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Using a Chelator-Buffered Nutrient System to Study Phosphorus, Manganese and Zinc Interactions in Russet Burbank Potato

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USING A CHELATOR – BUFFERED NUTRIENT SYSTEM TO STUDY PHOSPHORUS, MANGANESE AND ZINC INTERACTIONS IN RUSSET BURBANK POTATO

by

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GRADUATE COMMITTEE APPROVAL

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This thesis has been read by each member of the following graduate committee and by majority vote has been found to be satisfactory.

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As chair of the candidate's graduate committee, I have read the thesis of Steven A. Barben in its final form and have found that (1) its format, citations, and bibliographical style are consistent and acceptable and fulfill university and department style requirements; (2) its illustrative materials including figures, tables, and charts are in place; and (3) the final manuscript is satisfactory to the graduate committee and is ready for submission to the university library.

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ABSTRACT

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Potato production requires high phosphorus (P) application with potential negative environmental or nutritional consequences for potato as well as for subsequent crops. Impacts of high available P on yield and plant nutrition of species in potato cropping rotations are inadequately understood, and could result in antagonistic interactions with cationic micronutrients such as zinc (Zn) and manganese (Mn). Three hydroponic experiments were conducted with Russet Burbank potato to elucidate P and Zn relationships and associated interactions with other nutrients. In the first experiment, P solution concentration was constant at 256 µM while Zn concentration varied: 0.1, 2, 6, 18, 54, 162 and 456 µM Zn. In the second, Zn solution concentration was constant at 6 µM while P concentration varied: 32, 64, 128, 256, 512, 1024 and 2048 µM P. In the third, three levels of P and Zn varied in all possible combinations: 32, 128 and 1024 µM P and 0.1, 54 and 486 µM Zn. As expected, Zn increased in all plant parts with increasing

old shoots while root P increased. This suggests a P-Zn complex formation in roots preventing movement of P to the shoots of plants under high Zn. This was confirmed under variable P and Zn. Contrary to expectations, a direct impact of increased solution P on Zn uptake or distribution in potato was not observed except at 486 µM Zn in the third experiment. Increased solution P at low Zn levels resulted in a steep increase of P in new and old shoot growth and an accumulation of Mn in potato roots—factors that might indirectly impact Zn nutrition in potato. Although high P levels in potato did not directly reduce Zn content or cause Zn deficiency, excessive P accumulation with insufficient Zn may reduce the activity of Zn by interacting with other micronutrients such as Mn.

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Figure 4. Concentration of Zn in new shoots, old shoots, and roots of Russet Burbank potato grown for 17 days at seven levels of solution Mn (0.05, 3.2, 9.5, 28.5, 85.5, 256.5, and 769.5 µM Mn; and 128 µM P). Points along the same line for new shoots, old shoots or roots with the same letter are not significantly different at $p < 0.05$ level, Duncan-Waller K Ratio Test. To avoid confusion when lines overlap, NS means not significant at $p < 0.05$ level for roots). X axis is log scale.

Figure 5. Shoot, root and total dry weight of Russet Burbank potato grown for 17 days at three levels of Mn (0.05, 9.5, 769.5 µM Mn) and three levels of P (32, 128, and 1024 µM P; weights shown are averaged over P levels). For roots, shoots, or total dry weight, columns with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. Note: letters indicating significance are: above the root for comparing root, inside the top for comparing shoot, and above the top for comparing total yield for each column.

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MANUSCRIPT #1 - OPTIMIZING PHOSPHORUS AND ZINC CONCENTRATIONS IN HYDROPONIC CHELATOR-BUFFERED NUTRIENT SOLUTION FOR RUSSET BURBANK POTATO

(prepared for submission to Journal of Plant Nutrition)

OPTIMIZING PHOSPHORUS AND ZINC CONCENTRATIONS IN HYDROPONIC CHELATOR-BUFFERED NUTRIENT SOLUTION FOR RUSSET BURBANK POTATO

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ABSTRACT

Potato (*Solanum tuberosum* L.) production requires relatively high phosphorus (P) application with potential negative environmental or nutritional consequences for potato, as well as for subsequent crops. Impacts of high available P on yield and plant nutrition of species in potato cropping rotations are inadequately understood, and could result in antagonistic interactions with cationic micronutrients, such as zinc (Zn),. Two hydroponic experiments were conducted with Russet Burbank potato to elucidate optimum P and Zn concentrations in a chelator-buffered nutrient solution to enable study of the interactions of these nutrients in subsequent studies. In the first experiment, P solution concentration was constant at 256 µM while Zn concentration varied: 0.1, 2, 6, 18, 54, 162 or 456 µM. In the second, Zn concentration was constant at 6 µM while P concentration varied: 32, 64, 128, 264, 512, 1024 or 2048 µM. Results of the first experiment showed that low concentrations of solution Zn (0.1 and 2 µM) promoted low dry matter yield and Zn deficiency symptoms. High solution Zn concentrations (162 and 456 µM Zn) produced visual symptoms and nutrient Zn contents consistent with Zn toxicity, even though yields were not strongly affected. From these data, the optimal

range of Zn for potato grown in chelator-buffered nutrient solution is from 6 to 54 µM Zn (at 256 µM P). Results of the second experiment showed that dry matter yields, P concentration, and visual observations of plants grown in low solution concentration of P (32 µM) exhibited P deficiency and high solution P (1024 and 2048 µM) exhibited toxicity. Thus, the optimal solution P range for potato in this chelator-buffered solution is from 64 to 512 µM (at 6 µM Zn). In addition to defining the ranges of deficient, sufficient and toxic levels of P and Zn, a strong impact of increasing solution Zn on P content of potato was observed, while only minor impacts of increasing solution P on potato Zn content was observed. New and old shoot and root Mn concentrations were also affected by solution P. The ranges of solution P and Zn observed herein facilitate further study of the P-Zn interaction using the chelator-buffered solution system without the complication of soil interference.

INTRODUCTION

Potato (*Solanum tuberosum* L.) demands high nutrient availability relative to most crops (Hopkins et al., 2008). As a result, recommended rates of major nutrients are substantially higher than other common crops grown in rotation with potato. This is especially true for phosphorus (P) (Stark, Westermann, and Hopkins, 2004; Westermann, 2005; Westermann and Kleinkopf, 1985). For instance, the University of Idaho recommendations for P fertilizer are approximately double for potato (Stark, Westermann, and Hopkins, 2004) compared to spring wheat (Brown, Stark, and Westermann, 2001). This high P demand in potato is exacerbated by factors such as a

shallow and inefficient rooting system and low plant availability of P under high pH and calcium carbonate concentrations of semi-arid and arid zone soils and has led to elevated P fertilization in potato cropping systems, especially in the Pacific Northwest where the majority of U.S. potato production is concentrated (Hopkins et al., 2008, Marschner, 1986; Moraghan and Mascagni, 1991; Potash and Phosphate Institute, 2001; Stark Westermann and Hopkins, 2004; Westermann, 2005; Yamaguchi and Tanaka, 1990;).

Elevated P applications critical to high yield and quality in potato may result in negative environmental, plant nutritional and economic consequences (Hopkins et al., 2007, 2008). Yet resulting high residual soil P has not slowed P fertilizer application to potato despite these potentially negative consequences. Increasing regulatory pressures are mounting to decrease P loading into surface waters associated with excessively high P application, but little attention is being paid to the potentially negative impacts of excessive P essential for high yield potato production on other nutrients. Phosphorus reportedly interacts with many cationic micronutrients such as copper (Cu), iron (Fe), manganese (Mn) and zinc (Zn) (Beer et. al., 1972; Brown and Tiffin, 1962; Safaya, 1976; James, Hurst, and Tindall, 1995). The P-Zn interaction is among the most widely reported and studied of these interactions.

Phosphorus-induced Zn deficiency is well documented in maize (*Zea mays* L.; Brown and Tiffin, 1962; Christensen, 1972; Friesen, Miller, and Juo, 1980; Leece, 1978a; Safaya, 1976; Terman et al., 1972) and to a lesser degree in potato (Christensen, 1972; Christensen and Jackson, 1981; Hopkins et al., 2003; Idaho Potato Commission, 1997; Soltanpour, 1969) and crops commonly grown in rotation with potato, such as barley, wheat, oat, and alfalfa (*Hordeum vulgare* L., *Triticum aestivum* L. Thell., *Avenae*

byzantina K. Koch. and *Medicago sativa* L., respectively; Brown and Tiffin, 1962; Fageria and Baligar, 1989; Lindsey, 1974; MacLean, 1974; Moraghan, 1984; Moraghan and Mascagni, 1991; Singh, Karamanos, and Stewart, 1986; Torun et al., 2001; Webb and Loneragan, 1988; James, Hurst, and Tindall, 1995). Excessive P fertilizer application to potato reportedly reduces Zn uptake, yield and tuber size (Christensen, 1972; Christensen and Jackson, 1981; Hopkins et al., 2003; Idaho Potato Commission, 1997; Soltanpour, 1969).

Both soil and plant activities have been used to explain P-Zn interactions. Soil based explanations include precipitate formation (Gilkes and Sadleir, 1981) and reduced mycorrhizal infection under high P nutrition (Tinker, 1980). Plant related explanations under high P nutrition include reduced translocation of Zn from roots to shoots due to cell wall binding or chelation by organic ligands (Terman et al., 1972; Leece, 1978b; Singh, Karamanos, and Stewart, 1988), increased physiological Zn requirement (Cakmak and Marschner, 1987), physiological inactivation of Zn (Leece, 1978a), or growth dilution (Loneragan et al., 1979; Singh, Karamanos, and Stewart, 1988).

Apparent P-induced Zn deficiencies may occur without measured plant Zn declines (Boawn and Leggettt, 1964; Bingham, 1963). Even in closely controlled conditions of hydroponics, increasing P availability to cotton (*Gossypium barbadense* L.) did not affect Zn uptake, but did increase visual Zn deficiency symptoms (Cakmak and Marschner, 1987). Lack of impact of increasing P on Zn uptake, however, is also reported (Bingham, 1963).

Hydroponic studies can isolate plant response by eliminating soil impacts, and a few studies using hydroponic methodology relating to P-Zn interactions have been performed

(Cakmak and Marschner,1987; Lu and Miller, 1989). Improved chelator-buffered techniques in managing micronutrients in hydroponic solutions enhance the ability to study P-micronutrient interactions independent of soil (Yang et al., 1994; Hopkins et al., 1998). The technique depends upon maintaining micronutrients in solution with equal molar levels of chelates and micronutrients plus a slight excess of chelate [50 µM Trisodium N-(2-hydroxyethyl)ethylenediamine-N,N',N'-triacetate hydrate (NaHEDTA)] to sequester contaminant metals. Using this chelator-buffered nutrient solution allows identification of deficient, sufficient and excessive concentrations of micronutrients for plants with a focus on the plant aspects of the P-micronutrient interactions

Few research based guidelines are available for predicting P-Zn interactions in the field and only circumstantial management guidelines are available. In preparation for subsequent study of P-Zn interactions, our goals were to determine the optimum concentrations of P and Zn for potato grown in chelator-buffered nutrient solutions. Two controlled experiments were conducted with variable levels of P or Zn from which to identify deficient, sufficient, and excessive levels of these nutrients for potato. Results of these studies will form the basis for additional hydroponic research in potato and may play a vital role in developing P and micronutrient management guidelines for the potato cropping system.

METHODS

Two experiments were conducted in hydroponic conditions with potato (Russet Burbank). For all experiments, a complete-nutrient pretreatment solution was made using

a modified Steinberg solution (Steinberg, 1953). Each of the experimental nutrient solutions were made using a modified chelator-buffered nutrient solution (Hopkins et al., 1998; Yang et al., 1994) with the following concentrations (either P or Zn varied as described below): mM concentrations were 2.0 MES pH buffer (2-Morpholinoethanesulphonic acid (MES hydrate), 2.53 K_2SO_4, 1.43 NH_4NO_3, 1.64 $MgSO_4 \cdot 7H_2O$, 1.0 $CaCl_2 \cdot 2H_2O$; µM concentrations were 110 KCl, 100 $FeSO_4 \cdot 7H_2O$, 9.5 $MnSO_4 \cdot H_2O$, 2 $CuSO_4 \cdot 5H_2O$, 0.70 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 1.9 H_3BO_4. Solution pH was maintained at 6.0 ± 0.2 with 6 N KOH. The base concentration of trisodium N-(2-hydroxyethyl) ethylenediamine-N,N',N'-triacetate hydrate (Na-HEDTA) was 161.5 µM, with equivalent µM concentrations of Na-HEDTA added for each variable Zn level.

The experiments consisted of seven treatments of four plants each with four replications in a complete random block design. In the first experiment, P solution concentration was constant at 256 µM, while Zn concentration varied: 0.1, 2, 6, 18, 54, 162 or 456 µM Zn. Na-HEDTA (in addition to the base concentration of 161.5 µM) was added to each treatment at the same molar concentration as Zn in order to maintain a 50 µM chelate excess. In the second experiment, solution concentration Zn was constant at 6 µM (with Na-HEDTA at 167.5 µM) while P concentration varied: 32, 64, 128, 256, 512, 1024 or 2048 µM P. Nutrient solutions were completely replaced every 8 to 10 days. Concentration integrity was confirmed by nutrient content analysis at initial, mid-growth and final harvest periods.

Five- to eight-cm length potato plantlets (propagated asexually by tissue culture with a nutrient rich agar provided by the University of Idaho Potato Tissue Culture Lab, Moscow, ID) were transferred into 14 L of complete nutrient solution (pretreatment) and

grown for 17 days prior to placement into 14 L of treatment solution for 14 days. Growth chamber temperatures were maintained at 25° ± 1°C during the 14 hour light period and at 19° ± 1°C during the 10 hour dark period. Plants were observed in their respective treatments for relative health and appearance and then harvested at the end of the treatment periods by separating into three parts, namely: new shoots (new growth leaves and petioles), old shoots (old growth leaves, petioles, and stems), and roots. For some treatments, poor growth required combining new and old shoots to have adequate plant material for analysis. When combined, they will be labeled shoots. Plant tissue was oven dried at 65°C for a minimum of 48 hours, weighed, ground to pass a 1 mm screen, digested in nitric-perchloric acid, and analyzed by inductively coupled plasma (ICP, Thermo Electron Corporation, Franklin, Maryland) spectroscopy for nutrient concentrations. Results were statistically analyzed with SAS (Version 9.1, SAS Institute, 2003, Cary, North Carolina, USA) using ANOVA with Duncan mean separation tests. When appropriate, multiple or linear regression was used to confirm the significance of observed relationships.

RESULTS AND DISCUSSION

Variable Zn

Visual observations placed plants into three general categories under variable solution Zn levels. Plants grown in low level treatments (0.1 and 2 µM Zn) were stunted and exhibited reduced growth in both shoots and roots. Those grown in mid level treatments

(6, 18 and 54 µM Zn) were healthy and vigorous in growth similar to other findings (Boawn and Leggettt, 1964, Broadley et al., 2007, Chatterjee and Khurana, 2007). Plants grown in upper level treatments (162 and 486 µM Zn) generally exhibited rapid growth but also exhibited unhealthy symptoms of interveinal chlorosis, mottling, curling, burning at leaf edges and early leaf drop in older leaves similar to other reports (Broadley et al., 2007; Chatterjee and Khurana, 2007; Kaya and Higgs, 2001). Zinc deficiency resulted in decreased top and total dry matter yields at the lowest levels of solution Zn (Fig. 1). Increasing solution Zn produced significant improvements in dry matter. Although visual symptoms indicated toxicity at the highest level of solution Zn, the apparent decreased dry matter yield was not statistically significant (Fig. 1). Root yields were unpredictably affected by Zn level.

As expected, Zn concentrations of all plant parts increased as solution Zn levels rose (Fig. 2). Root P concentration increased with increasing solution Zn (Fig. 3), probably due to binding of these two elements within the root tissue and preventing P transport to shoots (Terman et al., 1972; Leece, 1978b; Singh, Karamanos, and Stewart, 1988). This binding likely is the reason for P concentrations decreasing in shoots, although only significant for the old growth, with increasing Zn activity in solution (Fig. 3). These results are similar to field observations for Russet Burbank potato (Boawn and Leggettt, 1964) and for a sand culture mustard (Chatterjee and Khurana, 2007) experiments.

Although observed Zn and P concentrations with increasing solution Zn were expected, the high levels of Mn in all three plant parts at the two highest levels of Zn was surprising (Fig 4). Root Mn concentration is depressed at intermediate Zn levels (6, 18 and 54 µM Zn) but high at both deficient and excessive solution Zn levels (0.1, 2.0, 162

and 486 µM Zn). Mn contents in new shoots and old shoots were generally similar as Zn increased up to 54 µM Zn, but massive accumulation of Mn in both new and old shoots were observed at 162 and 486 µM solution Zn levels. Thus, high Zn appears to strongly influence Mn distribution in potato. Previous research has given little explanation for the observed Zn impact on Mn uptake and concentration in potato.

From these data and visual observations, the optimal range of Zn was determined to be from 6 to 54 µM Zn for potato plants grown in this chelator-buffered nutrient solution at 256 µM P. The low (0.1 and 2 µM Zn) concentrations were definitely deficient based on plant matter yield, plant nutrient concentration and visual observation. Although the high (162 and 456 µM Zn) concentrations did not show significant declines in dry matter yield, the combination of visual observations and significant impacts on nutrient concentrations in the tissue show that these levels are excessive and borderline toxic.

Variable P

Visual observations placed plants into three general categories under variable solution P levels. Potato plants grown at low P treatments (32 and 64 µM) were stunted and had dark green, upturned leaves and a general purpling on the undersides of young leaves. Plants grown in the mid-level P treatments (128 and 264 µM) appeared completely healthy and vigorous. Growth appeared to be slightly inhibited in the upperlevel P treatments (1024 and 2048 µM), as compared with mid-level P treatments. Chlorosis, mottling, curling, leaf edge necrosis, and leaf drop were observed in the high solution P treatments, similar to P toxicity symptoms reported by Cakmak and Marschner (1987), as

well as being similar to symptoms observed with the upper-level Zn treatments in variable Zn experiment reported above. New shoot and total dry matter yields were negatively impacted by P deficiency at 32 µM and toxicity at the 1024 and 2048 µM P rates (Fig. 5). Root yield was relatively unaffected by solution P, although significantly depressed at 1024 µM P.

A general increase in P concentration was observed in all shoots and roots with increasing solution P; however, P concentrations plateau above 512 µM P (Fig. 6). In contrast with some previous reports (Christensen, 1972; Christensen and Jackson, 1981; Soltanpour, 1969), but in agreement with Boawn and Leggett (1964), Bingham (1963) and Cakmak and Marschner (1987), where plant leaf Zn concentration remained mostly unchanged as solution P increased, there were no clear impacts on Zn concentrations in new shoots and roots with these dramatic changes in solution P activity (Fig. 7). However, Zn concentration was significantly higher at both low and high solution P for older shoots, and all plant parts followed a similar trend of lower Zn at optimum P levels (128 and 256).

As in the variable Zn study, one of the surprising impacts of variable P was the effect on Mn. Manganese concentrations in new and old shoots were generally depressed by the first increment of solution P (32 µM P) and remained relatively constant thereafter, probably due to a dilution effect (Fig 8). There was a consistent and dramatic increase in Mn concentration in the roots with increasing P concentration (Fig. 8).

From these data and visual observations, it was determined that the optimal range of solution P is from 64 to 512 µM P for potato plants grown in this chelator-buffered nutrient solution at 6 µM Zn. Based on yield, plant nutrient concentration and visual

observation, the low P (32 µM) concentration was deficient and the high P (1024 and 2048 µM) concentrations were excessive and borderline toxic.

CONCLUSIONS

From visual observation, potato dry matter yield and plant part nutrient concentration data, deficient, sufficient and excessive levels of solution Zn and P were determined in these studies. Deficiency of Zn developed in potato grown in 0.1 and 2 µM Zn and deficiency of P in 32 µM P. The optimal range for potato plants grown in this chelator-buffered nutrient solution was from 6 to 54 µM Zn (at 256 µM P) and from 64 to 512 µM P (at 6 µM Zn). Evidence for defining excess or toxic levels of Zn and P was observed in potato grown at 162 and 456 µM Zn and 1024 and 2048 µM P concentrations.

Increasing solution Zn impacted potato by increasing Zn concentration in all plant parts, decreasing P in old shoots with a concomitant P increase in roots, and depressing root Mn at sufficient solution Zn relative to deficient and excessive Zn levels with both new and old shoot Mn increasing only at the higher Zn levels. Solution P increase resulted in a consistent increase in potato P in all plant parts, little direct impact on Zn concentration in potato tissues, but a strong increase in root Mn with shoot Mn only slightly affected. These studies establish chelator-buffered nutrient solution concentrations of P and Zn for potato to facilitate further P-Zn interaction studies without soil factor influences.

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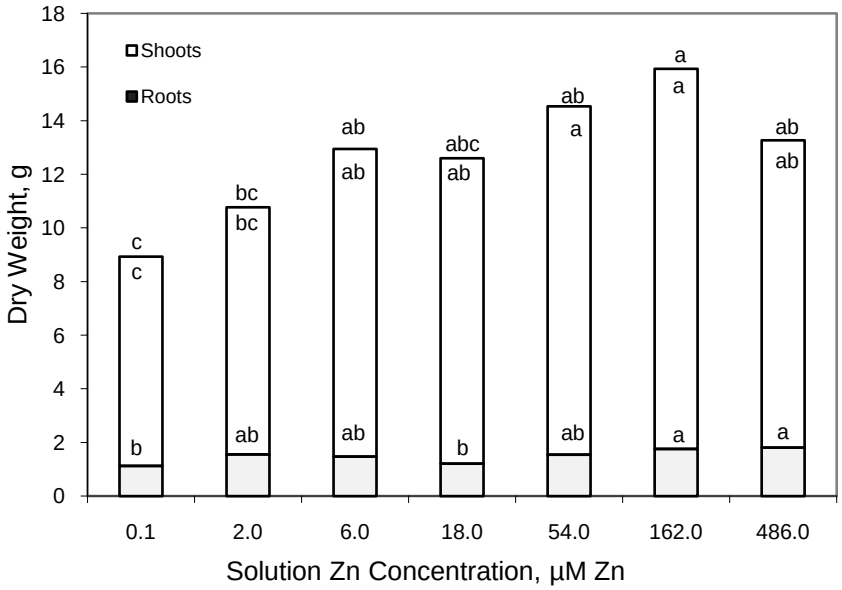

Figure 1. Shoot, root and total dry weight of Russet Burbank potato grown for 14 days at seven levels of Zn solution (0.1, 2, 6, 18, 54, 162 and 486 µM Zn; and 256 µM P). For roots, shoots, or total dry weight, columns with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. Note: letters indicating significance are: above the root bar for root interpretation, below the shoot bar for shoot interpretation, and above the shoot bar for total yield interpretation.

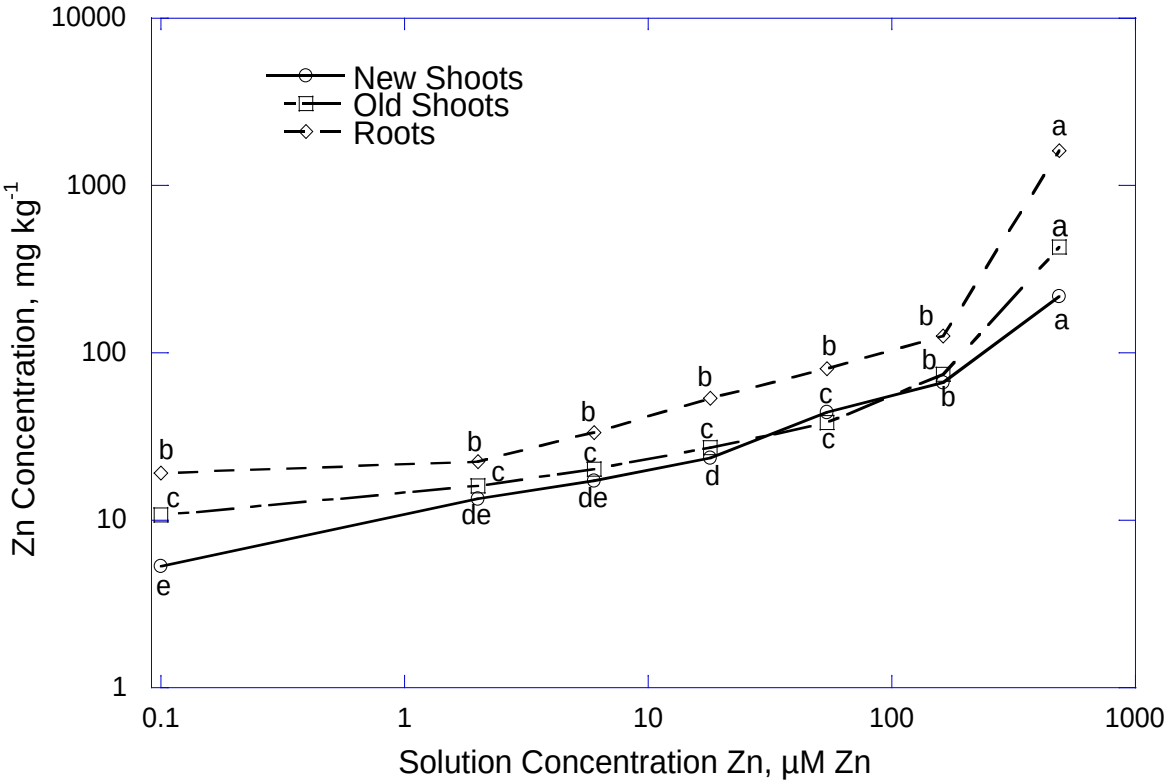

Figure 2. Concentration of Zn in new shoots, old shoots, and roots of Russet Burbank potato grown for 14 days at seven levels of solution Zn (0.1, 2, 6, 18, 54, 162 and 456 µM Zn; and 256 µM P). Points along the same line for new shoots, old shoots and roots with the same letters are not significantly different at $p < 0.05$ level, Duncan-Waller K Ratio Test. X and y axes are log scale.

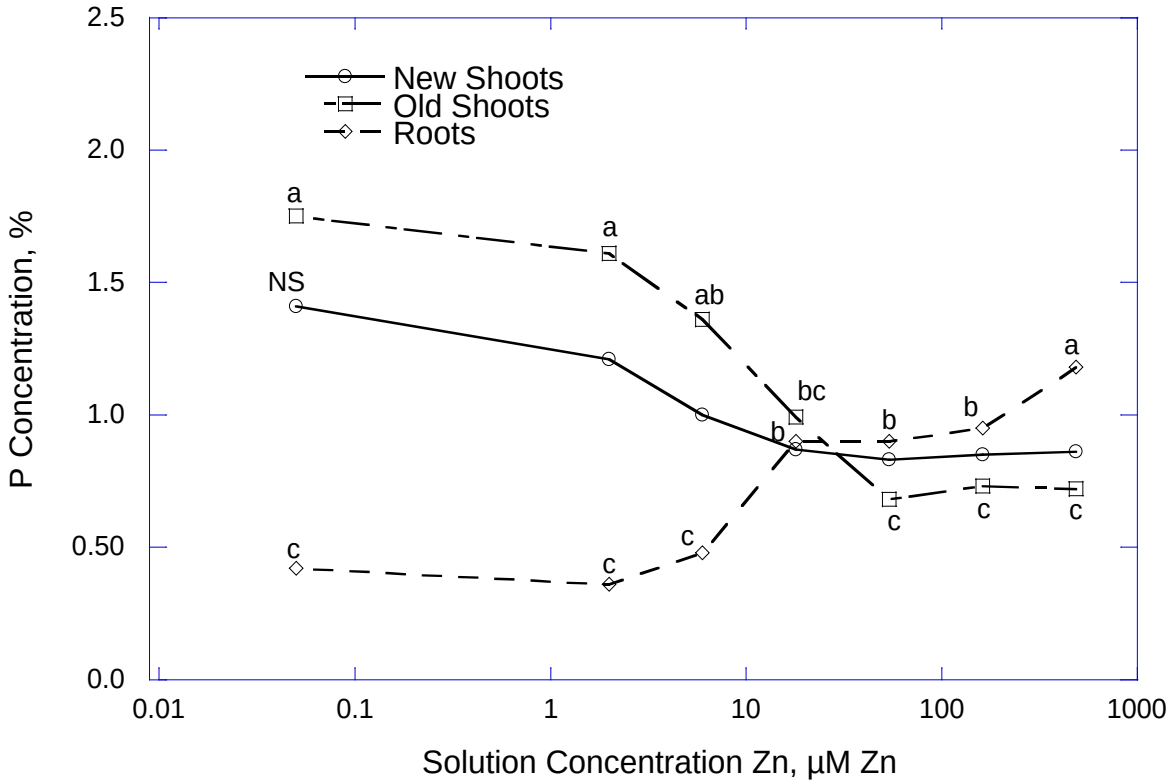

Figure 3. Concentration of P in new shoots, old shoots, and roots of Russet Burbank potato grown for 14 days at seven levels of Zn (0.1, 2, 6, 18, 54, 162 and 456 µM Zn; and 256 µM P). Points along the same line for new shoots, old shoots or roots with the same letters are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test . NS is not significant at $p < 0.05$ level (new shoots). X axis is log scale.

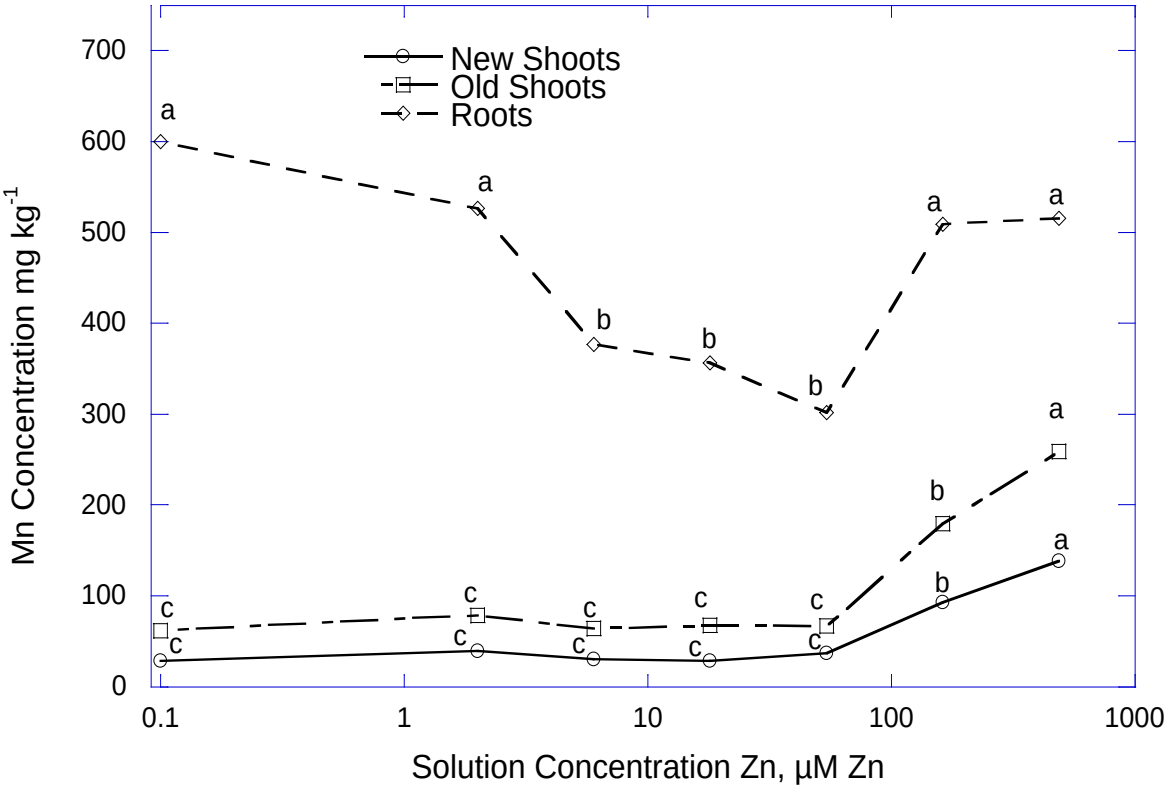

Figure 4. Concentration of Mn in new shoots, old shoots, and roots of Russet Burbank potato grown for 14 days at seven levels of solution Zn (0.1, 2, 6, 18, 54, 162 and 456 µM Zn;and 256 µM P). Points along the same line for new shoots, old shoots or roots with the same letters are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. X axis is log scale. .

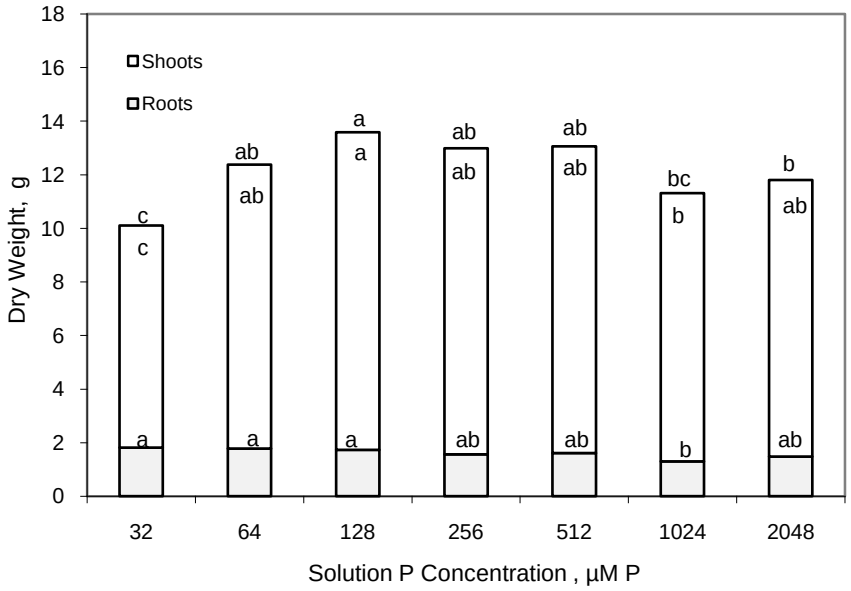

Figure 5. Shoot, root and total dry weight of Russet Burbank potato grown for 14 days at seven levels of solution P (32, 64, 128, 256, 512, 1024 and 2048 µM P; and 6 µM Zn). For roots, shoots, or total dry weight, columns with the same letter are not significantly different at $p < 0.05$ level, Duncan-Waller K Ratio Test. Note: letters indicating significance are: above the root bar for root interpretation, below the shoot bar for shoot interpretation, and above the shoot bar for total yield interpretation.

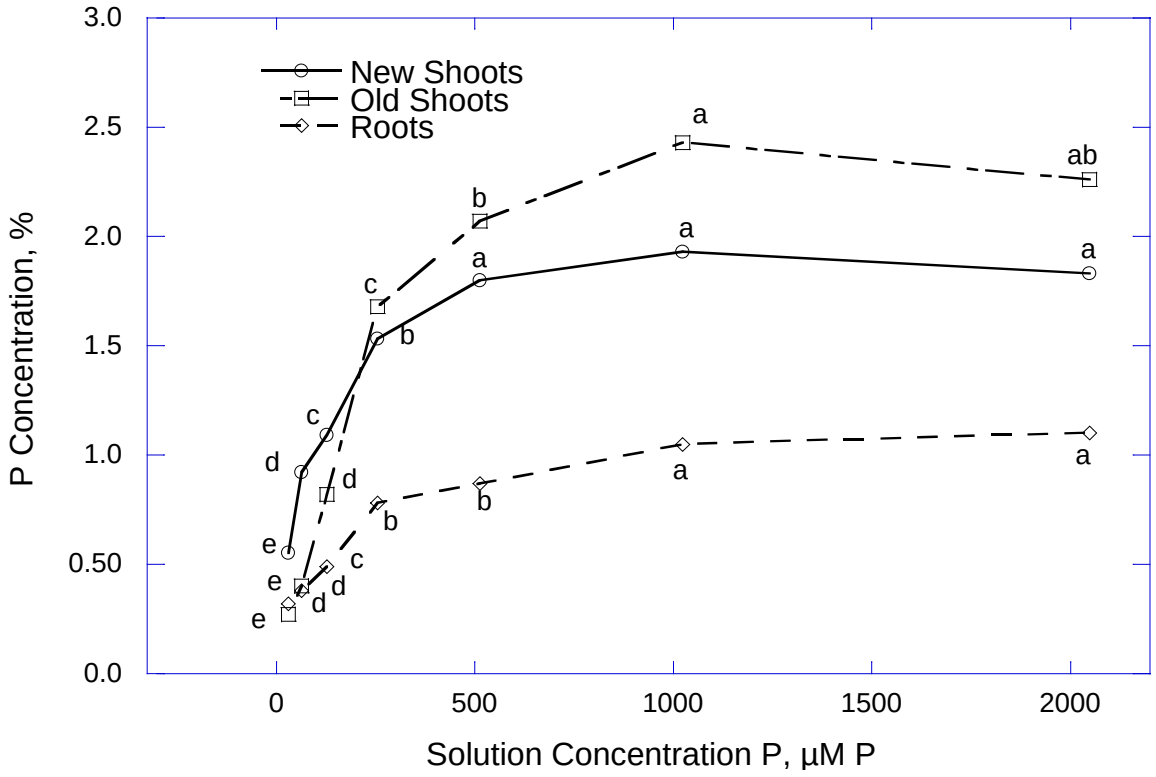

Figure 6. Concentration of P in new shoots, old shoots, and roots of Russet Burbank potato grown for 14 days at seven levels of solution P (32, 64, 128, 256, 512, 1024 and 2048 µM P; and 6 µM Zn). Points along the same line for new shoots, old shoots or roots with the same letters are not significantly different at $p < 0.05$ level, Duncan-Waller K Ratio Test.

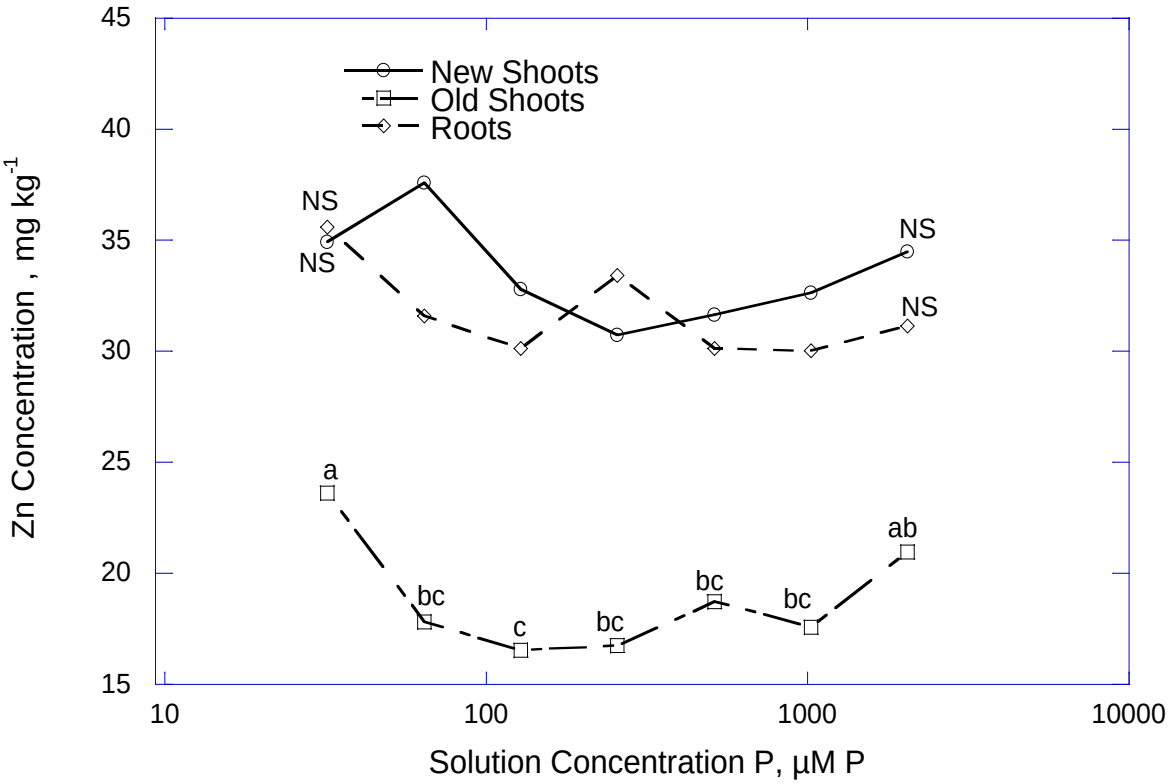

Figure 7. Concentration of Zn in new shoots, old shoots, and roots of Russet Burbank potato grown for 14 days at seven levels of solution P (32, 64, 128, 256, 512, 1024 and 2048 µM P; and 6 µM Zn). Points along the same line with the same letters are not significantly different at $p < 0.05$ level, Duncan-Waller K Ratio Test. NS is not significant at $p < 0.05$ level (new shoots and roots). X axis is log scale.

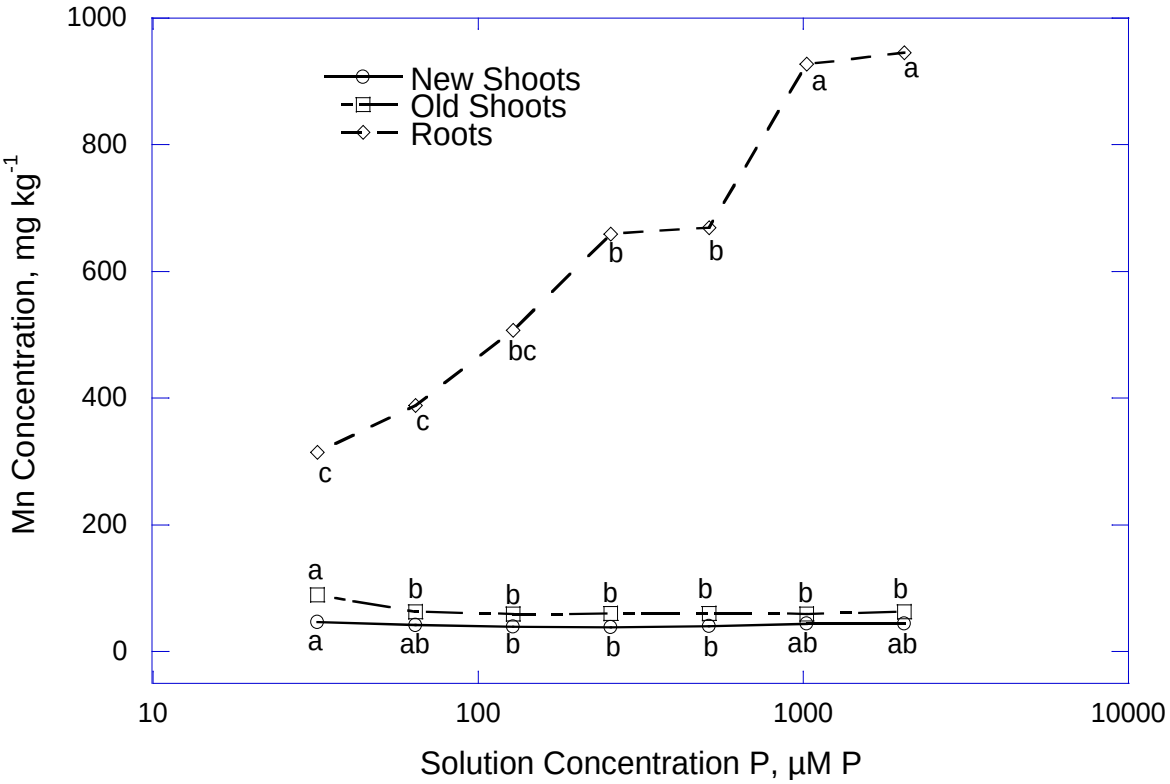

Figure 8. Concentration of Mn in new shoots, old shoots, and roots of Russet Burbank potato grown for 14 days at seven levels of solution P (32, 64, 128, 256, 512, 1024 and 2048 µM P; and 6 µM Zn). Points along the same line for new shoots, old shoots or roots with the same letters are not significantly different at $p < 0.05$ level, Duncan-Waller K Ratio Test. X axis is log scale.

MANUSCRIPT #2 - PHOSPHORUS AND ZINC INTERACTIONS IN HYDROPONIC CHELATOR-BUFFERED NUTRIENT SOLUTION GROWN RUSSET BURBANK POTATO

(prepared for submission to Journal of Plant Nutrition)

PHOSPHORUS AND ZINC INTERACTIONS IN HYDROPONIC CHELATOR-BUFFERED NUTRIENT SOLUTION GROWN RUSSET BURBANK POTATO

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ABSTRACT

Potato (*Solanum tuberosum* L.) production requires high phosphorus (P) application with potential negative environmental or nutritional consequences for potato, as well as for subsequent crops. Impacts of high available P on yield and plant nutrition of species in potato cropping rotations are inadequately understood, but antagonistic interactions with cationic micronutrients such as zinc (Zn) and manganese (Mn) could result. A hydroponic experiment was conducted with Russet Burbank potato to elucidate P and Zn relationships and associated interactions with other nutrients. Nine treatments included three levels of P and Zn in all possible combinations: 32, 128 and 1024 µM P and 0.1, 54 and 486 µM Zn. As expected, Zn increased in all plant parts with increasing solution Zn. As potato Zn content rose, P concentration declined in both top leaves and middle leaves and stems while root P increased. This suggests a P-Zn complex formation in roots preventing movement of P to the new shoots of plants under high Zn. This was confirmed under variable P and Zn. Contrary to expectations, a direct impact of increased solution P on Zn uptake or distribution in potato was not observed except at 486 µM Zn in the final experiment. Increased solution P at low Zn levels resulted in a steep increase of P in new and old shoot growth and an accumulation of Mn in potato roots—a factor

that might indirectly impact Zn nutrition in potato. Although high P levels in potato did not directly reduce Zn content or cause Zn deficiency, without sufficient Zn, excessive P accumulation may reduce the activity of Zn by interacting with other micronutrients such as Mn.

INTRODUCTION

A combination of high phosphorus (P) requirement, a shallow and inefficient rooting system in potato (*Solanum tuberosum* L.), and low plant availability of P under high pH and calcium carbonate concentrations of semi-arid and arid zone soils has stimulated elevated P fertilization in potato cropping systems (Hopkins et al., 2008, Marschner, 1986; Moraghan and Mascagni, 1991; Stark, Westermann, and Hopkins, 2004). High fertilizer P rates are considered critical to potatoes grown in alkaline, calcareous soils, but the trap of "if some is good, more is better" can lead to negative environmental and nutritional consequences (Hopkins et al, 2007 and 2008). A majority of U.S. potato production is concentrated in the Pacific Northwest and, as a result, soil test P levels have become very high (Hopkins et al., 2008, Potash and Phosphate Institute, 2001). Yet, high residual soil P has not slowed P fertilizer application to potato, even though this could lead to deterioration of water quality from surface runoff and erosion, reductions in yield and quality, and reductions in revenue in potato cropping systems (Hopkins et al., 2007 and 2008). Antagonistic interaction of P with other nutrients (Brown and Tiffin, 1962; James, Hurst, and Tindall, 1995) could explain many of these negative impacts on crop yields.

Zinc is absorbed by plants as Zn^{2+}, and P is absorbed as $H_2PO_4^{-1}$ or HPO_4^{-2}. These oppositely charged ions exhibit an electrical attraction that facilitates the formation of chemical bonds either in soil or within plant tissues. The relative strength of the P-Zn bond is robust and does not readily separate without dramatic changes in the physical or chemical environment. If excess soil or plant P binds Zn normally available to the plant, the result will be a P-induced Zn deficiency (Brown and Tiffin, 1962; Cakmak and Marschner, 1987; Christensen, 1972; Lindsey, 1974; Singh, Karamanos, and Stewart, 1986 and 1988).

Phosphorus-induced Zn deficiency is well documented in maize (Brown and Tiffin, 1962; Christensen, 1972; Friesen, Miller, and Juo , 1980; Leece, 1978a; Safaya, 1976; Terman, Giordano, and Allen, 1972). Although not as commonly studied in potato, this interaction has also been reported (Christensen, 1972; Christensen and Jackson, 1981; Hopkins et al., 2003; Idaho Potato Commission, 1997; Soltanpour, 1969) and in crops commonly grown in rotation with potato, such as barley, wheat, oat, and alfalfa (*Hordeum vulgare* L., *Triticum aestivum* L. Thell., *Avenae byzantina K. Koch*. and *Medicago sativa* L., respectively; Brown and Tiffin, 1962; Fageria and Baligar, 1989; Lindsey, 1974; MacLean, 1974; Moraghan, 1984; Moraghan and Mascagni, 1991; Singh, Karamanos, and Stewart, 1986; Torun et al., 2001; Webb and Loneragan, 1988; James, Hurst, and Tindall, 1995). Excessive P fertilizer application to potato reportedly reduces Zn uptake, yield and tuber size (Christensen, 1972; Christensen and Jackson, 1981; Hopkins et al., 2003; Idaho Potato Commission, 1997; Soltanpour, 1969).

Both soil and plant relationships have been suggested explanations of P-Zn interactions. Precipitates of Zn and P such as $Zn_3(PO_4)_2$ and $ZnNH_4PO_4$ formed in high

pH soils (7.0-8.5) could partially explain reduced Zn uptake by plants grown in the presence of excess P. Gilkes and Sadleir (1981) reported that 90% of Zn incorporated with ordinary superphosphate was in water-soluble form and dissolved within seven days after application and after one year the small remaining fraction of Zn was found with ordinary superphosphate granules. Thus, these P-Zn precipitates are relatively soluble and are not in control of Zn activity in most soils (Pasricha et al., 1987, Saeed, 1977). Another possible soil explanation is that high P may reduce mycorrhizal infection which in turn could reduce Zn uptake and lead to P-induced Zn deficiency (Tinker, 1986). Such was proposed as an explanation in a P-induced Zn deficiency of field-grown wheat (Singh, Karamanos, and Stewart, 1986), but Lu and Miller (1989) reported the opposite in maize. However, Soltanpour (1969) found that Zn and P applied in separate bands in the soil resulted in reduced Zn uptake by potato, and because the Zn and P bands were not in direct contact with each other in the soil, the P-Zn interaction seemed related more to plant physiology than to soil reactions.

A P-Zn physiological coupling has also been shown at the root-soil interface in maize (Zea *mays* L.; Safaya, 1976). Terman, Giordano, and Allen (1972) showed that P reduced Zn translocation from the roots to the leaves and stems in maize. Increased Zn concentration in the root cell walls as a function of added P could explain the reduced translocation of Zn. Singh, Karamanos, and Stewart (1988) showed that Zn deficiency was at least partially induced by reduced translocation of Zn from roots to shoots in bean (*Phaseolis vulgaris* L.). Others suggested that Zn may be bound to cell walls or chelated by organic ligands as a function of increased P (Leece, 1978b). In contrast to an excess P promotion of Zn deficiency, an accumulation of P in aerial parts of plants at reduced Zn

levels has been observed (Boawn and Leggett, 1964; Bingham, 1963). Loneragan et al. (1979) and Webb and Loneragan (1988) suggested that Zn deficiency symptoms are partially caused by a combination of Zn deficiency and P toxicity symptoms as a function of enhanced P uptake on low Zn soils.

Other possible plant related explanations of P-Zn interaction include: a dilution effect observed when added P increases shoot growth while Zn uptake rate remains constant, thus resulting in reduced tissue Zn concentration—an observation most likely to occur when both soil P and Zn are low (Loneragan et al., 1979; Singh, Karamanos, and Stewart, 1988) an increased physiological requirement for Zn (Cakmak and Marschner, 1987) or physiological inactivation of Zn (Leece, 1978a) observed at excess P levels. Additionally, P reportedly interacts with other cationic micronutrients such as manganese (Mn), iron (Fe), and copper (Cu) (Beer et. al., 1972; Brown and Tiffin, 1962; Safaya, 1976; James, Hurst, and Tindall, 1995).

In contrast with these studies, Friesen, Miller, and Juo (1980) observed an increased total Zn uptake with the addition of P fertilizer in maize. They credited this observation to increased root growth due to improved P nutrition. Boawn and Leggettt (1964) reported that even though Zn deficiency symptoms in potato plants were apparent with increased P application, Zn concentration in tissues was not reduced or found deficient with additional P. In a multiple plant study of tomato (*Lycopersicon esculentum* L., kidney bean (*Phaseolus vulgaris francais* L.) and sour orange (*Citrus aurantium*), Bingham (1963) found “no indication of Zn deficiency in any plant even though P varied from 1 to 100 ppm”, and that “growth was not depressed, Zn contents were normal” even under the highest P concentration (similar in magnitude as soils excessively fertilized

with P). Cakmak and Marschner (1987) also found that increasing solution P concentration of hydroponically grown cotton plants (*Gossypium barbadense* L.) did not affect Zn uptake, but did increase visual Zn deficiency symptoms.

To understand these complex interactions among soils and species may require study under carefully controlled conditions. Interactions observed through hydroponic studies can help to differentiate between direct P-micronutrient associations and the effects of variable soil factors. A few studies using hydroponic methodology relating to P-Zn interactions have been referred to previously (Cakmak and Marschner, 1987; Lu and Miller, 1989), but improved techniques in managing micronutrient contents in hydroponic solutions refine and enhance the ability to study P-micronutrient interactions independent of soil (Yang et al., 1994; Hopkins et al., 1998). The technique depends upon maintaining micronutrients in solution with equal molar levels of chelates and micronutrients plus a slight excess of chelate to sequester micronutrient contaminants. Barben et al. (2009) recently established deficient, sufficient and excess levels of solution P and Zn for Russet Burbank potato using a modification of this chelator-buffer technique. This knowledge makes it possible to study the P-Zn interaction with greater control and without interference from soil-borne activities. Research based guidelines are currently not available for predicting P-Zn interactions in the field and only circumstantial management guidelines are available. The interactions both in the plant and soil must be understood separately to comprehend the combination effects and, thus, management implications. Our goal was to determine P-Zn interactions in potato grown hydroponically in a chelator-buffered nutrition solution without interference from conflicting variables present in soil environments. A controlled nutrient hydroponic

experiment was conducted with variable levels of P and Zn from which to identify interactions with these two nutrients on potato dry matter yield and nutrient concentrations. Results of this study could play a vital role in developing phosphorus and micronutrient management guidelines for the potato cropping system.

METHODS

The experiment was conducted in hydroponic conditions in a complete random block design with potato (Russet Burbank). Five- to eight-cm length potato plantlets (propagated asexually by tissue culture with a nutrient rich agar provided by the University of Idaho Potato Tissue Culture Lab, Moscow, ID) were transferred into 14 L of complete nutrient solution (pretreatment) and grown for 17 days prior to placement into 14 L of treatment solution for 17 days. For all experiments, plants were initially grown in a complete-nutrient pretreatment consisting of a modified Steinberg solution (Steinberg, 1953). Each of the experiment treatment nutrient solutions were made using a modified chelator-buffered nutrient solution (Hopkins et al., 1998; Yang et al., 1994) with the following concentrations: mM concentrations were 2.0 MES pH buffer (2-Morpholinoethanesulphonic acid (MES hydrate), 2.53 K_2SO_4; 1.43 NH_4NO_3, 1.64 $MgSO_4 \cdot 7H_2O$, 1.0 $CaCl_2 \cdot 2H_2O$; µM concentrations were 110 KCl, 100 $FeSO_4 \cdot 7H_2O$, 9.5 $MnSO_4 \cdot H_2O$, 2 $CuSO_4 \cdot 5H_2O$, 0.70 $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$, and 1.9 H_3BO_4. The base concentration of trisodium N-(2-hydroxyethyl) ethylenediamine-N,N',N'-triacetate hydrate (Na-HEDTA) was 161.5 µM, with equivalent µM concentrations of Na-HEDTA added for each variable Zn level. This study consisted of nine treatments of four plants

each with three replications. Based on results of a previous studies in which deficient, optimum, and excessive levels of both Zn and P were identified (Barben et al., 2009), potato plants were grown in all combinations of three levels of P (32, 128 or 1024 µM P) and Zn (0.1, 54 or 486 µM Zn). Additional Na-HEDTA was added to each treatment at the same molar concentration as Zn in order to maintain a 50 µM chelate excess. Solution pH was maintained at 6.0 ± 0.2 with 6 N KOH. By daily assessment, newly mixed nutrient solutions replaced old solutions every 8 to 10 days. Concentration integrity was confirmed by nutrient content analysis at initial, mid-growth and final harvest periods. Growth chamber temperature was maintained at 25° ± 1°C during the 14-hour light period and at 19° ± 1°C during the 10-hour dark period. Plants were observed in their respective treatments for relative health and appearance, harvested at the end of the treatment periods, separated as new shoots (top leaves and petioles), old shoots (bottom leaves, petioles and stems), and roots, then oven dried at 65°C for a minimum of 48 hours, weighed, ground and digested in nitric-perchloric acid and analyzed by inductively coupled plasma (ICP, Thermo Electron Corporation, Franklin, Maryland) spectroscopy for nutrient concentrations. Some treatments produced minimal growth and separation into new and old shoots was not possible. In these cases results are expressed as shoots. Also new and old shoot weights are assumed in reporting shoot weight. Results were statistically analyzed with SAS (Version 9.1, SAS Institute, 2003, Cary, North Carolina, USA) using ANOVA with Duncan mean separation tests. When appropriate, multiple or linear regression was used to confirm the significance of observed relationships.

RESULTS AND DISCUSSION

Visual Symptoms and Dry Matter Yield

Observationally, Zn deficiency and toxicity had a relatively greater impact than did P with regard to general potato plant health and appearance. At 0.1 µM Zn (deficient Zn level), over all three P levels, plants showed severe stunting with small upturned leaves and consistently short and thin roots with a coarse barbed texture. At 54 µM Zn (optimal Zn level), shoots and roots were generally healthy, except at 32 µM P (deficient P level) in which roots appeared less dense and shorter. At 486 µM Zn (excessive Zn level) leaves had interveinal chlorosis, chlorosis at leaf margins and tips, mottling and down cupping and roots had brown/black tips with all three solution P levels. These observations with Zn were similar to those observed in previous studies to establish deficient, sufficient and excess levels of Zn (Barben et al., 2009). Although the stunted, dark green, upturned leaves and general purpling of the undersides of leaves were visible at 32 µM P, visual symptoms associated with P were milder than those observed in the previous studies. There were no clear visual symptoms associated with potato roots and solution P levels.

With regard to dry matter yield, no interactive effect between P and Zn was measured in this study; with the results following the same general trends observed in previous studies for both Zn and P (Barben et al., 2009). Potato shoot yields for variable Zn level and variable P level both follow a similar trend shifting from deficient to optimum levels, with variable Zn clearly the more dominant nutrient (Figs. 1 and 2). For example, the shoot yield of Zn deficient potato plants was 35% of optimal Zn (averaged

across P levels) while P-deficient shoot yield was 64% of optimal P (averaged across Zn levels; percentages calculated from data in Fig. 1 and 2). Yields of potato shoots for excessive levels of Zn and P were 83% and 92% of yields obtained at the optimum levels, respectively, but the decline in shoot yield shifting from optimal to excessive was significant only for Zn. Solution P level had little effect on root yields (Fig. 2), which was similar to previous findings (Barben et al., 2009). However, a consistent increase in root dry weight is seen with increasing Zn levels, even into the excessive level of Zn (Fig. 1), which is generally consistent with what was found in previous findings (Barben et al., 2009).

Summing root and shoot yields to calculate dry matter yield suggests slight toxicity at the high rates of both P and Zn, although the total yields did not significantly decline when increasing from the optimum to the high rates of P or Zn. Toxicity is confirmed through visual observations and the fact that the best fit models are quadratic for Zn. In regression analysis of Zn data in a previous study, R^2 values ranged from 0.69 to 0.71 for shoot, root and total dry weight (Barben et al., 2009). In the case of Zn, the significant increase that was observed with potato roots at 486 µM Zn counteracted the shoot yield decrease, thus resulting in null effect for total yield (Fig. 1). Barben et al. (2009) also found significant root yield increase with increased solution Zn concentration, with a shoot yield decrease, indicating that the negative impact of Zn is stronger on shoot yield.

Nutrient Concentrations

Although P and Zn interactions for dry matter yield were not observed, interactions were found with some tissue nutrient concentrations. A significant interaction occurs with increasing solution P on plant Zn content (Fig. 3). At optimal solution Zn (54 µM), tissue Zn concentration is unaffected with increasing solution P in all tissues. At low solution Zn (0.1 µM), increasing solution P increases Zn concentration in shoots, but not in roots. At 486 µM, a distinct opposite effect occurs with a consistent decrease in plant Zn concentration for all plant parts with increasing solution P. Contrary to expectations, a P-induced decline in shoot Zn concentration at low or optimal Zn levels and consequently a P-induced Zn deficiency were not observed as P levels increased. Instead, the data suggest a moderating or balancing effect of increased P on Zn uptake and concentration in all plant parts. Only at the 486 µM Zn, did Zn decline in concentration as P levels increased.

An interaction was also significant for shoot P concentration, but not for root P. Root P concentrations have the same predictable pattern, regardless of changing solution Zn concentrations, with P increasing significantly as solution P increases from 32 to 128 µM and then leveling off through the 1024 µM P level (Fig. 4). Shoot P follows this same general pattern for the optimum and excessive solution Zn levels, but increasing solution P promoted massive transport of P to potato shoots at the low Zn (0.1 µM) level. Thus, P uptake generally plateaus at 128 µM P, indicating a saturating effect at higher levels. This is similar to observations in a previous study (Barben et al., 2009), as well as those of Boawn and Leggett (1964), Bingham (1963), Loneragan et al. (1979) and Webb and Loneragan (1988) who reported enhanced uptake of P at low soil Zn. These data suggest that P uptake in potato is partially controlled by Zn, and without sufficient Zn, an

accumulation of P occurs in top growth. Roots exposed to high P and low Zn simultaneously did not have a decrease in tissue Zn concentration and shoots grown under these conditions actually had a significant increase in Zn concentration. These data confirm that at deficient levels of Zn, P uptake is enhanced resulting in excess P which may result in a Zn-deficiency induced P toxicity. Huang et al. (2000) in a study on Zn-deficient barley explained that "Zn appears to play a specific role in the signal transduction pathway involved in the regulation of genes encoding high affinity P transporters in plant roots. Zinc-deficient plants appear to have lost the capacity to down-regulate expression of genes encoding high-affinity P transporters in plant roots. This results in continued accumulation of high concentrations of P in the plant."

Hopkins et al. (2003) observed that application of P in the field often impacted both petiole Mn and Zn concentrations in potato. Both the current and a previous study, confirm that augmenting P impacts Mn concentrations. In the previous study, for example, root Mn increased from 314 mg kg^{-1} at 32 µM P to 507 and 927 mg kg^{-1} at 128 and 1024 µM P, respectively (Barben et al., 2009). In the current study, there was a significant, albeit smaller, gradual increase of Mn in roots , with Mn concentrations of 572 and 590 mg kg^{-1} for 32 and 128 µM P, respectively, significantly lower than 701 mg kg^{-1} for 1024 µM P (averaged over Zn levels). Also similar to the previous study, Mn in new and old shoots generally declined significantly with the first increment of P (new shoots declined from 90 to 84 mg kg^{-1} with a change from 32 to 128 µM P, respectively). Researchers have reported that high affinity phosphate transporters observed in *Arabidopsis* may also transport Mn (Luk, Jensen, and Culotta, 2003; Pitman, 2005). Activation of high affinity phosphate transporters under deficient Zn conditions would

increase P and Mn uptake and transport and could explain high concentrations of both P and Mn in shoots and roots under deficient Zn. Any increase in root Mn with increasing solution P could contribute to a Zn imbalance.

There was also a strong impact of variable solution Zn on Mn concentrations in all plant parts (Fig. 5; Barben et al., 2009). The optimum level of Zn (54 µM Zn) moderated Mn content of both shoots and roots. New shoot and root Mn were higher at Zn levels both below and above the optimum (0.1 and 486 µM Zn, respectively). These data suggest that optimal levels of Zn help control and maintain Mn by preventing excessive Mn uptake and accumulation in different plant parts. Welch and Norvell (1993) observed higher Mn concentration under deficient Zn in both roots and shoots of barley seedlings than at sufficient Zn supply despite ion leakage of Zn, Mn, Cu and Cl from roots being greater at deficient than sufficient Zn supply. They suggested that at low available Zn, a more rapid ion exchange mechanism engages to provide Zn to the plant to compensate for this leakage, which then explains increased uptake or accumulation of Mn. . In contrast to Zn, Mn has a broad range of transport pathways (Pittman, 2005; Hall and Williams, 2003), is preferentially coordinated with oxygen donors (Brown, 1963), and moves almost exclusively as a cation in plants (Tiffin, 1967). It is possible that Zn affects two or more of these potential biochemical pathways independently, which could help explain the effect of Zn on plant Mn observed in potato in these studies.

CONCLUSIONS

Deficient and excessive levels of Zn and P resulted in poor plant health, reduced dry matter yield and corresponding impacts on plant tissue Zn and P concentrations, and adequate Zn and P produced healthy, normal growing plants. None of the combinations of solution P and Zn supported the concept of a P-induced Zn deficiency, especially when solution Zn ranged from deficient to adequate--conditions under which P-Zn interaction traditionally is observed. At deficient solution Zn, increasing solution P boosted Zn concentration in shoots but not in roots. This trend reversed at the excessive solution Zn level with declining Zn in all tissues as solution P rose. Under adequate Zn and as solution P increased, no changes in plant Zn were observed. The effect of Zn on P uptake and accumulation was also somewhat surprising. At deficient solution Zn, increasing P in solution resulted in massive shoot P accumulation, whereas such increases in tissue P were more moderate at adequate and excessive solution Zn concentrations. Impacts of both Zn and P on Mn were also observed in this study and with further investigation might help explain P-Zn interaction. At adequate solution Zn, Mn was reduced in both shoots and roots but especially in roots. At deficient or excessive solution Zn, imbalances occur with both P and Mn. Therefore, although high P levels in potato did not directly reduce Zn content or promote Zn deficiency, high P may reduce the activity of Zn by interacting with other micronutrients such as Mn. The impact of Mn on P-Zn interaction requires further investigation.

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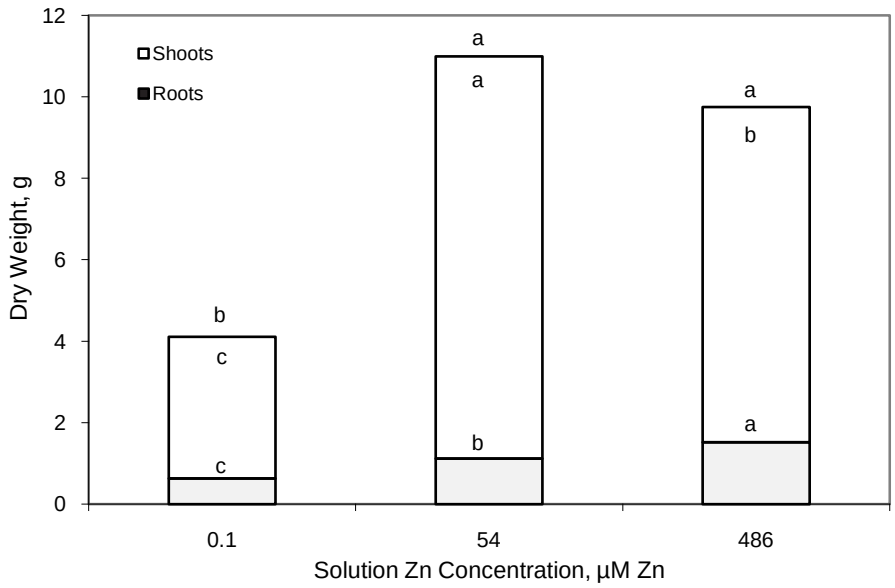

Figure 1. Shoot, root and total dry weight of Russet Burbank potato grown for 17 days at three levels of Zn (0.1, 54, 486 µM Zn) and three levels of P (32, 128, and 1024 µM P; weights shown are averaged over P levels). For roots, shoots, or total dry weight, columns with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. Note: letters indicating significance are: above the root for comparing root, inside the top for comparing shoot, and above the top for comparing total yield for each column.

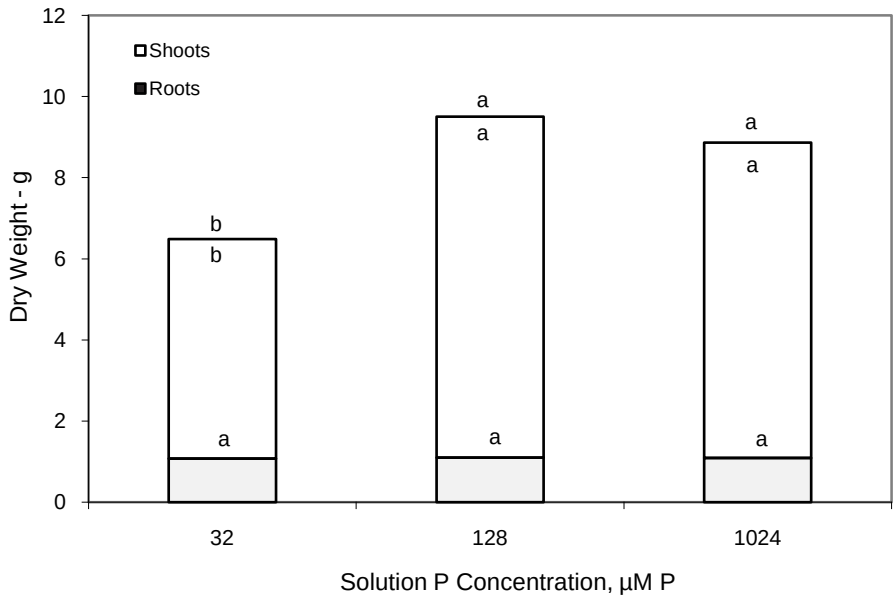

Figure 2. Shoot, root and total dry weight of Russet Burbank potato grown for 17 days at three levels of P (32, 128, and 1024 µM P) and three levels of Zn (0.1, 54, 486 µM Zn; weights are averaged over Zn levels). For roots, shoots, or total dry weight, columns with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test . Note: letters indicating significance are: above the root for comparing root, inside top for comparing shoot, and above the top for comparing total yield for each column.

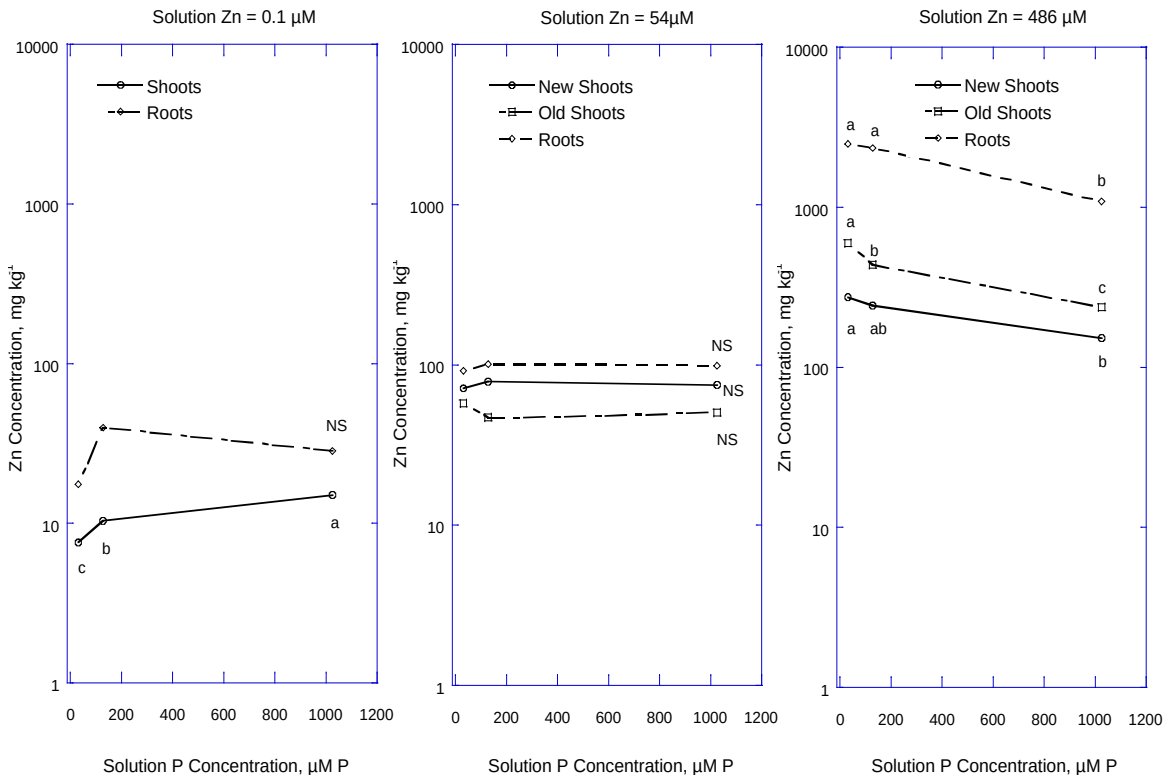

Figure 3. Concentration of Zn in new shoots, old shoots (total shoots for 0.1 µM Zn due to poor growth and limited plant material), and roots of Russet Burbank potato grown for 17 days at three levels of P (32, 128, and 1024 µM P) and three levels of Zn (0.1, 54, 486 µM Zn).. Points along the same line with the same letters are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. For a given line, NS is not significantly different at $p < 0.05$.

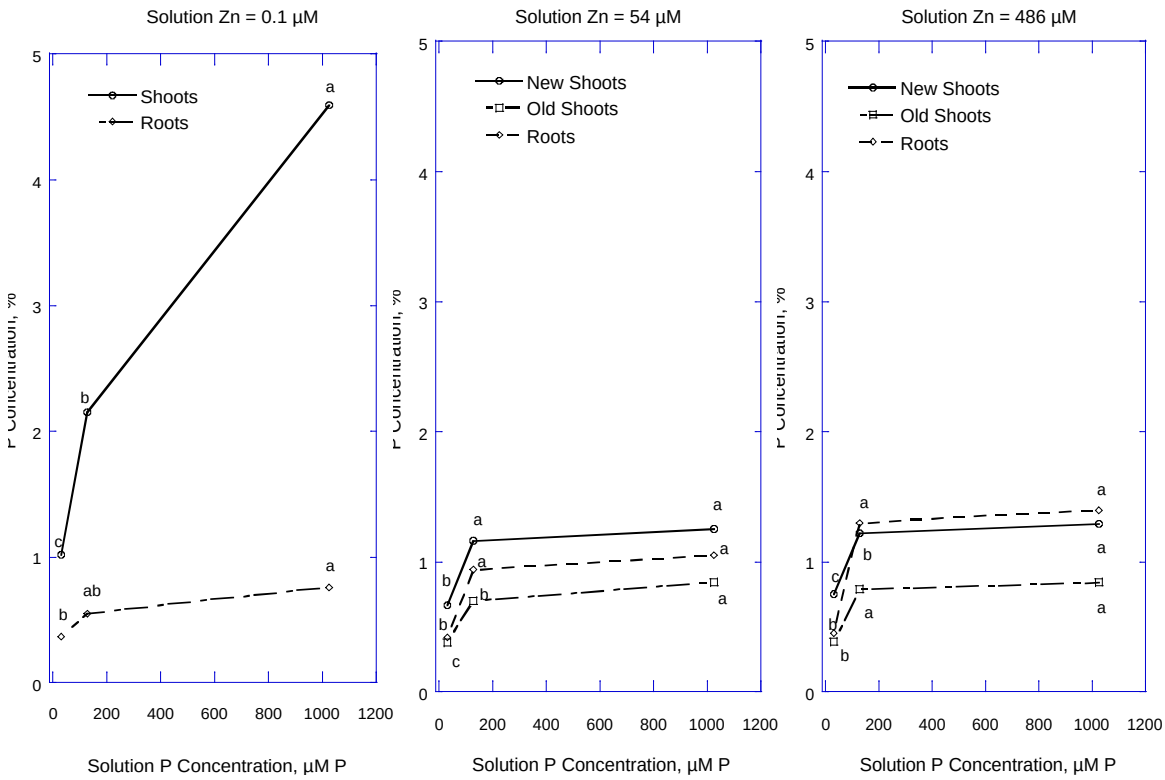

Figure 4. Concentration of P in new shoots, old shoots (total shoots for 0.1 µM Zn due to poor growth and limited plant material),, and roots of *Russet Burbank* potato grown for 17 days at three levels of P (32, 128, and 1024 µM P) and three levels of Zn (0.1, 54, 486 µM Zn) . Points along the same line with the same letters are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test.

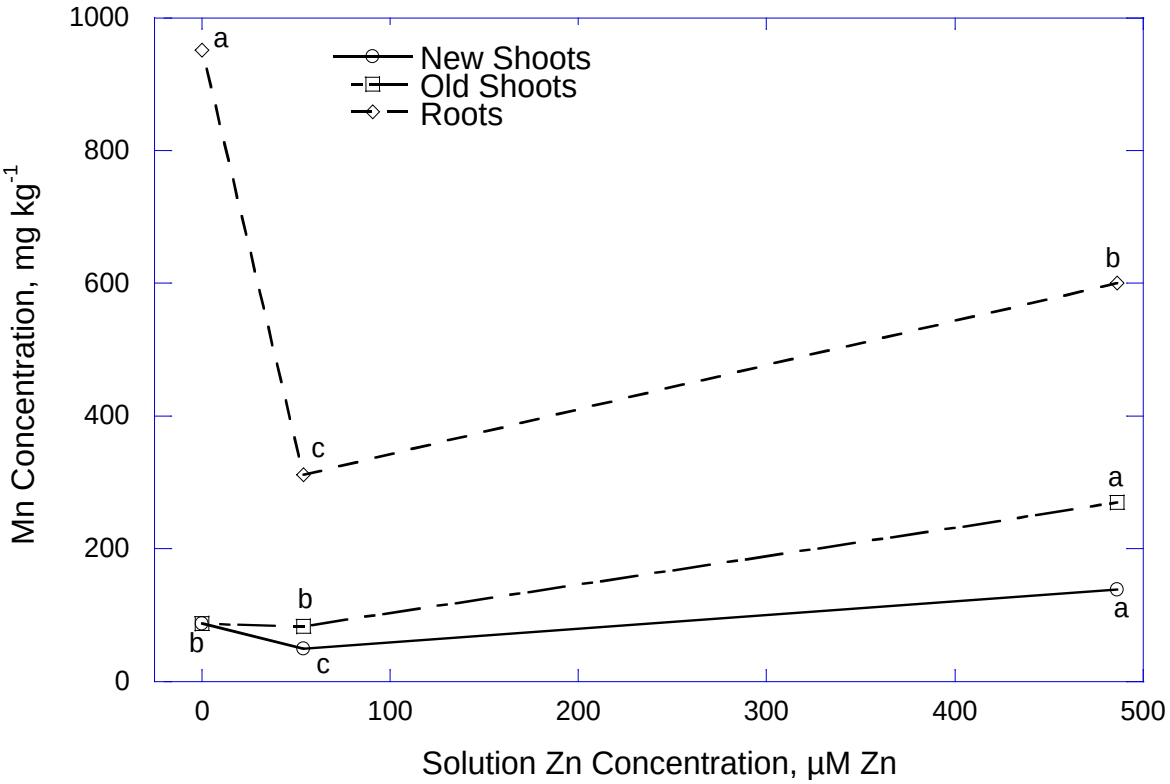

Figure 5. Concentration of Mn in new shoots, old shoots (shoots for 0.1 µM Zn due to poor growth and limited plant material), and roots of Russet *Burbank* potato grown for 17 days at three levels of Zn (0.1, 54, 486 µM Zn) and three levels of P (32, 128, and 1024 µM P; values are averaged over all P levels). Points along the same line with the same letters are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test.

MANUSCRIPT #3 - PHOSPHORUS AND MANGANESE INTERACTIONS AND THEIR RELATIONSHIPS WITH ZINC IN HYDROPONIC CHELATOR-BUFFER NUTRIENT SOLUTION GROWN RUSSET BURBANK POTATO

(prepared for submission to Journal of Plant Nutrition)

PHOSPHORUS AND MANGANESE INTERACTIONS AND THEIR RELATIONSHIPS WITH ZINC IN HYDROPONIC CHELATOR-BUFFER NUTRIENT SOLUTION GROWN RUSSET BURBANK POTATO

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ABSTRACT

Potato production requires high P availability with potential negative environmental and nutrient uptake effects. Impacts of high available P on species in potato cropping rotations are inadequately understood, but antagonistic interactions with cationic micronutrients, such as Zn and Mn could result. Two hydroponic experiments were conducted with Russet Burbank potato to elucidate P and Mn relationships and associated interactions with Zn. In the first experiment, P solution concentration was constant at 128 μM while Mn concentration varied: 0.05, 3.2, 9.5, 28.5, 85.5, 256.5, and 769.5 μM Mn. In the second, plants were grown at each of three levels of P and Mn with Mn at 0.05, 9.5 and 769.5 μM and with P at 32, 128 and 1024 μM. Potato yield maximized between 9.5 to 85.5 μM Mn and declined at deficient (0.05 and 3.2 μM) and excessive (256.5 and 769.5 μM) solution Mn levels and plant appearance reflected the observation for deficient, sufficient and excessive Mn values. As solution Mn concentration increased in the first experiment, concomitant Mn concentration increases in new shoots, old shoots, and roots followed. A P concentration decline in new shoots, old shoots, and roots resulted as solution Mn changed from deficient to sufficient and P concentration rose in

all plant parts as solution Mn changed to excessive levels. In the variable Mn experiment, Zn concentrations consistently increased in new shoots with increasing solution Mn. Old shoot Zn concentrations remained flat from 0.05 to 9.5 μM Mn solution levels, but increased significantly above 9.5 μM Mn levels. No significant changes were found in root Zn with variable solution Mn. The double variable experiment (three levels each of P and Mn) confirmed the observation of a decline and subsequent rise in plant P with increasing solution Mn levels. A strong increase in root Zn as solution P level increases is weakened as solution Mn levels increase from deficient to excessive with a concomitant decline of new and old shoot Zn. Our current observations with variable Mn and P support findings of variable P-Zn experiments, wherein high P accumulation in leaves and stems was seen under deficient Zn conditions and available Zn levels controlled plant Mn concentrations. In the current study, available Mn was observed to control plant P levels and to influence Zn uptake and translocation, thus Mn has considerable impact on uptake and distribution of P, Zn and on P-Zn interactions in potato.

INTRODUCTION

High phosphorus (P) requirement in potato (*Solanum tuberosum* L.) and low plant availability of P under high pH and calcium carbonate concentrations of arid-zone soils have led to elevated P fertilization in potato cropping systems (Marschner, 1986; Moraghan and Mascagni, 1991; Stark and Westerman, 2002). Consequently, many solis in the northwestern United States have developed extremely high soil tests (Potash and Phosphate Institute, 2001), and continuing P fertilizer application may lead to

deterioration of water quality from surface runoff and erosion, to micronutrient deficiencies, and to reductions in revenue. Additionally, excessive soil and/or fertilizer P may negatively affect crops grown in rotation with potato (Moraghan and Mascagni, 1991). An antagonistic interaction with other nutrients is a likely contributor (James et. al., 1995; Brown and Tiffin, 1962). While the most commonly observed and studied antagonistic interaction is with Zn, which can bind with P, resulting in excess P uptake under deficient soil Zn (Barben et al, 2009; Boawn and Leggettt, 1964; Bingham, 1963; Loneragan et al., 1979; and Webb and Loneragan, 1988) or a P-induced Zn deficiency (Christensen, 1972; Christensen and Jackson, 1981; Soltanpour, 1969), P reportedly also interacts with other cationic micronutrients such as manganese (Mn), iron (Fe), and copper (Cu) (Beer et. al., 1972; Brown and Tiffin, 1962; James et. al., 1995; Safaya, 1976). Hopkins et al. (2003) reported that Mn was the only micronutrient in potato besides Zn that was consistently impacted by high P soils of south eastern Idaho, but no additional field or greenhouse study followed their observation.

Cationic micronutrient impacts the P-Zn interaction are real (Buniak and Dziezycowa, 1976). Interactions between Mn and Zn have been reported in several studies. Although Gunes et al., (1998) found that plant Mn was not affected by increased Zn levels, many studies have shown significant to remarkably significant reductions in plant Mn with increased available Zn (Adiloglu, 2006; Welch and Norvell, 1993; Singh and Steenberg 1974). Barben et al. (2009) saw decreased Mn concentration in potato (for all plant parts) from deficient to optimal solution Zn but a subsequent rise in plant Mn concentrations from optimal to toxic solution Zn, indicating that optimal available Zn reduces Mn accumulation in potato and aids in controlling Mn toxicity. Ducic and Polle (2007) in a

Mn toxicity experiment on Douglas fir (*Pseudotsuga menziesii*) found that Mn stress symptoms were reduced under low available P. Zhu et al. (2002) reported slightly increased root Mn concentration with increased P availability in seven barley (*Hordeum vulgare*) genotypes. In both soil and nutrient solution experiments, several studies have reported P stimulating increased plant uptake and even toxic levels of Mn in potato (Sharma and Arora, 1987; Rhue et al., 1981; Marsh et al., 1989) and tomato (*Lycopersicon esculentum* L.) (Gunes et al., 1998). In a chelator-buffered hydroponic study of P and Zn relationships in potato, Barben et al., (2009) observed massive increases in root Mn and a slight Mn increase in shoot Mn as solution P increased--but, opposite effects have also been reported. Over a period of seven cuttings of perennial ryegrass (*Lolium perenne*), plant Mn rose with increased available soil P in four of the cuttings in one of the two soils used, but no effect of P on Mn was observed for other cuttings or in the second soil (Le Mare, 1977). A study on Mn toxicity in potato reported a synergistic effect of reduced Mn toxicity despite an accumulation of plant Mn as available P increased, (Sarkar et al., 2004). Increased P reduced Mn in soybean (*Glycine max*) shoots and roots and also alleviated Mn toxicity symptoms (Nogueira et al., 2004). In wheat (*Triticum aestivum*), leaf tissue Mn concentrations were reduced in high P soils (Neilsen et al., 1992). Thus, P effects on Mn uptake are contradictory under both insufficient and excess available Mn.

Only a few studies have reported the effects of Mn on P, with variable results. Reductions in plant P with increasing Mn were observed in both tomato (Gunes et al., 1998) and potato (Sarkar et al., 2004), while a rise in P was seen in all plant parts with increasing Mn in sorghum (*Sorghum vulgare*) (Galvez et al., 1989).

The effect of plant available Mn on plant Zn concentration has not been as well investigated as the impact of plant available Zn on plant Mn concentration. Findings in former studies of little or no direct influence of high levels of available Mn on plant Zn concentration (Ghasemi-Fasaei et al., 2005; Lombnaes and Singh, 2003; Singh and Steenberg 1974; Quartin et al., 2001) have likely led to fewer new studies. However, increased solution Mn was observed to decrease plant Zn concentration in two species of annual medic (de Varennes, Carneiro, and Gross, 2001). Under deficient Mn conditions, Zn was found to increase in two species of barley (Lombnaes and Singh, 2003). And while no significant differences were seen in whole plant Zn with increased available Mn in nine cultivars of triticale and one of wheat, Quartin et al. (2001) observed Mn-Zn shoot-root imbalances and Zn concentration increases in the roots of all cultivars.

While the impact of Zn is a very strong factor, Mn interactions likely influence P activity and/or P-Zn relationships in potato. Soil factors including pH (Borkert and Cox, 1999; Neilsen et al., 1992), mycorrhizae (Kothari, Marschner, and Romheld, 1991; Nogueira et al., 2007), soil moisture (Fox and Guerinot, 1998) and poor aeration can especially influence Mn uptake in plants. Interactions observed through hydroponic studies can help to differentiate between direct P-micronutrient associations and the effects of variable soil factors. Improved techniques in managing micronutrient contents in hydroponic solutions refine and enhance the ability to study P-micronutrient interactions independent of soil (Yang et al., 1994; Hopkins et al., 1998). Using chelator-buffered nutrient solution permits refined management of solutions and allows studies to identify deficient, sufficient and toxic concentrations of micronutrients and to focus on the plant aspects of P-micronutrient interactions.

With only circumstantial management guidelines available, improved research based guidelines are needed for predicting interactions among P, Zn and Mn and to unravel the complex nature of the chelator-buffered nutrient solution P and Mn concentrations associated with relationships in the field. The purposes of this study were 1) to identify sufficient, deficient or excess Mn levels for potato, and 2) to accurately determine P and Mn impacts on potato yield and micronutrient nutrition by using the chelator-buffered nutrient system to remove interference from conflicting variables present in soil environments. Two controlled nutrient hydroponic experiments were conducted to identify Mn-P, P-Mn and Mn-Zn relationships in potato tissue associated with deficient to toxic levels of P and Mn. Results of these studies could play a vital role in developing phosphorus and micronutrient management guidelines for the potato cropping system.

METHODS

Two experiments were conducted in hydroponic conditions in a complete random block design with Russet Burbank potato to elucidate P and Mn relationships and associated interactions with other nutrients. For both experiments, pretreatment solution was made using a modified Steinberg complete nutrient solution (Steinberg, 1953). Each of the experimental nutrient solutions were made using a modified chelator-buffered nutrient solution (Hopkins et al., 1998; Yang et al., 1994) with the following concentrations (either P or Mn varied as described below): mM concentrations were 1.0 MES pH buffer (2-Morpholinoethane-sulphonic acid (MES hydrate); 2.53 K_2SO_4; 1.43 NH_4NO_3; 1.64 $MgSO_4 \cdot 7H_2O$; $CaCl_2 \cdot 2H_2O$; μM concentrations were 110 KCl ;100 $FeSO_4 \cdot 7H_2O$; 54

$ZnSO_4 \cdot 7H_2O$; 2 $CuSO_4 \cdot 5H_2O$; 0.70 $(NH4)_8Mo_7O_{24} \cdot 4\ H_2O$; and 1.9 H_3BO_4. The base concentration of trisodium N-(2-hydroxyethyl)ethylenediamine-N,N',N'-triacetate hydrate (Na-HEDTA) was 206 µM with equivalent µM concentrations of Na-HEDTA added for each variable Mn level. Solution pH was maintained at 6.0 ± 0.2 with 6 N KOH. Nutrient solutions were renewed by complete change every 8 to 10 days. Solution concentration integrity was confirmed by nutrient content analysis at initial, mid-growth and final harvest dates. Growth chamber temperature was maintained at 25°C ± 1° during the 14 h light period and at19°C ± 1° during the 10 h dark period.

The first experiment consisted of seven treatments of four plants each with four replications. The second consisted of nine treatments of four plants each with three replications. Five to eight cm length potato plantlets (tops and roots) grown on agar provided by University of Idaho (Department of Plant, Soil and Entomological Sciences, Moscow, ID) were transferred into 14 L of complete nutrient solution (pretreatment) and grown for 17 days prior to placement into 14 L of treatment solution for another 17 days. Plants were observed in their respective treatments for relative health and appearance, harvested at the end of the 17-day treatment periods, separated as new shoots (upper leaves and stems), old shoots (lower leaves and stems), and roots, then oven dried at 65°C for a minimum of 48 hours, weighed, ground (Wiley mill, 1 mm sieve) and digested in nitric-perchloric acid and analyzed by inductively coupled plasma (ICP, Thermo Electron Corporation, Franklin, Maryland) spectroscopy for nutrient content. Results were statistically analyzed with SAS using ANOVA with Duncan mean separation tests.

In the first experiment, P solution concentration was constant at 128 µM while Mn concentration varied: 0.05, 3.2, 9.5, 28.5, 85.5, 256.5 and 769.5 µM Mn. In addition to

the base concentration of 206 µM Na-HEDTA (equivalent to the concentration of micronutrient metals plus 50 µM excess) additional Na-HEDTA was added equivalent to the Mn level in each treatment (0.05, 3.2, 9.5, 28.5, 85.5, 256.5 or 769.5 µM). From the first experiment, deficient, optimal and excess levels of Mn in a chelator-buffered environment were established for potato and selected levels were subsequently used in the second experiment (double variable P and Mn). In this second experiment, all possible combinations of three levels of P (32, 128 and 1024 µM P; determined from variable P and Zn experiments; Barben et al., 2009) and Mn (0.05, 9.5 and 769.5 µM Mn) were studied. Equivalent Na-HEDTA for each required treatment was provided as explained for experiment 1 (0.5, 9.5 and 769.5 µM).

RESULTS AND DISCUSSION

Variable Mn

Potato plants grown in mid-level treatments (9.5, 28.5 and 85.5 µM Mn) appeared most healthy based on visual observation. Those grown in low solution concentrations (0.05 and 3.2 µM Mn) exhibited general chlorosis, reduced shoot elongation and necrosis in older leaves, similar to some Mn deficiency symptoms observed by Lombnaes and Singh (2003) but did not exhibit symptoms of necrotic spotting with brown margins in younger leaves also reported in their study. Those grown at high solution Mn (256.5 and 769.5 µM Mn) exhibited unhealthy symptoms including excessive shoot elongation with weak spindly stems, down cupping and curling of leaves, leaf tip and margin chlorosis,

mottling, and necrosis in older leaves similar to those found in other studies (Sarkar et al., 2004; El-Jaqual and Cox, 1998), but necrotic spotting, stem streak necrosis and interveinal chlorosis also reported, were not observed. Decreased shoot yields (summation of new and old shoots) resulted at the lowest (0.05 µM) and highest (256.5 and 769.5 µM) levels of solution Mn. However, root dry matter yields were not significantly different among treatments (Fig. 1).

As expected, new shoot, old shoot, and root Mn concentrations increased dramatically as solution Mn levels rose (Fig. 2). Phosphorus concentrations were depressed in all plant parts as Mn solution increased from low to intermediate levels, and then consistently rose as solution Mn increases from intermediate to high levels (Fig.3). Also, a consistent increase of Zn in new shoots is seen as solution Mn increases (Fig. 4). In old shoots, little change in Zn concentration is seen from low to intermediate solution Mn, except for a sharp Zn reduction from 3.2 to 9.5 µM solution Mn. From intermediate to high solution Mn levels, a rather steep rise is observed in Zn concentration from 9.5 to 769.5 µM Mn. No significant impact of increasing solution Mn on Zn content of roots was seen (Fig 4).

Double Variable Mn and P

Observationally, the Mn variable exhibited a stronger influence in general potato plant health and appearance than variable P. Similar to the variable Mn experiment, plant grown at 0.05 µM Mn (over all three P levels) showed general yellowing in aerial plant parts, but with relatively rigorous growth, except in the 32 µM P treatment in which

plants were quite small with some necrotic edges and lower leaf drop. Poor root growth under 0.05 µM solution Mn concentration was generally observed as well. Except at the 32 µM solution P level where general stunting and dark green and purpling of leaves were apparent, the 9.5 µM Mn treatment (optimal level) noticeably improved leaf color and root mass. Shoots and roots were generally healthy and were characterized by large leaves of good color, supportive stems, full root systems and rigorous growth in both shoots and roots, At 769.5 µM Mn, toxicity symptoms of general chlorosis, leaf margin and tip chlorosis, mottling and down cupping in leaves, leaf drop, necrotic spotting and necrosis in older leaves as well as poor root growth with roots exhibiting a general yellow-brown coloration were observed regardless of solution P levels. Symptoms of P impact were not clearly observed and in some treatments were likely masked by more obvious Mn symptoms. Deficiency symptoms at the low P level (32 µM) were expressed as reduced and stunted growth with small dark green and purpling leaves. Plants grown at both 128 and 1024 µM P levels exhibited rigorous growth with large full leaves. Chlorosis and leaf edge necrosis reported as P toxicity symptoms in other studies (Cakmak and Marschner, 1987; Johnston, Gikaara and Edwards, 2006) were not apparent at high solution P in this study—the only abnormal symptoms apparently associated with the excess P of the 1024 µM P level were down cupped leaves and reduced growth in new shoots.

Both whole shoot and root potato yields rose as solution Mn changed from deficient (0.05 µM) to sufficient (9.5 µM), but then declined as solution Mn increased from sufficient to excess (769.5 µM; Fig. 5). With variable solution P, whole shoot yield rises continually from deficient (32 µM) to excess (1024 µM) with no significant difference

found between sufficient (128 µM) and excess solution P (Fig. 6). For 0.05 and 9.5 µM Mn, root yield declined from deficient to sufficient solution P followed by a minor rise from sufficient to excess solution P, but with 769.5 uM Mn, yield increased from deficient to sufficient P and plateaued at excess P (Fig 7)). Similar to the impact of Zn compared to P in a previous study (Barben et al., 2009), variable Mn clearly exhibits a more dominant impact on yield than P at either deficient or excess solution levels. Manganese deficient total yield (shoot plus root) was 66% that of optimal Mn while P-deficient yield was 79% of optimal P. Yields of potato shoots for excessive levels of Mn and P were 65% and 107%, respectively, of yields obtained at optimum levels. This suggests Mn has a stronger control on potato growth than P under this chelator-buffer growth system. This is likely because equilibrium reactions maintain available micronutrients (Mn in this study) at desired levels with the chelator-buffered solution system while P levels likely fluctuate more and are maintained by physically changing solutions regularly.

Nutrient concentrations of Mn and P from this experiment (data not presented) confirmed the observations of the previous Mn (Fig. 2) and P rate experiments (Barben et al., 2009a) which were increased P or Mn in solution resulted in concomitant increases in each respective element for all plant parts. Similar to the solution Mn effect on plant P concentration observed in the variable Mn experiment (Fig. 3), the trend was for a decline in P content in most tissues from low to optimal Mn with an an increase in P at excess levels of Mn. However, the trend was significant in this experiment for all three points only for new shoot P (Fig 7; 32 µM P). Other cases where P changed significantly as solution Mn increased occurred when solution Mn changed from optimum to excess (Fig.

8; 32 and 1024 µM P). When P was optimum, no impact of Mn on plant P occurred with any tissue. These results agree with findings of Sarkar et al. (2004) at low Mn supply and Galvez et al. (1989) at high Mn supply. In contrast, Sarkar et al. (2004) and Gunes et al. (1998), both observed reduced plant P concentrations with high Mn supply.

The influence of increasing solution Mn on plant Zn follows a similar trend as the effect of solution Mn on plant P, but consistently only with older shoots regardless of P background P level (Fig. 9)—namely, generally a decline in old shoot Zn from low (0.05 µM) to optimal (9.5 µM) Mn,and a subsequent increase from optimal to excess Mn (1024 µM). Roots grown at the lowest (32 µM P) also followed this pattern. New shoot Zn was never affected at any solution P level. Previous studies have found only small and inconsistent impacts of Mn on Zn. In sand culture, Quartin et al. (2001) observed increased Zn content in roots of triticale and wheat with increased Mn availability in field experiments, while no effect of available Mn on plant Zn was seen in chickpea (Ghasemi-Fasaei et al., 2005), maize (*Zea mays* L.) and barley (Singh and Steenberg, 1974), reduced plant Zn concentrations were found in annual medic (de Varennes, Carneiro, and Gross, 2001), peanut (Moussa, Dahdoh, and Shehata, 1996), barley and oat (Lombnaes and Singh, 2003). However, Lombnaes and Singh, (2003) attributed Zn concentration decline to reduced biomass production at deficient Mn, so also concluded no observed Mn impact on plant Zn except at the lowest Mn supply level.

Regardless of solution Mn level, root Zn consistently increased as solution P increased from low (32 µM) to optimum P (1024 µM) with a concomitant decrease in both new and old shoots (Fig. 10; one exception was at 0.05 µM P and old shoots) confirming again the binding of Zn in roots at high P levels. This results in a strong

separation between both new and old shoots compared to roots as P increases. In combination with increasing Mn (0.05 to 9.5 to 769.5 µM), the difference between shoot and root Zn concentration narrows, especially at low P and optimum to excess Mn levels, indicating that Mn may improve Zn translocation into shoots by reducing P-Zn binding in roots (Fig. 10). Other studies confirm that Mn affects shoot to root partitioning and translocation of Zn, but the effect is not limited to Zn as Fe, Ca, Mg and K are also impacted (Quartin et al., 2001; de Varennes, Carneiro, and Gross, 2001). Ducic and Polle (2007) found no effect of P supply on plant Mn accumulation in Douglas fir. But Marsh, Peterson and McCown (1989) reported increased uptake of Mn and development of Mn toxicity symptoms with increasing P level in potato.

In our current study, available Mn impacted potato P content more than available P impacted Mn. In our previous study (Barben et al., 2009a), holding Mn constant and increasing solution P dramatically increased the concentration of Mn in root tissue with minor impacts on shoot Mn contents. This suggested a P-Mn complex that could indirectly impact Zn. While no direct effect of increasing solution P on plant Mn concentration was observed in the current study (data not presented), total removal of Mn as solution P increased was significant at optimum (9.5 µM) and excess (769.5 µM) solution Mn (Fig. 11). Examining removal by roots and shoots separately instead of totaling the two (Fig. 12), increasing P from deficient (32 µM) to optimum (128 µM) to excess (1024 µM) at optimum solution Mn level (9.5 µM Mn) consistently and significantly increased total Mn uptake in both roots and shoots. This strongly suggests that P influences Mn uptake and transport when adequate Mn is available. These

increases could easily interact in the plant to reduce Zn movement or activity and produce P-induced Zn deficiency indirectly.

Suggested Mn and P interactions and impacts on Zn are complex and likely result from a combination of reported explanations. Plants require Mn for enzyme activation (Ducic and Polle, 2007; El-Jaqual and Cox, 1998; Welch, 1995) oxygen evolution in photosynthesis, detoxification of oxygen-free radicals, CO_2 fixation (Fox and Guerinot, 1998; Welch, 1995), auxin catabolism (Marsh, Peterson and McCown, 1989) ribosome structure and disease resistance (Welch, 1995). These processes can be disturbed not only from direct availability of Mn, but also from indirect effects of Mn and P relationships and the influence of Zn on Mn and P. In a previous study (Barben et al. 2009b), solution P affected Zn synergistically by improving Zn uptake at low Zn availability and reducing Zn uptake at high Zn availability. Sufficient Zn in solution was observed to restrict plant Mn from rising to toxic levels and both Mn and P rose to toxic levels when solution Zn was deficient (Barben et al., 2009). In this study, sufficient solution Mn reduced plant P, preventing P from climbing to toxic levels and while plant Zn was only slightly affected by solution Mn level, the P-Zn relationship appeared to be influenced as increasing solution Mn reduced the difference between shoot and root Zn, especially with sufficient P in solution. Considering plant removal of Mn as P level augmented, supports a role for Mn, in the P-induced Zn deficiency triangle as total as well as root and shoot Mn increased at optimum Mn concentration with increasing solution P. These results may help explain observed plant Mn, P or Zn deficiency and toxicity symptoms not explained by direct soil availability or tissue levels of Mn, P or Zn.

Physiological explanations have given some insight into the behavior of Mn, P and Zn interactions in plants. High affinity phosphate transporters observed in *Arabidopsis* may also transport Mn (Luk et al., 2003; Pitman, 2005). This may explain accumulation of both plant P and Mn concentrations in shoots and roots as solution Mn increases from sufficient to excessive levels observed in the current study. Compared with other transition metals, Mn transport is efficient. It has a broad range of transport pathways (Pittman, 2005; Hall and Williams, 2003), can be absorbed through transport mechanisms independent of other micronutrients (Bowen, 1968), is preferentially coordinated with oxygen donors (Brown, 1963), and while Zn commonly moves as an anion (Zn-citrate or malate), Mn moves almost exclusively as a cation in plants (Tiffin, 1967; Grusak et al., 1999). Furthermore, while Zn appears to be the most mobile of all micronutrients, Mn is not easily remobilized (Grusak et al., 1999). These factors could influence plant Mn accumulation and toxicity at high Zn and P solution concentrations. Enzymatic activity and genetic aspects in plants also play important roles in plant P, Zn and Mn relationships. Enzyme activation and balancing associated with Mn impacts plant growth and function and could help explain symptoms related to deficiencies or toxicities of Mn as well as other nutrients. Increased activity of indoleacetic acid, peroxidase, and polyphenol oxidase and decreased activity of catalase, ascorpic acid oxidase and glutathione oxidase have been associated with Mn toxicity (El-Jaqual and Cox, 1998). Nicotianamine synthesized from unidentified enzymes and cofactors is suggested to play a regulatory role in plant uptake of Fe^{2+}, Cu^{2+}, Mn^{2+}, and Zn^{2+} (Welch, 1995). Multiple genes may also control the operation of regulatory mechanisms for metal availability in plants (Hall and Williams, 2003).

CONCLUSIONS

Levels of deficient, sufficient and excessive Mn in a chelator-buffered nutrient solution were determined by the first experiment of this study from visual observation, potato yield and plant part nutrient concentration data. At 128 µM P, potato grown in 0.05 and 3.2 µM solution Mn developed Mn deficiency, solution concentrations for optimal potato health and appearance ranged from 9.5 to 85.5 µM Mn, and excess levels of Mn were determined in potato grown at 256.5 and 769.5 µM Mn. Deficient, optimum and excessive Mn solution concentrations chosen for further study were 0.05, 9.5 and 769.5 µM Mn, respectively, and deficient, optimum and excess solution P concentrations of 32, 128 and 1024 µM P were determined from previous experiments (Barben et al., 2009). A reduction of plant P at optimal solution Mn levels compared with either deficient of excessive Mn levels was found in both experiments of this study suggesting that sufficient Mn balances plant P uptake and distribution, preventing P accumulation and potentially toxic effects from high P levels. Although slight, a similar trend was seen with the impact of Mn on Zn in old shoots, indicating some Mn influence on plant Zn as well. A direct impact of solution P on plant Mn concentration was not found, but after total removal analysis, an increase in plant Mn with increasing P was revealed at optimum solution Mn. Similar to previous experiments, a Zn concentration separation between shoots and roots was observed as solution P increased, with root Zn rising while shoot Zn declined. Increasing Mn in solution promoted smaller differences between shoot and roots slightly. The results of this study suggest that P, Zn and P-Zn relationships in potato

are impacted by Mn and these impacts of Mn should be further studied and evaluated for developing improved nutrient management guidelines for potato cropping systems.

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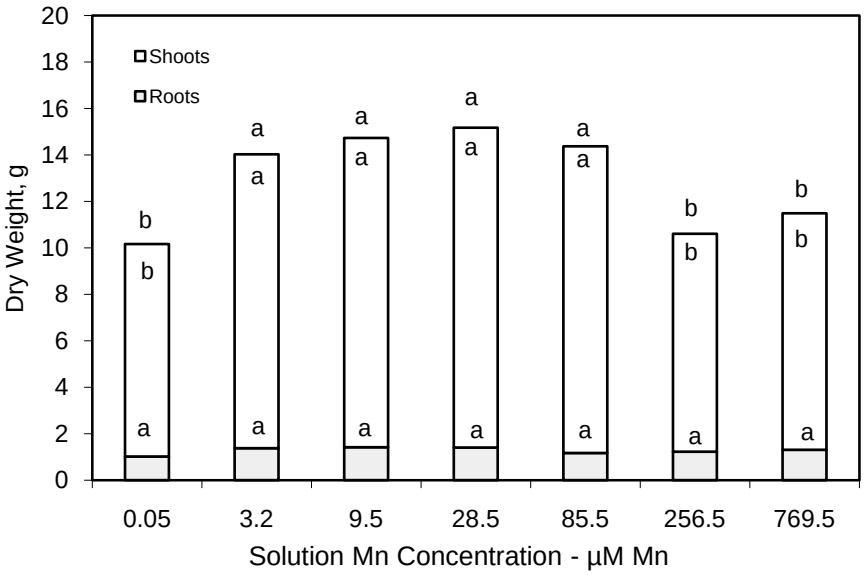

Figure 1. Shoot, root and total dry weight of Russet Burbank potato grown for 17 days at seven levels of solution Mn (0.05, 3.2, 9.5, 28.5, 85.5, 256.5, and 769.5 µM Mn; and 128 µM P). For roots, shoots, or total dry weight, columns with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. Note: letters indicating significance are: above the root bar for root interpretation, below the shoot bar for shoot interpretation, and above the shoot bar for total yield interpretation.

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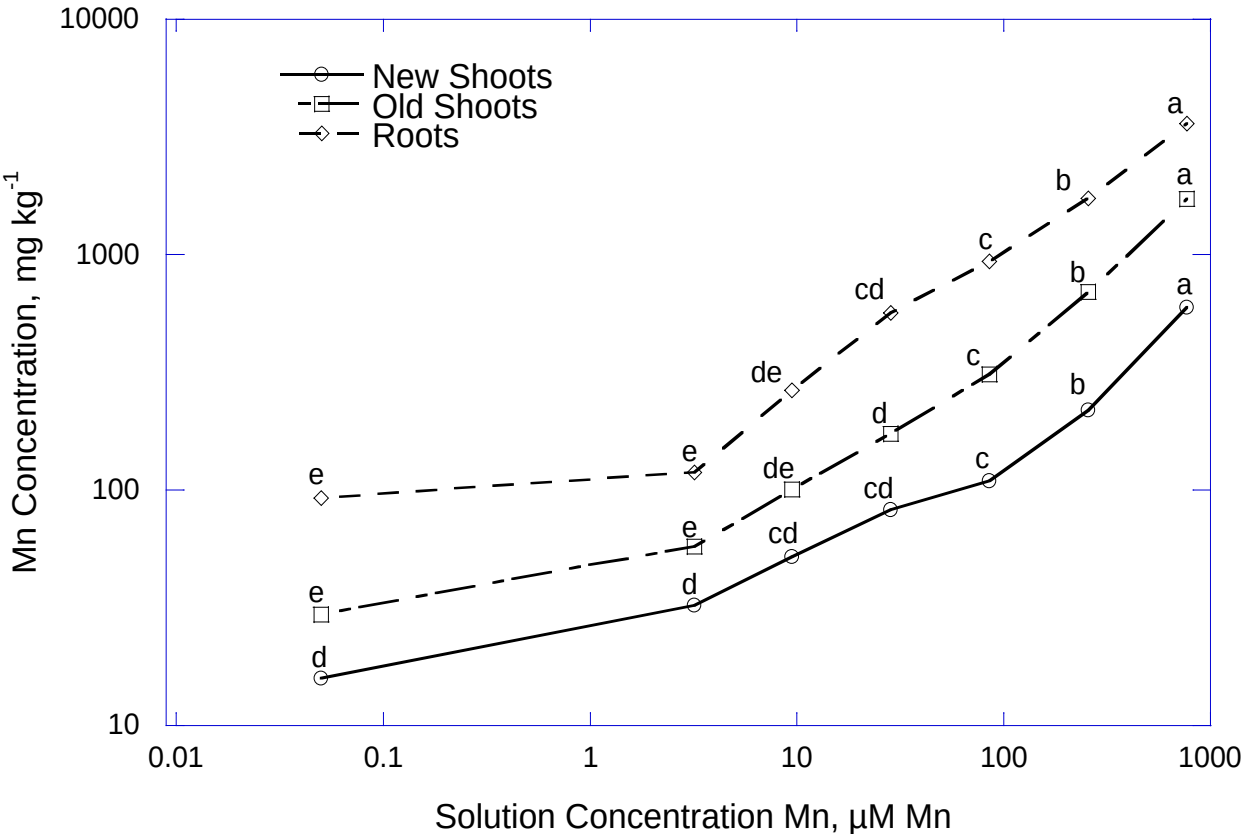

Figure 2. Concentration of Mn in new shoots, old shoots, and roots of Russet Burbank potato grown for 17 days at seven levels of solution Mn (0.05, 3.2, 9.5, 28.5, 85.5, 256.5, and 769.5 µM Mn; and 128 µM P). Points along the same line for new shoots, old shoots or roots with the same letter are not significantly different at $p < 0.05$ level, Duncan-Waller K Ratio Test. X and y axes are log scale.

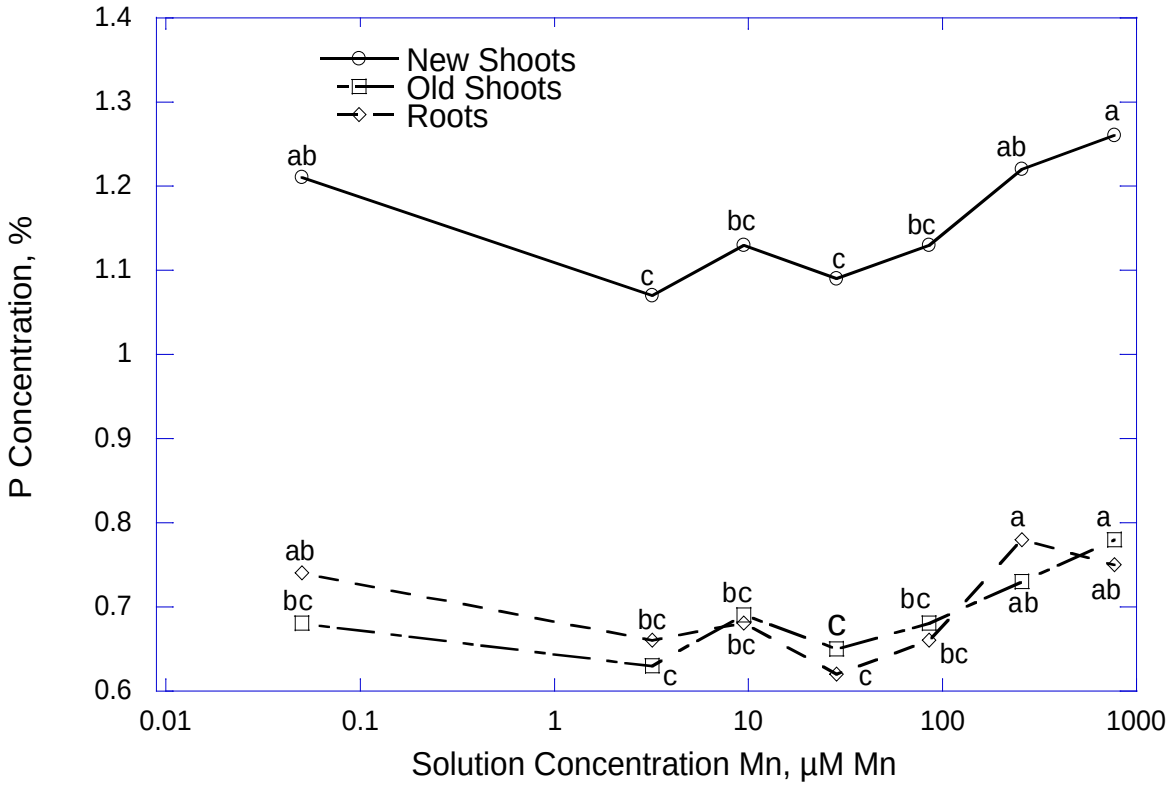

Figure 3. Concentration of P in new shoots, old shoots, and roots of Russet Burbank potato grown for 17 days at seven levels of solution Mn (0.05, 3.2, 9.5, 28.5, 85.5, 256.5, and 769.5 µM Mn; and 128 µM P). Points along the same line for new shoots, old shoots or roots with the same letter are not significantly different at $p < 0.05$ level, Duncan-Waller K Ratio Test. X axis is log scale.

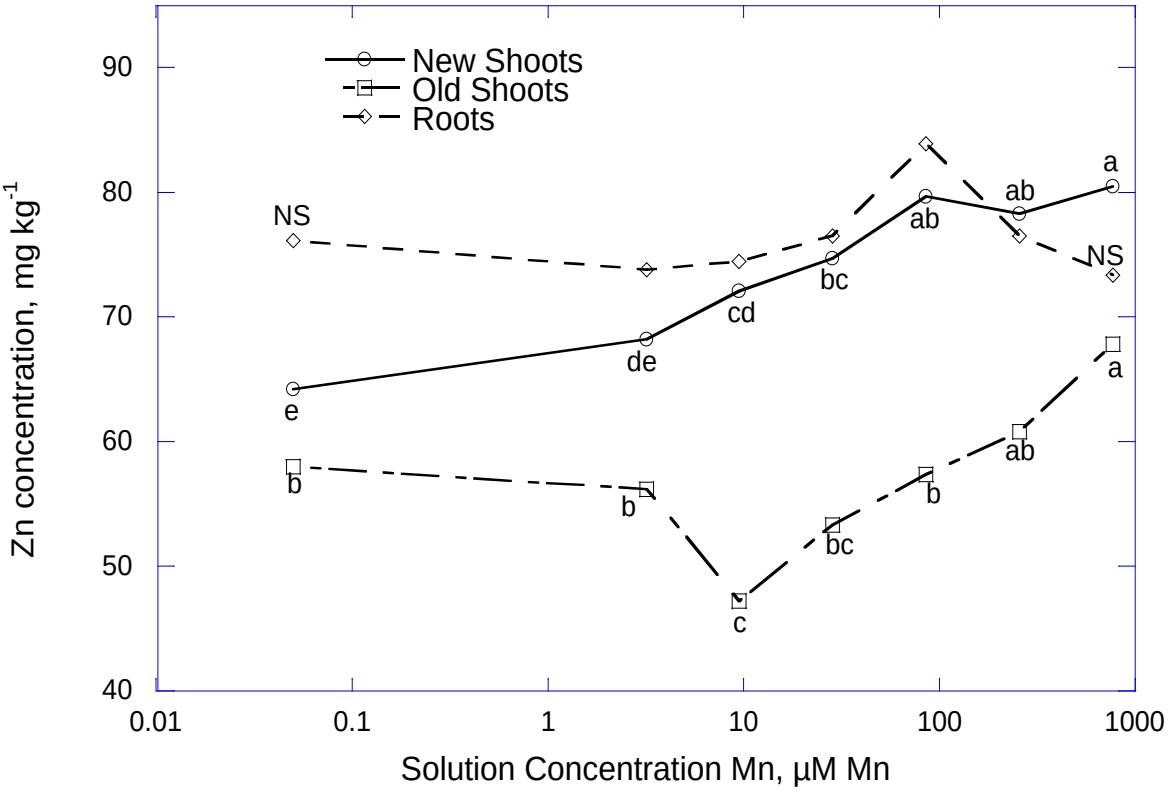

Figure 4. Concentration of Zn in new shoots, old shoots, and roots of Russet Burbank potato grown for 17 days at seven levels of solution Mn (0.05, 3.2, 9.5, 28.5, 85.5, 256.5, and 769.5 µM Mn; and 128 µM P). Points along the same line for new shoots, old shoots or roots with the same letter are not significantly different at $p < 0.05$ level, Duncan-Waller K Ratio Test. To avoid confusion when lines overlap, NS means not significant at $p < 0.05$ level for roots). X axis is log scale.

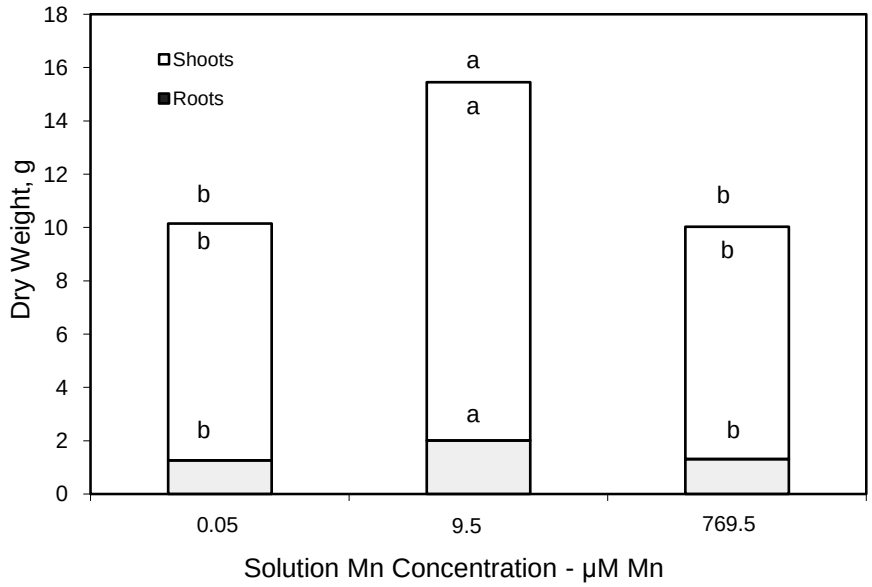

Figure 5. Shoot, root and total dry weight of Russet Burbank potato grown for 17 days at three levels of Mn (0.05, 9.5, 769.5 µM Mn) and three levels of P (32, 128, and 1024 µM P; weights shown are averaged over P levels). For roots, shoots, or total dry weight, columns with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. Note: letters indicating significance are: above the root for comparing root, inside the top for comparing shoot, and above the top for comparing total yield for each column.

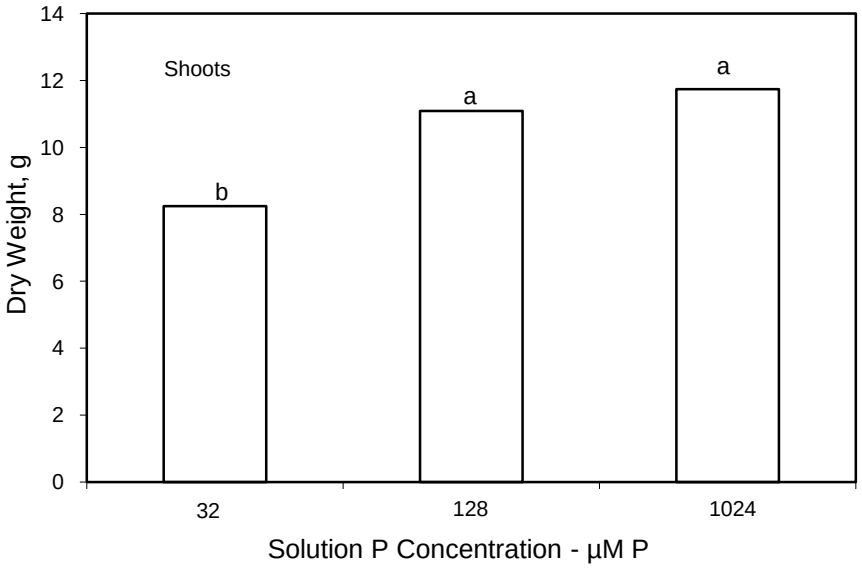

Figure 6. Shoot dry weight of Russet Burbank potato grown for 17 days at three levels of Mn (0.05, 9.5, 769.5 µM Mn) and three levels of P (32, 128, and 1024 µM P; Averaged over all Mn levels. Columns with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test.

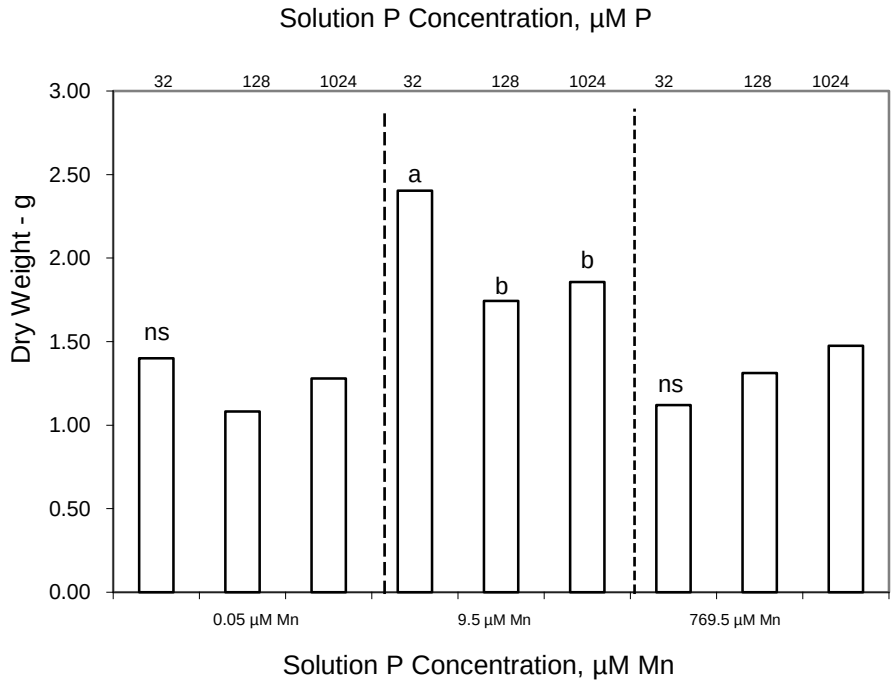

Figure 7. Root dry weight of Russet Burbank potato grown for 17 days at three levels of Mn (0.05, 9.5, 769.5 µM Mn) and three levels of P (32, 128, and 1024 µM P; A P by Mn interaction required presentation of all data for roots). Columns with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. NS means not significant at $p < 0.05$ level.

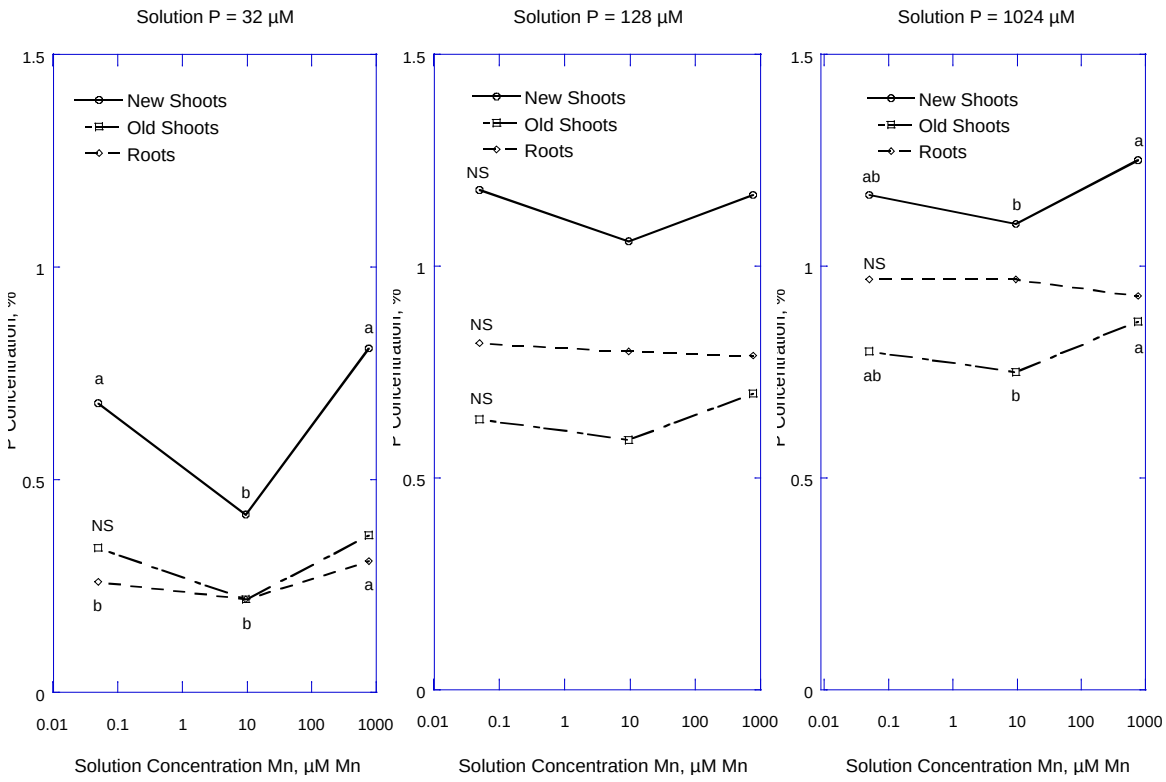

Figure 8. Concentration of P in new shoots, old shoots, and roots of Russet Burbank potato grown for 17 days at three levels of P (32, 128, and 1024 µM P) and three levels of Mn (0.05, 9.5, 769.5 µM Mn). Each graph shown as Mn varies. Points along the same line with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. NS is not significant at $p < 0.05$ level.

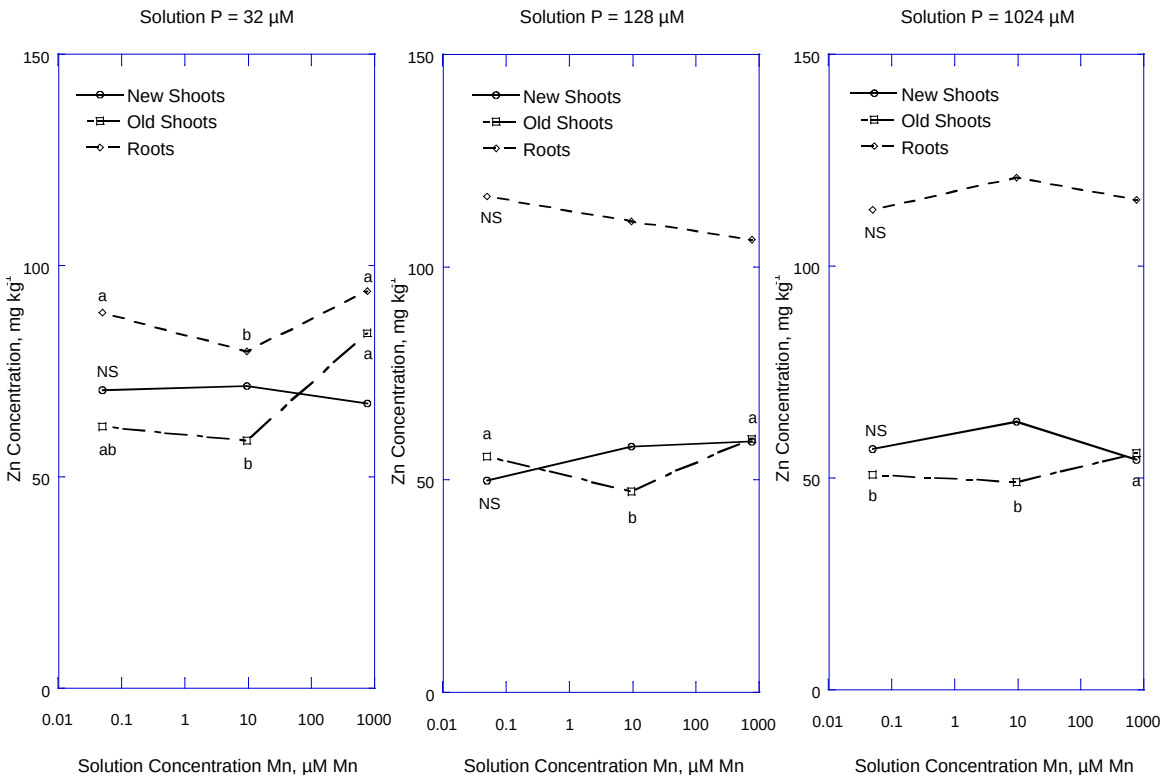

Figure 9. Concentration of Zn in new shoots, old shoots, and roots of Russet Burbank potato grown for 17 days at three levels of P (32, 128, and 1024 µM P) and three levels of Mn (0.05, 9.5, 769.5 µM Mn). Each graph shown as Mn varies. Points along the same line with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. NS is not significant at $p < 0.05$ level.

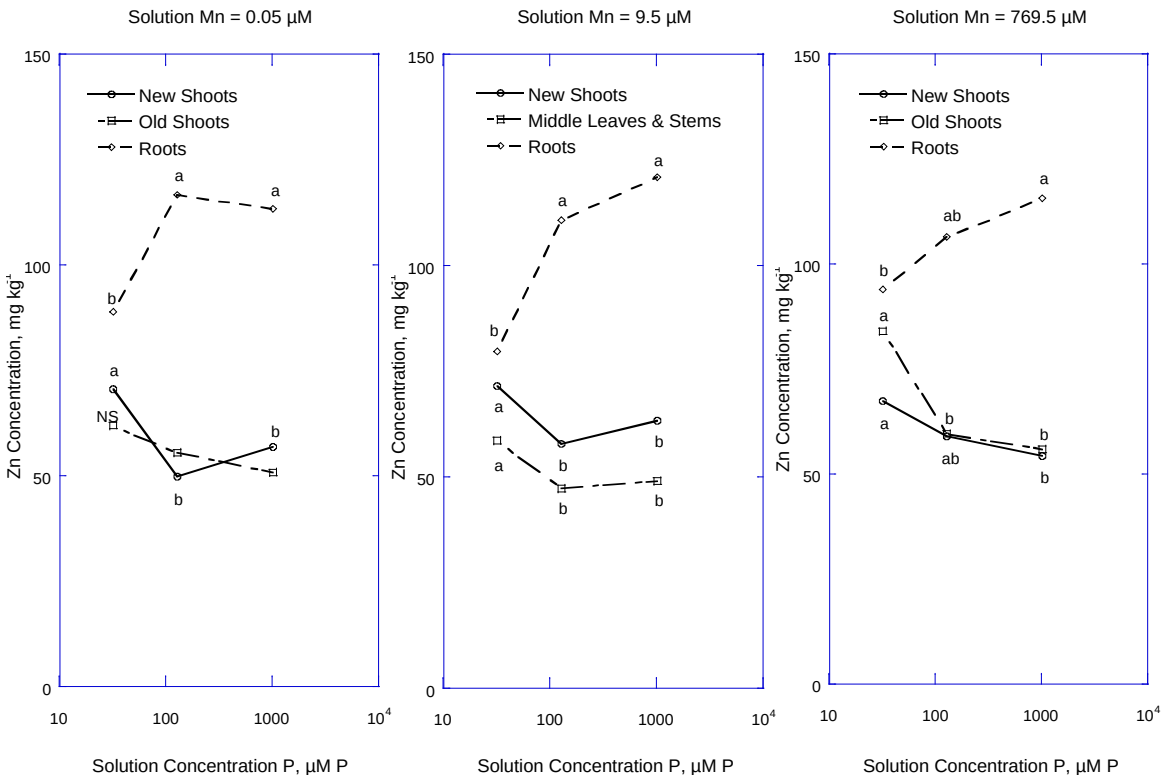

Figure 10. Concentration of Zn in new shoots, old shoots, and roots of Russet Burbank potato grown for 17 days at three levels of P (32, 128, and 1024 µM P) and three levels of Mn (0.05, 9.5, 769.5 µM Mn). Each graph shown as P varies. Points along the same line with the same letter are not significantly different at $p < 0.05$, Duncan-Waller K Ratio Test. NS is not significant at $p < 0.05$ level.

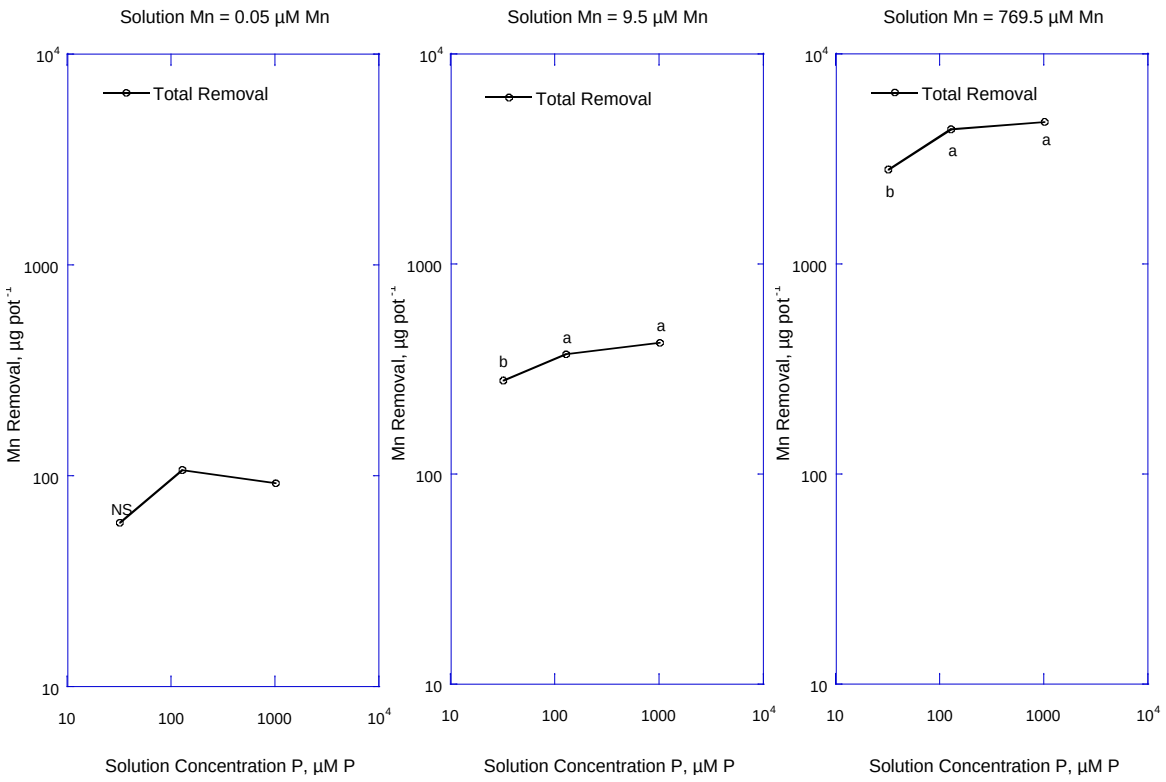

Figure 11. Total removal of Mn in Russet Burbank potato (whole plant or new and old shoots and roots) grown for 17 days at three levels of P (32, 128, and 1024 µM P) and three levels of Mn (0.05, 9.5, 769.5 µM Mn). Each graph shown as P varies. Points with the same letter are not significantly different at 0.05 level, Duncan-Waller K ratio test. NS is not significant at $p < 0.05$ level.

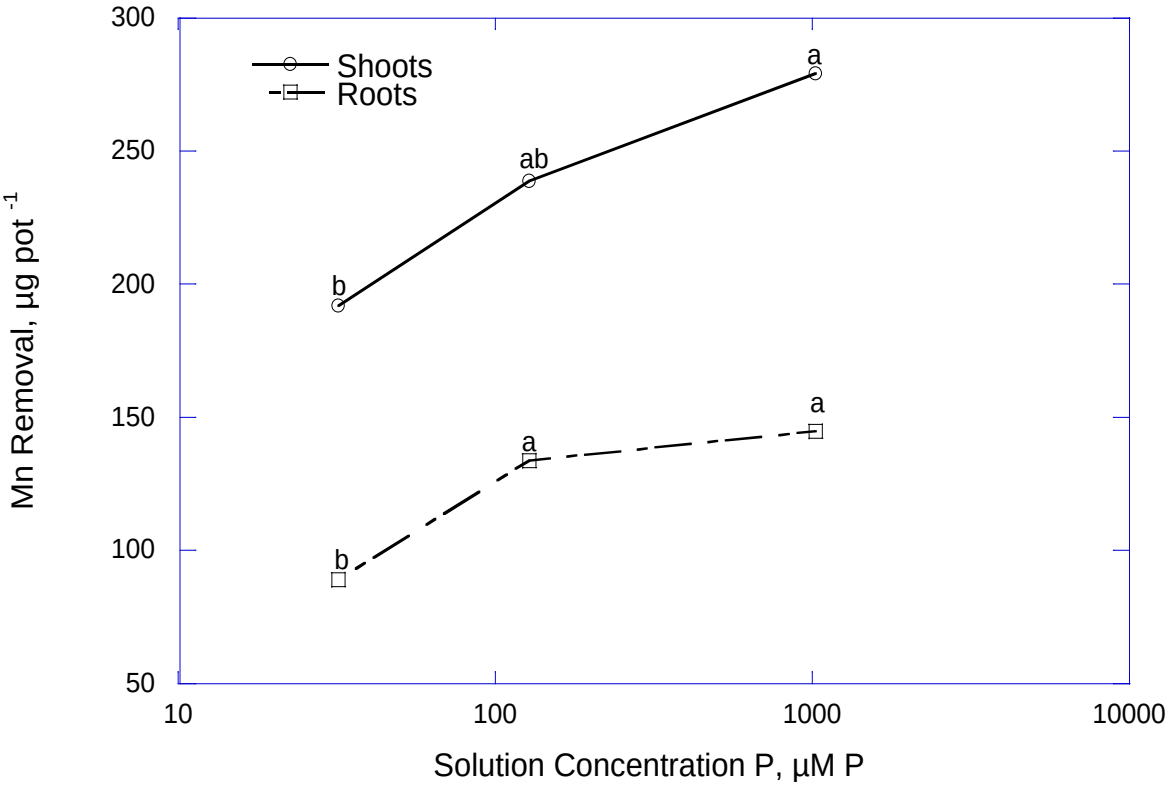

Figure 12. Total removal of Mn in Burbank potato shoots (new and old shoots) and roots grown for 17 days at 9.5 µM Mn in solution at each of three levels of P (32, 128, and 1024 µM P). Points with the same letter are not significantly different at 0.05 level, Duncan-Waller K Ratio Test.

APPENDIX A

Nutrient Concentration Tables

Chemicals for treatments for Constant P variable Zn-Experiment A06

Element symbol	Element F.W. g/mole	Chemical	Chemical F.W. g/mole	Desired Micromolar	Stock g/L	Stock Soln. M	Element M	**Stock Soln. ml to add/trt**	element mole/ compound mole	ml per 28 trts
MES	195.24	MES	195.24	2000	150	0.77	0.77	**36.44**	1	1020
K	39.0983	KCl	74.55	110	74.56	1.00	1.00	**1.54**	1	43
K	39.0983	K_2SO_4	174.27	2530	87.14	0.50	1.00	**35.42**	2	992
NH4-N	14.0067	NH_4NO_3	80.04	1430	80.04	1.00	1.00	**20.02**	1	561
Mg	24.305	$MgSO_4{*}7H_2O$	246.47	1640	246.47	1.00	1.00	**22.96**	1	643
Ca	40.008	$CaCl_2{*}2H_2O$	147.02	1000	73.51	0.5	0.5	**28.00**	1	784
Fe	55.847	$FeSO_4{*}7H_2O$[a]	278.02	100	48.64	0.17	0.17	**8.00**	1	224
Mn	54.938	$MnSO_4{*}H_2O$	169.01	9.5	2.82	0.017	0.017	**8**	1	223
Cu	63.546	$CuSO_4{*}5H_20$	249.68	2	0.874	0.00350	0.004	**8**	1	224
MoO_4		$NH_4MoO_4{*}4H_2O$	235.9	0.525	0.215	0.00091	0.001	**8**	1	226
BO_4		H_3BO_4	61.83	1.9	0.205	0.00332	0.003	**8**	1	225
HEDTA-acid form-added 8 g NaOH/L		HEDTA	278.26	161.5	69.5	0.24976641	0.250	**9.05**	1	253
P	30.973	H_3PO_4	85% H_3PO_4=14.7 M	**256**		1	1	**3.58**	1	100

14.7 M X68 mL/L=1M H_3PO_4 or 1M P

[a]When mixing iron sulfate add 10 mL of H_2SO_4 to keep in solution

Anions included in above solutions:	
SO_4-S	3016.5
NO_3-N	1430
Cl	2110

Treatment volume, L
14

Element symbol	Element F.W. g/mole	Chemical	Chemical F.W. g/mole	**Desired Micromolar**	Stock Soln. M/L	Element M	**Stock Soln. ml to add/trt**	160	Additional uM HEDTA needed	Additional ml 0.2497 HEDTA
$ZnSO_4{*}7H_2O$	65.38	$ZnSO_4 * 7H_2O$	287.54	**0.05**	0.028		0.025		0.05	0.0028
				2	0.028		1		2	0.11
				6	0.028		3		6	0.34
				18	0.28		0.9		18	1.01
				54	0.28		2.7		54	3.03
				162	0.28		8.1		162	9.08
				486	0.28		24.3		486	27.25

Bottles of Zn labeled as follows:

Zn_1 =0.0028 M (3.06 g $ZnSO_4{*}7H_20$ per 3.8 L and 4.14 g HEDTA (Na) per 3.8 L or 44.8 ml 0.247 M HEDTA per 3.8 L

Zn_2 =0.028 M (30.6 g $ZnSO_4{*}7H_20$ per 3.8 L and 41.4 g HEDTA (Na) per 3.8 L or 448.0 ml 0.247 M HEDTA per 3.8 L

Zn3=0.28 M (306.0 g ZnSO4*7H20 per 3.8 L and 414.0 g HEDTA (Na) per 3.8 L or ____ ml of stock 1.0 M stock Solution

Another possible source

Ca	40.078	$CaCl_2$	110.98	1000	27.745	0.25	0.25	**56.00**	1	1568
HEDTA (Na form) has no more than 1		HEDTA	344.21	161.5	85.972	0.24976613	0.250	**9.05**	1	253
Zn(low)		$ZnSO_4 * 7H_2O$	287.54	2	0.805	0.00279961	0.003	**10.00**	1	280

Chemicals for treatments for Constant Zn variable P-Experiment B06

Element symbol	Element F.W. g/mole	Chemical	Chemical F.W. g/mole	Desired Micromolar	Stock g/L	Stock Soln. M	Element M	**Stock Soln. ml to add/trt**	element mole/ compound mole	ml per 28 trts
MES	195.24	MES	195.24	2000	150	0.77	0.77	**36.44**	1	1020
K	39.0983	KCl	74.55	110	74.56	1.00	1.00	**1.54**	1	43
K	39.0983	K_2SO_4	174.27	2530	87.14	0.50	1.00	**35.42**	2	992
NH4-N	14.0067	NH_4NO_3	80.04	1430	80.04	1.00	1.00	**20.02**	1	561
Mg	24.305	$MgSO_4*7H_2O$	246.47	1640	246.47	1.00	1.00	**22.96**	1	643
Ca	40.008	$CaCl_2*2H_2O$	147.02	1000	73.51	0.5	0.5	**28.00**	1	784
Fe	55.847	$FeSO_4*7H_2O$[a]	278.02	100	48.64	0.17	0.17	**8.00**	1	224
Mn	54.938	$MnSO_4*H_2O$	169.01	9.5	2.82	0.017	0.017	**8**	1	223
Cu	63.546	$CuSO_4*5H_20$	249.68	2	0.874	0.00350	0.004	**8**	1	224
MoO_4		$NH_4MoO_4*4H_2O$	235.9	0.525	0.215	0.00091	0.001	**8**	1	226
BO_4		H_3BO_4	61.83	1.9	0.205	0.00332	0.003	**8**	1	225
HEDTA-acid form-added 8 g NaOH/L		HEDTA	278.26	161.5	69.5	0.24976641	0.250	**9.39**	1	263
Zn	65.38	$ZnSO_4 * 7H_2O$	287.54	**6**	8.05 #	0.028	1	3	1	84

14.7 M X68 mL/L=1M H_3PO_4 or 1M P

[a]When mixing iron sulfate add 10 mL of H_2SO_4 to keep in solution

Anions included in above solutions:						Treatment volume, L				
SO_4-S				3016.5		14				
NO_3-N				1430						
Cl				2110						

Chemical	Chemical F.W. g/mole	**Desired Micromolar**	Stock g/L	Stock Soln. M	Element M	**Stock Soln. ml to add/trt**	ompound mole			228
H3PO4		**32**		1	1	**0.45**				
		64		1	1	**0.90**				
		128		1	1	**1.79**				
		256		1	1	**3.58**				
		512		1	1	**7.17**				
		1024		1	1	**14.34**				
		2048		1	1	**28.67**				

85% H3PO4 is 14.7 M

14.7 M X68 mL/L=1M H3PO4 or 1M P

see p. 54

Bottles of Zn labeled as follows:

Zn_1 =0.0028 M (3.06 g $ZnSO_4*7H_20$ per 3.8 L or 0.85 g/L

Zn_2 =0.028 M (30.6 g $ZnSO_4*7H_20$ per 3.8 L or 8.05 g/L

Zn3=0.28 M (306.0 g ZnSO4*7H20 per 3.8 L or 80.53 g/L

Another possible source

Ca	40.078	$CaCl_2$	110.98	1000	27.745	0.25	0.25	**56.00**	1	1568
HEDTA (Na form) has no more than :		HEDTA	344.21	161.5	85.972	0.24976613	0.250	**9.05**	1	253
Zn(low)		$ZnSO_4 * 7H_2O$	287.54	2	0.805	0.00279961	0.003	**10.00**	1	280

Chemicals for treatments for variable P variable Zn-Experiment C06

Element symbol	Element F.W. g/mole	Chemical	Chem. F.W. g/mole	Desired Micromolar	Stock g/L	Stock Soln. M	Element M	**Stock Soln. ml to add/trt**	element mole/ compound mole	ml per 28 trts
MES	195.24	MES	195.24	2000	150	0.77	0.77	**36.44**	1	1020
K	39.0983	KCl	74.55	110	74.56	1.00	1.00	**1.54**	1	43
K	39.0983	K_2SO_4	174.27	2530	87.14	0.50	1.00	**35.42**	2	992
NH4-N	14.0067	NH_4NO_3	80.04	1430	80.04	1.00	1.00	**20.02**	1	561
Mg	24.305	$MgSO_4{*}7H_2O$	246.47	1640	246.47	1.00	1.00	**22.96**	1	643
Ca	40.008	$CaCl_2{*}2H_2O$	147.02	1000	73.51	0.5	0.5	**28.00**	1	784
Fe	55.847	$FeSO_4{*}7H_2O$[a]	278.02	100	48.64	0.17	0.17	**8.00**	1	224
Mn	54.938	$MnSO_4{*}H_2O$	169.01	9.5	2.82	0.017	0.017	**8**	1	223
Cu	63.546	$CuSO_4{*}5H_20$	249.68	2	0.874	0.00350	0.004	**8**	1	224
MoO_4		$NH_4MoO_4{*}4H_2O$	235.9	0.525	0.215	0.00091	0.001	**8**	1	226
BO_4		H_3BO_4	61.83	1.9	0.205	0.00332	0.003	**8**	1	225
HEDTA-acid form-added		HEDTA	278.26	161.5	69.5	0.2497664	0.250	**9.05**	1	253

[a]When mixing iron sulfate add 10 mL of H_2SO_4 to keep in solution

Anions included in above solutions:		Treatment volume, L
SO_4-S	3016.5	14
NO_3-N	1430	
Cl	2110	

Treatments Number	P Level Micromolar	Zn Level	Stock Solution Concentration P Conc. M/L	Zn Conc. M/L	Designation	Stock Solution added ml to add per treatment P	Zn	ml per 28 trts P	ml per 28 trts Zn	uM HEDTA needed	Additional ml 0.2497 M HEDTA
1 2 3	32	0.1	1	0.028	Zn2	**0.45**	0.05	4.032	0.45	0.05	0.0056
4 5 6	32	54	1	0.28	Zn3	**0.45**	2.7		24.3	54	3.03
7 8 9	32	486	1	0.28	Zn3	**0.45**	24.3		218.7	486	27.25
10 11 12	128	0.1	1	0.028	Zn2	**1.79**	0.05	16.128		0.05	0.0056
13 14 15	128	54	1	0.28	Zn3	**1.79**	2.7			54	3.03
16 17 18	128	486	1	0.28	Zn3	**1.79**	24.3			486	27.25
19 20 21	1024	0.1	1	0.028	Zn2	**14.34**	0.05	129.024		0.05	0.0056
22 23 24	1024	54	1	0.28	Zn3	**14.34**	2.7			54	3.03
25 26 27	1024	486	1	0.28	Zn3	**14.34**	24.3			486	27.25
		14.7 M X68 mL/L=1M H_3PO_4 or 1M P						149.184	243.45		

Bottles of Zn labeled as follows:

Zn_1 =0.0028 M (3.06 g $ZnSO_4{*}7H_20$ per 3.8 L and 4.14 g HEDTA (Na) per 3.8 L or 44.8 ml 0.247 M HEDTA per 3.8 L

Zn_2 =0.028 M (30.6 g $ZnSO_4{*}7H_20$ per 3.8 L and 41.4 g HEDTA (Na) per 3.8 L or 448.0 ml 0.247 M HEDTA per 3.8 L

Zn3=0.28 M (306.0 g ZnSO4*7H20 per 3.8 L and 414.0 g HEDTA (Na) per 3.8 L or _____ ml of stock 1.0 M stock Solution

Other possible sources of above chemicals

Ca	40.078	$CaCl_2$	110.98	1000	55.49	0.50	0.50	**28.00**	1	784	
HEDTA (Na form) has nc		HEDTA	344.21	161.5	85.972	0.2497661	0.250	**9.05**	1	253	
Zn(low)		$ZnSO_4 * 7H_2O$	287.54	2	0.805	0.0027996	0.003	**10.00**	1	280	

Chemicals for treatments for Constant P variable Mn-Experiment A07

Element symbol	Element F.W. g/mole	Chemical	Chemical F.W. g/mole	Desired Micromolar	Stock g/L	Stock Soln. M	Element M	**Stock Soln. ml to add/trt**	element mole/ compound mole	ml per 28 trts		
MES	195.24	MES	195.24	2000	150	0.77	0.77	**36.44**	1	1020		
K	39.0983	KCl	74.55	110	74.56	1.00	1.00	**1.54**	1	43		
K	39.0983	K_2SO_4	174.27	2530	87.14	0.50	1.00	**35.42**	2	992		
NH4-N	14.0067	NH_4NO_3	80.04	1430	80.04	1.00	1.00	**20.02**	1	561		
Mg	24.305	$MgSO_4{*}7H_2O$	246.47	1640	246.47	1.00	1.00	**22.96**	1	643		
Ca	40.008	$CaCl_2{*}2H_2O$	147.02	1000	73.51	0.5	0.5	**28.00**	1	784		
Fe	55.847	$FeSO_4{*}7H_2O$[a]	278.02	100	48.64	0.17	0.17	**8.00**	1	224		
Zn	65.38	$ZnSO_4 * 7H_2O$	287.54	16?	8.05	0.028	0.028	**8**	1	224		
Cu	63.546	$CuSO_4{*}5H_20$	249.68	2	0.874	0.00350	0.004	**8**	1	224		
MoO_4		$NH_4MoO_4{*}4H_2O$	235.9	0.525	0.215	0.00091	0.001	**8**	1	226		
BO_4		H_3BO_4	61.83	1.9	0.205	0.00332	0.003	**8**	1	225		
HEDTA-acid form-added 8 g NaOH/L		HEDTA	278.26	161.5	69.5	0.24976641	0.250	**9.05**	1	253		
P	30.973	H_3PO_4	85% H_3PO_4=14.7 M	**256**		1	1	**3.58**	1	100		
		14.7 M X68 mL/L=1M H_3PO_4 or 1M P										

[a]When mixing iron sulfate add 10 mL of H_2SO_4 to keep in solution

Anions included in above solutions:						Treatment volume, L						
SO_4-S				3016.5		14						
NO_3-N				1430								
Cl				2110							Additional	
											uM HEDTA	Additional ml
Element symbol	Element F.W. g/mole	Chemical	Chemical F.W. g/mole	**Desired Micromolar**		Stock Soln. M/L	Element M	**Stock Soln. ml to add/trt**		77	needed	0.2497 HEDTA
Mn	54.938	$MnSO_4{*}H_2O$	169.01	**0.05**		0.017		0.042			0.05	0.0028
				3.2		0.17		0.27			2	0.18
				9.5		0.17		0.8			6	0.53
				28.5		0.17		2.4			18	1.60
				85.5		0.17		7.2			54	4.79
				256.5		1.7		2.16			162	14.38
				769.5		1.7		6.48			486	43.14

Bottles of Mn labeled as follows:

Mn_1 =0.017 M (2.82 g $MnSO_4{*}H_2O$ per L)

Mn_2 =0.17 M (28.2 g $MnSO_4{*}H_2O$ per L)

Mn_3 =1.7 M (282 g $MnSO_4{*}H_2O$ per L)

Another possible source

Ca	40.078	$CaCl_2$	110.98	1000	27.745	0.25	0.25	**56.00**	1	1568		
HEDTA (Na form) has no more than :		HEDTA	344.21	161.5	85.972	0.24976613	0.250	**9.05**	1	253		
Zn(low)		$ZnSO_4 * 7H_2O$	287.54	2	0.805	0.00279961	0.003	**10.00**	1	280		

Chemicals for treatments for variable P variable Mn-Experiment B07

Element symbol	Element F.W. g/mole	Chemical	Chem. F.W. g/mole	Desired Micromolar	Stock g/L	Stock Soln. M	Element M	**Stock Soln. ml to add/trt**	element mole/ compound mole	ml per 28 trts
MES	195.24	MES	195.24	2000	150	0.77	0.77	**36.44**	1	1020
K	39.0983	KCl	74.55	110	74.56	1.00	1.00	**1.54**	1	43
K	39.0983	K_2SO_4	174.27	2530	87.14	0.50	1.00	**35.42**	2	992
NH4-N	14.0067	NH_4NO_3	80.04	1430	80.04	1.00	1.00	**20.02**	1	561
Mg	24.305	$MgSO_4{*}7H_2O$	246.47	1640	246.47	1.00	1.00	**22.96**	1	643
Ca	40.008	$CaCl_2{*}2H_2O$	147.02	1000	73.51	0.5	0.5	**28.00**	1	784
Fe	55.847	$FeSO_4{*}7H_2O$[a]	278.02	100	48.64	0.17	0.17	**8.00**	1	224
Zn	54.938	$ZnSO_4{*}7H_2O$	287.54	54	27.17	0.094	0.094	**8**	1	224
Cu	63.546	$CuSO_4{*}5H_20$	249.68	2	0.874	0.00350	0.004	**8**	1	224
MoO_4		$NH_4MoO_4{*}4H_2O$	235.9	0.525	0.215	0.00091	0.001	**8**	1	226
BO_4		H_3BO_4	61.83	1.9	0.205	0.00332	0.003	**8**	1	225
HEDTA-acid form-added		HEDTA	278.26	206	69.5	0.2497664	0.250	**11.55**	1	323

[a]When mixing iron sulfate add 10 mL of H_2SO_4 to keep in solution

Anions included in above solutions:					Treatment volume, L	14
SO_4-S				3016.5		
NO_3-N				1430		
Cl				2110		

Treatments Number	P Level Micromolar	Mn Level	Stock Solution Concentration P Conc. M/L	Mn Conc. M/L	Designation	Stock Solution added ml to add per treatment P	Mn	ml per 28 trts P	ml per 28 trts Mn	uM HEDTA needed	Additional ml 0.2497 M HEDTA
1 2 3	32	0.05	1	0.017	Mn1	**0.45**	0.042	4.032	0.378	0.05	0.0028
4 5 6	32	9.5	1	0.17	Mn2	**0.45**	0.8		7.2	9.5	0.53
7 8 9	32	769.5	1	1.7	Mn3	**0.45**	6.48		58.32	769.5	43.14
10 11 12	128	0.05	1	0.017	Mn1	**1.79**	0.042	16.128		0.05	0.0028
13 14 15	128	9.5	1	0.17	Mn2	**1.79**	0.8			9.5	0.53
16 17 18	128	769.5	1	1.7	Mn3	**1.79**	6.48			769.5	43.14
19 20 21	1024	0.05	1	0.017	Mn1	**14.34**	0.042	129.024		0.05	0.0028
22 23 24	1024	9.5	1	0.17	Mn2	**14.34**	0.8			9.5	0.53
25 26 27	1024	769.5	1	1.7	Mn3	**14.34**	6.48			769.5	43.14
		14.7 M X68 mL/L=1M H_3PO_4 or 1M P						149.184	65.898		

Bottles of Mn labeled as follows:

Mn_1 =0.017 M (2.82 g $MnSO_4{*}H_20$ per L)

Mn2 =0.17 M (28.2 g $MnSO_4{*}H_20$ per L)

Mn3 =1.7 M (282 g $MnSO_4{*}H_20$ per L)

Other possible sources of above chemicals

Ca	40.078	$CaCl_2$	110.98	1000	55.49	0.50	0.50	**28.00**	1	784	
HEDTA (Na form) has nc		HEDTA	344.21	161.5	85.972	0.2497661	0.250	**9.05**	1	253	
Zn(low)		$ZnSO_4 * 7H_2O$	287.54	2	0.805	0.0027996	0.003	**10.00**	1	280	

APPENDIX B

Experiment Data and Information Files

Data and Information Files for Experiments A06 – B07

All Files Contained in Drive D "Steven Barben P-Zn-Mn file folder"

Experiment Folders: MS_Expt_Thesis→PZN_Potato_Project→Folder (A06, B06, etc.)

Experiment A06 (Constant P-Variable Zn) file names for:

Pictures: A06PZn_Zn_H2 Potato Pict.doc
Statistics: Anova files (many)
Solution Formula Charts: Chemical solutions forZnrateA06Sept06.xls or (xlsx)
Data and Analysis: ExptA06vZn_PlantData.xls

Experiment B06 (Constant Zn-Variable P) file names for:

Pictures: B06PZn_P_H1 Potato Pict.doc; B06PZn_ P_H2 Potato Pict.doc
Statistics: Anova files (many)
Solution Formula Charts: Chemical solutions for P rate B06Oct 2006.xls
Data and Analysis: ExptB06vP_PlantData.xls

Experiment C06 (Variable Zn-Variable P) file names for:

Pictures: C06 PZn_PZn Potato H1 Pict.doc; C06 PZn-PZn Potato H2 Pict.doc
Statistics: Anova files (many)
Solution Formula Charts: Chemical solutions for 3 level Zn P expt C06 December06.xls
Data and Analysis: ExptC06vP-Zn_PlantData.xls

Experiment A07 (Constant P-Variable Mn) file names for:

Pictures: Expt A07_vMn_P potato Pict.doc; A07 JPG Picts (file folder)
Statistics: Anova files (many)
Solution Formula Charts: Chemical solutions for Mn rateA07 Jan 2007.xls
Data and Analysis: ExptA07vMn_PlantData.xls

Experiment B07 (Constant P-Variable Mn) file names for:

Pictures: B07Expt.vPvMnPotatoPict.doc; B07Expt.vPvMnPotatoPict.02.doc; Jpeg Pictures B07 (file folder)
Statistics: Anova files (many)
Solution Formula Charts: Chemical solutions for 3 level Mn P expt B07.xls
Data and Analysis: ExptB07vP-Mn_PlantData.xls

Charts and Graphs for all Experiments:

KaleidaGraph: MS_Expt_Thesis → KGraphs (folder) → Choose file
or MS_Expt_Thesis → GraphSize.doc; GraphSize.02.doc;
GraphSize.02.docx (all graphs for copy and paste).

Excel: MS_Expt_Thesis PZN_Potato_Project Graphs A06-C06.xls; Graphs A06-C06.01.xlsm; Graphs A07-B07.xls; Graphs A07-B07-07.xlsx.