significantly different (p< 0.05, Tukey's pairwise comparisons).

Forage Quality Measurements

Wheat CP increased between 0 µM P treatment and other treatments while ADF increased from 200 and 800 µM P (Table 1). No differences were found in wheat aNDF due to P treatments (Table 1). Oat CP increased from 0 to 200 µM P, while ADF and aNDF decreased from 0 to 200 µM P, but then increased with higher P treatments (Table

2). Cereal rye CP decreased from 0 to 400 µM P (Table 3). Cereal Rye ADF increased

from 0 to 200 µM P, while aNDF increased from 0 to 200 µM P and 200 to 800 µM P

(Table 3).

Table 1. Wheat forage quality measurements of % crude protein (CP), % acid detergent fiber (ADF), and % neutral detergent fiber (aNDF) after 32 days of growth in various P treatments.

Treatment		CP (%)	ADF (%)	aNDF (%)
0 µM P		23.896±0.352a	24.668±0.388ab	45.260±0.570a
200 µM P		30.767±0.293c	23.693±0.162a	44.044±0.268a
400 µM P		30.403±0.372bc	24.542±0.302ab	44.452±0.331a
800 µM P		29.361±0.361b	25.314±0.295b	45.272±0.523a
	df	ANOVA *F* Value and (probability)		
Treatment	3	108.62 (<0.0001)	4.73 (0.0099)	2.59 (0.0764)
Block	2	3.45 (0.0482)	1.73 (0.1987)	3.79 (0.0371)
Treatment*Block	6	1.62 (0.1838)	0.46 (0.8283)	1.99 (0.1076)

Column means (± Std. Error) across P treatments that are not followed by the same letter are significantly different (p<0.05, using Tukey's pairwise comparisons).

Table 2. Oat forage quality measurements of % crude protein (CP), % acid detergent fiber (ADF), and % neutral detergent fiber (aNDF) after 32 days of growth in various P treatments.

Treatment		CP (%)	ADF (%)	aNDF (%)
0 µM P		19.542±0.277a	27.892±0.173c	47.262±0.329c
200 µM P		22.534±0.743b	23.839±0.362a	40.649±0.372a
400 µM P		21.374±0.830b	24.831±0.284ab	42.087±0.533b
800 µM P		21.353±0.675b	25.640±0.239b	42.623±0.165b
	df	ANOVA F Value and (probability)		
Treatment	3	12.26 (<.0001)	38.02 (<.0001)	64.24 (<.0001)
Block	2	30.67 (<.0001)	1.20 (0.3181)	1.41 (0.2641)
Treatment*Block	6	4.75 (0.0026)	0.68 (0.6665)	1.36 (0.2717)

Column means (± Std. Error) across P treatments that are not followed by the same letter are significantly different (p<0.05, using Tukey's pairwise comparisons).

Table 3. Cereal Rye forage quality measurements of % crude protein (CP), % acid detergent fiber (ADF), and % neutral detergent fiber (aNDF) after 32 days of growth in various P treatments.

Treatment		CP (%)	ADF (%)	aNDF (%)
0 µM P		29.407±0.404b	22.760±0.197a	40.472±0.195a
200 µM P		27.623±0.452ab	25.318±0.356b	44.464±0.296b
400 µM P		25.939±0.504a	25.992±0.416b	45.252±0.378bc
800 µM P		26.027±0.775a	26.374±0.368b	46.044±0.477c
	df	ANOVA F Value and (probability)		
Treatment	3	12.35 (<0.0001)	24.68 (<0.0001)	67.69 (<0.0001)
Block	2	3.17 (0.0601)	4.72 (0.0187)	4.90 (0.0164)
Treatment*Block	6	2.48 (0.0518)	0.32 (0.9196)	1.66 (0.1734)

Column means (± Std. Error) across P treatments that are not followed by the same letter are significantly different (p<0.05, using Tukey's pairwise comparisons).

DISCUSSION

Biomass

Increased biomass due to P treatments was expected, as found in other species by Proirier and Bucher (2002) and Hong Liao and Yan (2003). Wheat exhibited incremental growth with increased P availability, while oat and cereal rye did not show increased biomass above 200 µM P treatments. This suggests that for oat and cereal rye, the 200 µM P treatment is either adequate available P for biomass or that a treatments greater than 800 µM P would further increase growth. However, it is worth noting that increased leaf P concentrations at 800 µM P, compared to 200 and 400 µM P, did not further increase biomass or photosynthetic rates in oat or cereal rye. This suggests that leaf P from the 200 µM P treatment may be adequate for biomass in these species.

Mineral Nutrition

Increased P tissue concentrations were expected with treatments. These results are similar to that of Reinbott and Blevins (1999) in that higher P availability increases mobilization from root to leaves.

In this study, greater available P increased root Ca and Mg in oat and cereal rye, however leaf concentrations were not increased. While available P was adequate for uptake of Ca and Mg into the roots, it was not enough for remobilization into the leaf tissue. Greater quantities of available P may be needed to remobilize accumulated root Ca and Mg into leaf tissue.

Oat was the only species that showed healthy growth at 0 µM P. The increase in leaf Ca and Mg between 0 and 200 µM P and subsequent decrease in the grass tetany

ratio, suggests that when grown in P deficient conditions, an increase in available P, could result in a decreased risk of grass tetany.

At higher levels of available P, between 200 and 800 µM P, cereal rye increased in leaf Ca and Mg. The increases in leaf Ca and Mg at higher levels of available P resulted in a lower grass tetany ratio.

In wheat, the decrease in grass tetany ratio from 200 to 400 µM P was due to a decrease in K along with an increase in Mg. This decrease in K was not found at the 800 µM P level, so the grass tetany ratio is not improved.

In oat, the grass tetany ratio was decreased from 0 to 200 µM P due to increased leaf Ca and Mg with no change in K. Similarly in cereal rye, from 200 to 400 µM P, increases in leaf Ca and Mg without changes in K led to a decreased grass tetany ratio. Similar to the oat and cereal rye mixes in Chelliah et al. (2008), the oat in this study remained above the 2.2 grass tetany susceptibility threshold.

In wheat, it is possible to see increases in growth, leaf P, and an improved grass tetany ratio with higher levels of P availability. In oat and cereal rye, it is possible to see increased leaf P and improved grass tetany ratio with higher levels of P availability, despite not having increased growth. This suggests that increased P availability can increase leaf P and improve the grass tetany ratio, with or without increased yields in annual cereal forages.

In this study, plants were grown a relatively short period of time (32 days), and as the plants mature changes in macronutrients concentrations and forage quality could be found. McDowell (1985) noted that immature plants are typically higher in K than mature plants. It should be considered that the grass tetany ratio may decrease with time, if the

observed species were grown longer. Additionally, CP, ADF, and aNDF are greatly influenced by plant maturity. Effects of P availability on forage quality measures could also change with plant maturity.

Further Research

These finding suggest that winter annual forage species react similarly to increased P availability as tall fescue. This is important for the beef and dairy industry in Missouri, as winter feeding becomes more economical when dietary deficiencies can be avoided. These findings also create a basis for further research.

Further research should be conducted on the effects of P availability greater than 800 µM P on growth and leaf nutrient content. This could be done in a similar fashion as the current experiment and could result in the determination of toxicity zones of P availability in the species examined. Further hydroponic research on the effects of P availability on the macronutrient concentrations of winter annual forages could also be done where temperature and humidity would more closely reflect normal field soil and air temperatures during the winter months.

Also, field research should be conducted to examine how available soil P levels effects the macronutrient concentrations of winter annual forage species. Field studies would greatly benefit producers, as it could give an estimated fertilizer value to alleviate the risk of grass tetany in cattle grazing winter annual forage crops. Preliminary field research on the effects of P availability on wheat, oat, and cereal rye has been initiated at the Missouri State University Shealy Farm near Pleasant Hope, MO.

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