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# Phosphorus Improves Leaf Nutrient Concentrations in Wheat, Oat, and Cereal Rye

Jerri Lynn Henry, William E. McClain II, and Melissa A. Remley\*

## Core Ideas

- Increased P availability increases leaf P and Mg in wheat, oat, and rye.
- The grass tetany ratio is improved with greater P availability in these species.
- Unlike cereal rye and oat, wheat increases shoot growth with high P levels.

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## ABSTRACT

Winter annual species grown for forage are prone to mineral imbalances that could result in animal nutritional disorders, such as grass tetany. Adequate soil P has been found to be critical for the growth and adequate nutrient content of Mg, Ca, and K in other forages for grazing animals. This study examined the effect of P availability on growth and leaf nutrients in annual cereal grains commonly grown for winter forage. Winter wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), and cereal rye (*Secale cereal* L.) were grown hydroponically in greenhouse conditions in complete nutrient solutions with P treatments of 0, 200, 400, and 800  $\mu\text{mol L}^{-1}$  P. After 32 d, plants were harvested and analyzed for P, Mg, Ca, and K content. Shoot growth of all three species increased from 0 to 200  $\mu\text{mol L}^{-1}$  P; however, only wheat shoots increased incrementally with the other P treatment concentrations. Leaf P also responded incrementally to increased P treatments in all three species. Wheat and cereal rye exhibited increases in leaf Mg and improved grass tetany ratio from 200 to 400  $\mu\text{mol L}^{-1}$  P, whereas oat showed these improvements from 0 to 200  $\mu\text{mol L}^{-1}$  P treatments. This study suggests increased P availability could improve the grass tetany ratio, with or without increased shoot growth, in winter annual forage production on low P soils.

Abbreviations: DAP, days after planting; DI, deionized.

More pasture managers are utilizing annual cereal grain species, such as winter wheat (*Triticum aestivum* L.), oat (*Avena sativa* L.), and cereal rye (*Secale cereal* L.), as forage crops during the winter months to extend their growing and grazing seasons. These species can be planted late fall or early spring, allowing for forage biomass accumulation during months of reduced growth of perennial cool- and warm-season grasses (Ball et al., 2015). Although winter annual forage crops provide biomass during winter and early spring months, they are the most prone to mineral imbalances in the leaf tissue, which could result in nutritional disorders within animals consuming the forage (Chelliah et al., 2008; Han, 2010). One critical nutritional disorder in grazing cattle (*Bos taurus*) is grass tetany (hypomagnesemia). Grass tetany is typically linked to low levels of Mg in the blood serum of cattle (Stewart et al., 1981). The lack of Mg in blood serum is directly associated with a balanced ratio of certain nutrient concentrations of the plant tissues consumed by the animal. The grass tetany ratio is calculated as  $\text{K}^+ / (\text{Ca}^{2+} + \text{Mg}^{2+})$  on an equivalent basis, and cattle become susceptible to grass tetany when the ratio is greater than 2.2 in the diet (Kemp and Hart, 1957).

In both plants and animals the three macronutrients in the grass tetany ratio are interlinked in their absorption and translocation. In animals, high concentrations of K depresses the absorption of Mg through the rumen wall into the bloodstream (Bhanugopan et al., 2010; Reinhardt et al., 1988; Schonewille et al., 1999). Similarly in plants, K

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availability poses an antagonistic relationship with Mg in plant shoot tissue (Ohno and Grunes, 1985). High levels of soil K can depress Mg uptake (Wilkinson, 1983) and has been shown to depress leaf Mg and Ca in oat (Ragab, 1979). Ohno and Grunes (1985) found K fertilization resulted in decreased Mg concentrations in winter wheat thus creating greater grass tetany ratio values.

Phosphorus nutrition has also been linked with Ca and Mg levels in plant leaf tissue. Reinbott and Blevins (1999) found increased P availability increased Ca and Mg translocation from roots to shoots in hydroponically grown squash resulting in greater leaf concentrations of Mg, Ca, and K. However, greater increases in leaf Mg and Ca relative to K decreased the grass tetany ratio. In hydroponically grown winter wheat, increased P availability also resulted in increased shoot Mg and Ca, and decreased K concentrations (Reinbott and Blevins, 1991). The effect of P availability on the grass tetany ratio was also explored in field studies in tall fescue [*Schedonorus arundinaceus* (Shreb.) Dumort], a common cool-season perennial grass forage known to cause grass tetany in cattle (Sleper, 1979). On low P soils, Reinbott and Blevins (1994) found P fertilization to increase leaf P, Ca, and Mg, and decrease leaf K concentrations in tall fescue. Similarly, P fertilization of tall fescue on low-P soils was found to increase leaf Ca, Mg, and K in spring growth (Lock et al., 2002) and during the fall and winter stockpiling period (McClain and Blevins, 2007).

Common sources of P fertilizer, such as poultry litter and dairy manure, also contain high amounts of K, and may not provide the proper nutrient balance for improved leaf nutrients. Applications of poultry litter decreased leaf Mg and Ca concentrations over time in grasses (Kayser and Isselstein, 2005; McClain and Blevins, 2009). Similarly, dairy manure applications often cause an accumulation of soil K (Schonewille, 2013), resulting in grass tetany ratios remaining above 2.2 in dairy manured pastures (Cherney et al., 2002).

Winter annual forage crops have been reported to have nutrient imbalances that may result in increased risk for grass tetany. Chelliah et al. (2008) found cereal rye, oat, and ryegrass (*Lolium multiflorum* Lam.), grown separately and mixed, to have a grass tetany ratio above the critical 2.2 level. Han (2010) reported cool-season annual forage mixtures of ryegrass, wheat, oat, and cereal rye to have greater grass tetany ratios than warm-season annuals, warm-season perennials, and cool-season legumes. Phosphorus nutrition has been shown to improve grass tetany ratios in wheat and other species, but no studies to date have determined if other winter annual forage crops will have a similar response to increased P. This study examines the effects of increased P availability on leaf nutrient concentrations in hydroponically grown winter annual forage crops of wheat, oat, and cereal rye.

## MATERIALS AND METHODS

### Hydroponic Setup

Kingrazer winter wheat, Bob oat, and Winter King cereal rye were grown hydroponically in greenhouse conditions. For each species, a randomized complete block design was utilized with four P treatments and three blocks. In each block, three pots were assigned to each treatment and randomized within block. Each block was then assigned to available bench space within the greenhouse.

Plants were established in 5-cm diameter net pots filled with 1 cm<sup>3</sup> rockwool mineral fiber cubes and topped with two layers of cotton cheese cloth. Approximately 22 seeds were sown on top of the cheese cloth in each pot and the net pots were placed in

deionized (DI) water. Seven days after planting (DAP), seedlings were culled to 15 plants pot<sup>-1</sup> and each net pot was suspended in a 400 mL polypropylene container containing aerated DI water. One 110 L min<sup>-1</sup> aquatic air pump in each block of pots supplied continuous ambient air to solutions. Pots were then placed in a greenhouse (Missouri State University, Springfield, MO) on 6 Sept. 2016 (wheat), 11 July 2016 (oat), or 8 Aug. 2016 (cereal rye). Plants received natural light with an average daylength of 12.2, 14.1, and 13.3 h for wheat, oat, and cereal rye, respectively.

### Phosphorus Treatments

Eleven DAP, the DI water was replaced by treatment nutrient solutions containing 2 mmol L<sup>-1</sup> CaCl<sub>2</sub>, 0.5 mmol L<sup>-1</sup> MgSO<sub>4</sub>, 2.4 mmol L<sup>-1</sup> KCl, 2.5 mmol L<sup>-1</sup> NH<sub>4</sub>NO<sub>3</sub>, 12.5 μmol L<sup>-1</sup> Fe Sequestrene 330, 0.6 μmol L<sup>-1</sup> ZnSO<sub>4</sub>, 0.1 μmol L<sup>-1</sup> NaMoO<sub>4</sub>, 0.11 μmol L<sup>-1</sup> NiCl<sub>2</sub>, 0.01 μmol L<sup>-1</sup> CoCl<sub>2</sub>, 0.15 μmol L<sup>-1</sup> CuSO<sub>4</sub>, 2.3 μmol L<sup>-1</sup> H<sub>3</sub>BO<sub>3</sub>, and 0.9 μmol L<sup>-1</sup> MnSO<sub>4</sub> with P treatments of 0, 200, 400, and 800 μmol L<sup>-1</sup> P (using one part Na<sub>2</sub>HPO<sub>4</sub> to four parts NaH<sub>2</sub>PO<sub>4</sub>). Sodium chloride was added to equalize the amount of sodium across all P treatments, and pH was adjusted to 6.4 ± 0.2 using HCl. Nutrient solutions were replaced every 3 d.

### Data Collected

Greenhouse daily maximum and daily minimum temperature and relative humidity (RH) were recorded at plant height using a Hydro-thermometer (Thermo Fisher Scientific, Waltham, MA). Thirty-two DAP, instantaneous photosynthetic rates were measured on a fully expanded leaf with 400 μmol CO<sub>2</sub> m<sup>-2</sup> s<sup>-1</sup> and 1500 μmol m<sup>-2</sup> s<sup>-1</sup> photosynthetic photon flux density using a Li-6400XT (LI-COR, Lincoln, NE). Each pot was then divided into one composited shoot and one composited root sample. Samples were dried in a forced-air oven at 55°C, weighed, and ground to pass a 1-mm<sup>2</sup> screen. Tissues were digested in trace grade nitric acid using a MARS 6 microwave (CEM Corp., Matthews, NC). Leaf P concentrations were determined by an acid molybdate colorimetric assay at 660 nm (Murphy and Riley, 1962), and leaf Ca, Mg, and K concentrations were determined by atomic absorption spectrophotometry.

### Statistical Analysis

Data were analyzed using a general linear model (PROC GLM) in SAS v. 9.4 (SAS Institute, Cary, NC). To test for statistical significance of P treatment, as well as interactions, P treatment and block were considered fixed effect factors. All effects and interactions were considered significant when means differed at  $p < 0.05$ . When  $F$  tests showed significance ( $p < 0.05$ ), means were separated by Tukey's pairwise comparison.

## RESULTS AND DISCUSSION

During the 20-d growth period with treatment solutions in the greenhouse, average daily maximum and average daily minimum temperature were 36 and 21°C for wheat, 35 and 23°C for oat, and 36 and 22°C for cereal rye, respectively. Average daily maximum and average daily minimum RH were 85 and 43% for wheat, 96 and 60% for oat, 90 and 44% for cereal rye.

Shoot biomass increased in each species from 0 to 200 μmol L<sup>-1</sup> P treatments, with a 1.7, 1.5, and 3.8-fold increase in wheat, oat, and cereal rye, respectively (Fig. 1). However, only wheat exhibited further incremental shoot growth and declining root growth with

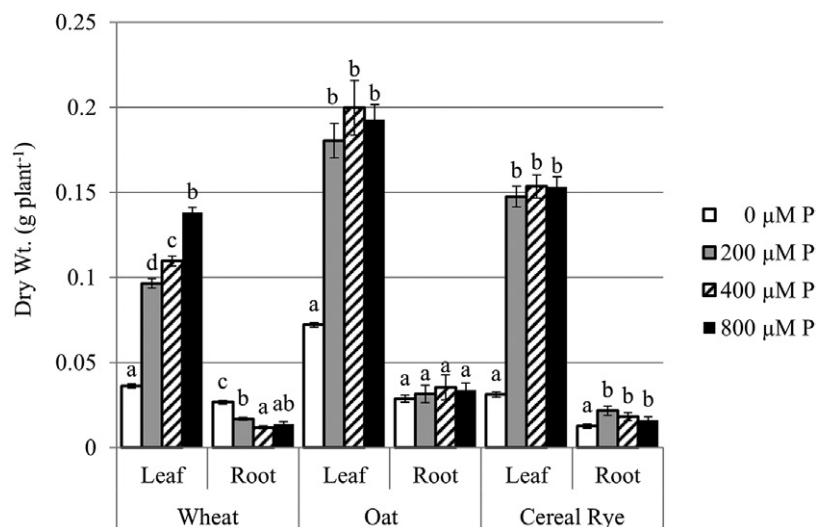


Fig. 1. Dry wt. of shoots and roots after 32 d of growth of wheat, oat, and cereal rye grown in various P treatments. Values are treatment means  $\pm$  SE,  $n = 9$ . Within species and tissue type, values not followed by the same letter are significantly different ( $p < 0.05$ , Tukey's pairwise comparisons).

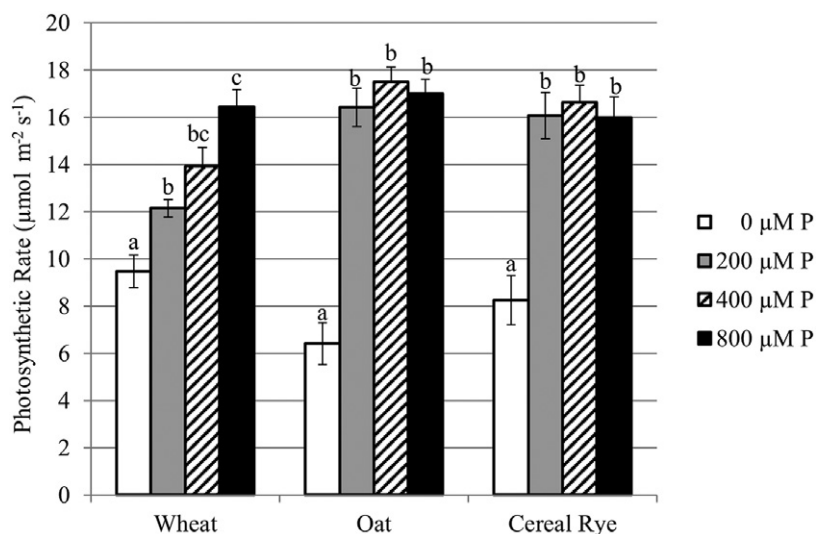


Fig. 2. Photosynthetic rates after 32 d of growth of wheat, oat, and cereal rye grown in various P treatments. Values are treatment means  $\pm$  SE,  $n = 9$ . Within species, values not followed by the same letter are significantly different ( $p < 0.05$ , Tukey's pairwise comparisons).

increasing P treatments (Fig. 1). This growth response of wheat to P availability has previously been reported (Reinbott and Blevins, 1991, 1994). Oat and cereal rye shoot biomass did not improve above 200  $\mu\text{mol L}^{-1}$  P and root growth was not affected by higher P levels (Fig. 1). Similar to shoot growth responses, photosynthetic rates of wheat increased incrementally with P concentrations, and oat and cereal rye remained the same at 200  $\mu\text{mol L}^{-1}$  P or greater (Fig. 2).

Leaf P concentrations incrementally increased with P treatment concentrations in all three species (Fig. 3). From 0 to 800  $\mu\text{mol L}^{-1}$  P there was a 5.5-, 6.7-, and 4.3-fold increase of leaf P in wheat, oat, and cereal rye, respectively (Fig. 3). Wheat leaf Ca and Mg decreased from 0 to 200  $\mu\text{mol L}^{-1}$  P treatments (Fig. 4 and 5). However, wheat leaf Mg increased 17% with added P from 200 to 800  $\mu\text{mol L}^{-1}$  P treatments (Fig. 5). Similarly, cereal rye leaf Mg decreased from 0 to 200  $\mu\text{mol L}^{-1}$  P (Fig. 5), and both leaf Mg and Ca increased 15 and 27%, respectively, from 200 to 800  $\mu\text{mol L}^{-1}$  P (Fig. 4 and 5). In both wheat and cereal rye, leaf K increased from 0 to 200  $\mu\text{mol L}^{-1}$  P, and there were no differences in leaf K with greater P treatments (Fig. 6).

Unlike wheat and cereal rye, oat exhibited a 24% increase in leaf Ca and Mg from 0 to 200  $\mu\text{mol L}^{-1}$  P treatments, with no change in

greater P treatments (Fig. 4 and 5), and leaf K remained the same across all P treatments (Fig. 6).

The grass tetany ratio decreased with increased P availability in all three species; however, this occurred at different levels of P, from 200 to 400  $\mu\text{mol L}^{-1}$  P in wheat and cereal rye, and from 0 to 200  $\mu\text{mol L}^{-1}$  P in oat (Fig. 7).

Both wheat and cereal rye expressed severe stunting ( $< 0.05 \text{ g plant}^{-1}$ ) and necrosis of the shoots when grown in 0  $\mu\text{mol L}^{-1}$  P (Fig. 1). This reduced growth could have resulted in the higher concentrations of leaf Ca and Mg found in these shoots compared with those grown in 200  $\mu\text{mol L}^{-1}$  P (Fig. 4 and 5), and in the resulting increase of the grass tetany ratio from 0 to 200  $\mu\text{mol L}^{-1}$  P treatments (Fig. 7). When wheat and cereal rye were provided with some P for growth ( $> 0 \mu\text{mol L}^{-1}$  P treatments), the response to plant growth and leaf nutrients resulted in an improved (reduced) grass tetany ratio between 200 and 400  $\mu\text{mol L}^{-1}$  P treatments. These results suggest that when wheat and cereal rye are grown in low available P, the leaf nutrients and the grass tetany ratio of wheat and cereal rye may be improved with increased P availability. Oat, however, maintained growth ( $> 0.05 \text{ g plant}^{-1}$ ) in 0  $\mu\text{mol L}^{-1}$  P treatments, and exhibited

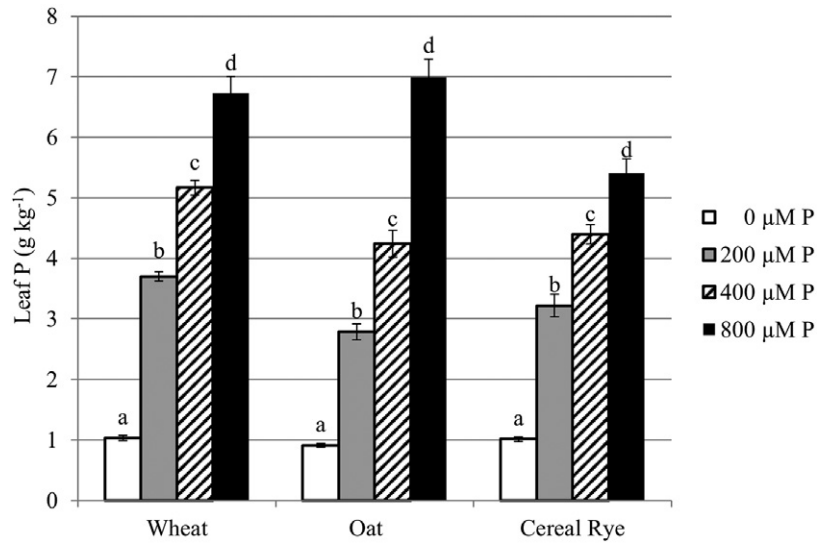


Fig. 3. Leaf P content of wheat, oat, and cereal rye after 32 d of growth in various P treatments. Values are treatment means  $\pm$  SE,  $n = 9$ . Within species, values not followed by the same letter are significantly different ( $p < 0.05$ , Tukey's pairwise comparisons).

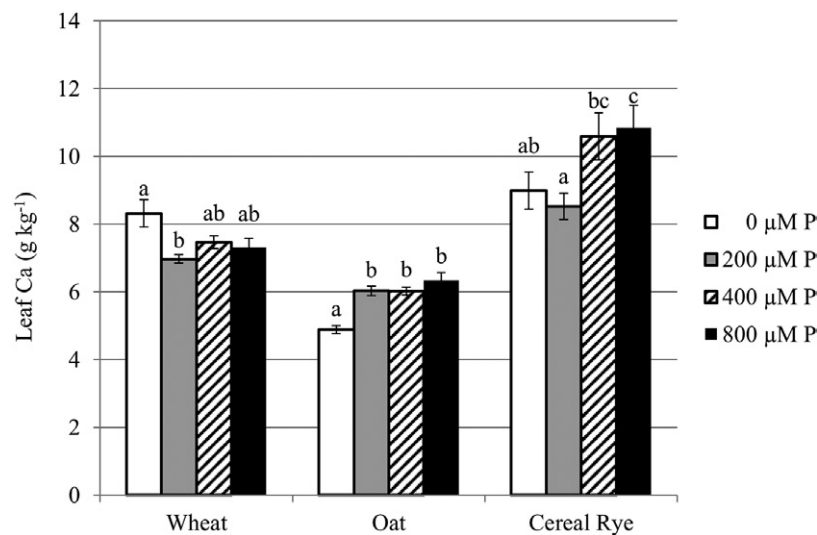


Fig. 4. Leaf Ca content of wheat, oat, and cereal rye after 32 d of growth in various P treatments. Values are treatment means  $\pm$  SE,  $n = 9$ . Within species, values not followed by the same letter are significantly different ( $p < 0.05$ , Tukey's pairwise comparisons).

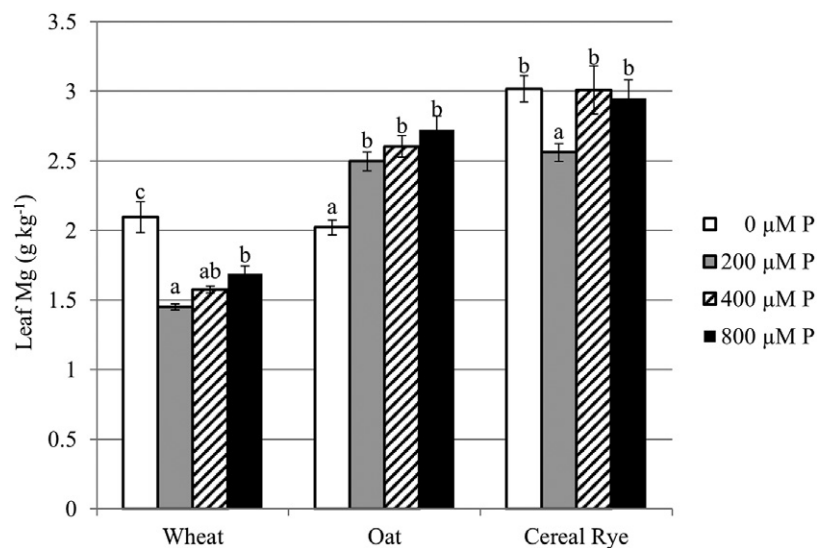


Fig. 5. Leaf Mg content of wheat, oat, and cereal rye after 32 d of growth in various P treatments. Values are treatment means  $\pm$  SE,  $n = 9$ . Within species, values not followed by the same letter are significantly different ( $p < 0.05$ , Tukey's pairwise comparisons).



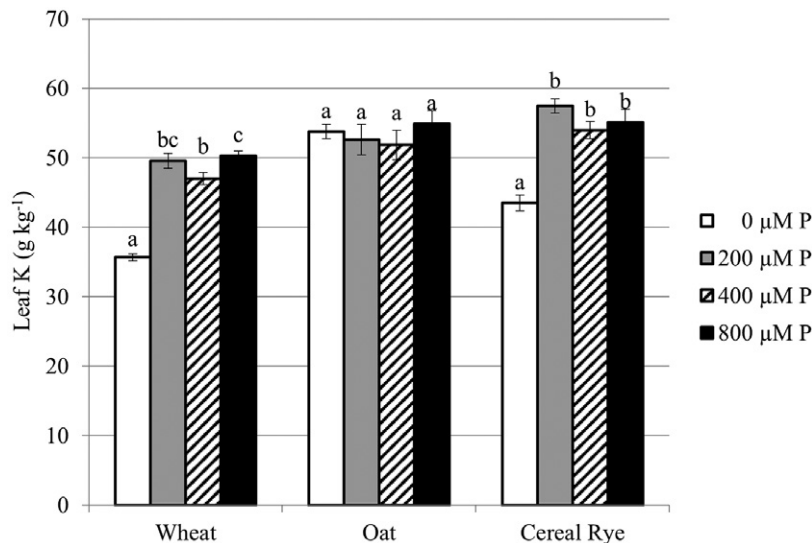


Fig. 6. Leaf K content of wheat, oat, and cereal rye after 32 d of growth in various P treatments. Values are treatment means  $\pm$  SE,  $n = 9$ . Within species, values not followed by the same letter are significantly different ( $p < 0.05$ , Tukey's pairwise comparisons).

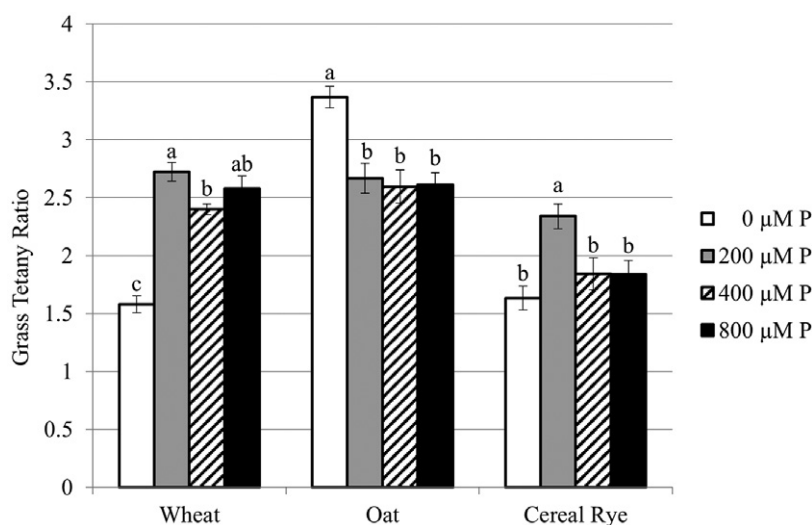


Fig. 7. Grass tetany ratio of wheat, oat, and cereal rye after 32 d of growth in various P treatments. Values are treatment means  $\pm$  SE,  $n = 9$ . Within species, values not followed by the same letter are significantly different ( $p < 0.05$ , Tukey's pairwise comparisons).

improved leaf nutrient concentrations and decreased grass tetany ratio when grown in lower levels of available P (0–200  $\mu\text{mol L}^{-1}$  P). 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 (Fig. 7). These results suggest that oat shoot growth and leaf nutrient levels may only be suppressed in very low available P conditions, and improvements in leaf nutrients and the grass tetany ratio of oat may only be observed when grown in very low P conditions.

This study shows that higher levels of P availability may incrementally improve growth, photosynthetic rates, and leaf P of wheat. Growth and photosynthetic rates of oat and cereal rye may not respond incrementally to greater levels of P availability and leaf P content, but the grass tetany ratio may be improved. Based on these findings, responses to improved soil P of annual cereal grain species grown for winter forage production on low P soils could be dependent on initial P availability. Oat may respond only in very low initial soil P conditions, whereas wheat and cereal rye may respond in greater levels of available soil P. Additionally, increased P availability could improve the grass tetany ratio, with or without increased yields

in annual cereal forages. Field-based experiments are needed to determine if these species grown over winter will respond similarly to soil P availability, and to determine the amount of available P needed to reduce the risk of dietary nutritional deficiencies in winter feeding.

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