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# Effectiveness of Phosphorus Fertilizers in Hydroponics and Glasshouse Settings with Moderate and High Organic Matter Soils

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Effectiveness of Phosphorus Fertilizers in Hydroponics and Glasshouse Settings with  
Moderate and High Organic Matter Soils

Jeffrey Sean Summerhays

A thesis submitted to the faculty of  
Brigham Young University  
in partial fulfillment of the requirements for the degree of  
Master of Science

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## ABSTRACT

### Effectiveness of Phosphorus Fertilizers in Hydroponics and Glasshouse Settings with Moderate and High Organic Matter Soils

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Master of Science

Phosphorus (P) is poorly soluble in most soils and, thus, has poor plant uptake efficiency. AVAIL® and Carbond P (CBP) are new fertilizer products shown to increase P use efficiency (PUE) and increase crop yields when grown in P limiting soils. Carbond P has specifically been seen to increase P uptake and crop yields in soils low in P, although effectiveness in regards to soil organic matter is unknown. The objectives of these studies were to determine if the mode of action for these products is related to physiological response, to determine if Carbond P is toxic to plant roots when in direct contact at high rates, and determine the limitations of Carbond P in regards to biomass (yield), P uptake and concentration.

We used a hydroponic study to compare CBP to AVAIL in evaluating plant toxicity and plant physiological response. AVAIL and CBP were also compared to ammonium polyphosphate (APP) at pH 6 or 8 for hydroponically grown maize (*Zea mays* L.). Additionally, a glasshouse study evaluated the PUE of CBP with soil in which maize was grown. Soils were moderate or high in organic matter, with 0, 5, 15, 45, or 135 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> applied as either APP or CBP. Both studies showed that CBP is a suitable PUE enhancing fertilizer. In the greenhouse study, the high organic matter soil revealed that both CBP and APP fertilization resulted in similar increases in biomass yield and P concentration and uptake. However, in the moderate organic matter soil, biomass and total P uptake was significantly greater for CBP than APP at the two lowest P rates of fertilization and significantly higher for APP than CBP at the highest P application rate. In the hydroponic study, neither AVAIL nor CBP had any positive or adverse effects on the plants as compared to APP.

These results, coupled with this and previous soil-based greenhouse and field studies with AVAIL and CBP, show that the increase in PUE is not a physiological growth stimulant response, but rather likely the result of impacts on P solubility in the soil. However, the presence of high organic matter in the soil seemed to negate the effects of the organic acid bonded P used in Carbond P. We conclude that CBP, and possibly other organic acid based fertilizers, can assist in furthering agricultural goals, as well as environmental responsibility with these known limits.

#### Keywords:

AVAIL, Carbond P, copolymer, dicarboxylic acid, fertilizer, glasshouse, hydroponic, maize, organic acid, organic matter, P, pH, phosphorus, *Zea mays*

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## Chapter 1

### **No Benefits or Toxicities Associated with Use of Enhanced Phosphorus Fertilizers (Carbond P® and AVAIL®) Supplied to Maize in Hydroponics**

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## **ABSTRACT**

Phosphorus is poorly soluble in most soils and, thus, has poor plant uptake efficiency. AVAIL® and Carbond P (CBP) are new fertilizer products shown to increase P use efficiency (PUE) and increase crop yields when grown in P limiting soils. The objectives of this study were to determine if the mode of action for these products is related to physiological response, and to determine if they are toxic when in direct contact with plant roots at high rates. AVAIL and CBP were compared to ammonium polyphosphate (APP) at pH 6 or 8 for hydroponically grown maize (*Zea mays* L.). Not surprisingly, biomass and P concentration increased with increases in P concentration in the nutrient solutions. However, neither AVAIL nor CBP had any positive or adverse effects on the plants as compared to APP. These results, coupled with previous soil-based greenhouse and field studies with AVAIL and CBP, show that the increase in PUE observed are not physiological growth stimulant responses, but rather likely the result of impacts on P solubility in the soil. Furthermore, the presence of these materials in direct contact with plant roots is not toxic—even at relatively high concentrations.

## **INTRODUCTION**

Phosphorus is an essential nutrient required by plants in every phase of growth and in every plant part (Bennett, 1993). The most important role of P is as a component of adenosine mono/di/triphosphate (AMP/ADP/ATP) used in photosynthesis to convert light to chemical energy and in respiration. Consequently, adequate P is important for all energy requiring reactions. In addition, P is used to modify the activity of various



enzymes by phosphorylation and for cell signaling. Furthermore, phosphate esters are structural components of DNA, RNA, and phospholipids.

Providing adequate food, fiber, and fuel for society requires efficient P fertilization (Hopkins et al., 2008). Phosphorus is needed in relatively large quantities for crop production. Phosphorus is among the top four mineral nutrients in regard to plant concentration (Havlin et al., 2005) but is often applied at rates nearly equal to N and K due to poor solubility in soils. Unfortunately, P is so poorly soluble that uptake by plant roots can be problematic (Foth and Ellis, 1996; Hopkins et al., 2008). Therefore, plant root growth and diffusion promote P uptake. Consequently, P deficiencies are relatively more common in the early part of the growing season when plant roots are developing and soil temperatures are relatively cool (Hopkins et al., 2008).

Growers often apply high rates of manure and/or fertilizer to supply P to plants and, in some cases, this rate of application becomes an environmental concern (Mueller and Dennis, 1996; Sharpley et al., 2003). Phosphorus can be transported to surface water bodies through overland flow, especially if soluble P concentrations in soil are exceptionally high (Mueller and Dennis, 1996). As with N, high concentration of P in surface water bodies is potentially negative. Although N is usually the limiting factor for plant growth in soil-based systems, P is generally the limiting factor in fresh water systems (Mueller and Dennis, 1996; Sharpley et al., 2003). As a result, high concentrations of P in surface water bodies can lead to algae blooms that can deplete oxygen and cause death of aquatic organisms. These hypoxic conditions can be unsightly and can have a pungent odor (Mueller and Dennis, 1996). Additionally, this loss of P to water body flow represents a loss in potential nutrients to crops and a waste of grower

and mineral resources. Consequently, improved efficiency in the utilization of applied P fertilizers is critical yet elusive.

Increasing the percentage of P from fertilizer that is utilized by plants (P-use efficiency or PUE) is critical for maximizing grower resources, reducing environmental impacts, and reducing consumption of non-renewable P mineral resources. However, improving PUE is challenging due to inherent inefficiencies in the soil-plant system that generally lead to fertilizer P recoveries of near zero to a maximum of less than 30% (Randall et al., 1985). Recovery of P from fertilizer is impacted by soil constituents (cations) and pH (Sposito, 2008). For example, solubility of P minerals is dramatically less in strongly alkaline and acidic soils compared to soils of slightly acidic to neutral pH. In acidic soil, the negatively charged phosphate bonds with Al, Fe, and Mn and forms poorly soluble mineral precipitates. A similar reaction occurs in alkaline soil, as phosphate bonds with Ca and Mg. Additionally, alkaline soils often contain free excess calcite (limestone), which further decreases P solubility through adsorption (Sposito, 2008). In the case of neutral pH soils, the P dissolved in soil solution bonds with cations similarly as in acid/alkaline soil, but the solubility and, therefore, the dissolution rate of soil minerals are relatively greater.

A number of rate, timing, and placement options can be used to improve PUE (Foth and Ellis, 1996; Hopkins et al., 2010a, 2010b, 2010c). In addition to these cultural practices that may enhance P uptake and utilization, fertilizer manufacturers have sought to engineer materials to enhance PUE (Hopkins et al., 2008). Two P fertilizer additives that have been theoretically engineered to keep the P more plant available are: AVAIL®

(AVAIL, Specialty Fertilizer Products, Leawood, KS, USA) and Carbond P® (CBP, Landview Fertilizer, Rupert, ID, USA).

The proposed mode of action for AVAIL is that its high-charge density sequesters interfering cations, such as calcium or aluminum in the microsite surrounding the fertilizer prill. AVAIL is unique in that it is a water soluble long chain dicarboxylic acid (DCA) copolymer but only slightly mobile from point of contact. According to Dunn and Stevens (2008), this material is specific to adsorption of divalent and trivalent cations and is minimally affected by temperature, pH or ionic strength. Thus, when applied to soils, AVAIL theoretically minimizes the concentration of potentially reactive cations in the immediate vicinity of the P fertilizer. More detail regarding the impacts of AVAIL on production of a variety of crops is reviewed by Hopkins (2012) and Stark and Hopkins (2013). In brief, there is evidence that the AVAIL molecule impacts soil P chemistry and apparently increases solubility but also produces mixed field results. Not surprisingly, positive results with crop yield and quality were reported more often when soil test P levels and fertilizer application rates were relatively low i.e. there is no added benefit to applying AVAIL if plants already have ample P nutrition.

Carbond P is also designed to enhance P nutrition, but has a different mode of action than AVAIL. Carbond P has major compositional elements that are classified as organic acids (note that CBP also includes a cocktail of other proprietary additives beyond P and organic acids). The theoretical impact of CBP is based on chemical bonding of P with these organic acids. Organic acids and other fractions of soil play a major role in soil and plant nutrition. Humic substances (HS), especially humic and fulvic acids (HA, FA), make up the majority of soil organic material. These compositional

elements play a major role in the environmental fate of both organic and inorganic materials (Tan, 2003). Synthetic humic acids have proven beneficial to plant growth (Seyedbagheri, 2010). Various studies have shown positive bio-enhancement effects of organic acids. These studies vary in results and organic acid origins, but often have similar findings. Generally, root bio-enhancement has been observed via elongation, yield, and emergence (Canellas et al., 2002; Eyheraguibel et al., 2008; Piccolo et al., 1993). In addition to the claims of various growth responses, improvement of P nutrition is another documented impact of organic acids (Seyedbagheri, 2010; Tan, 2003).

As a relatively new product, testing of CBP has not been as widespread as AVAIL, but there is evidence that CBP impacts soil chemistry (Hill et al., 2013a, 2013b; Hopkins et al., 2013). As with AVAIL, positive results were reported more often when CBP is applied to soil with low plant available P levels and fertilizer application rates of P are also relatively low. Increased availability of P provided as CBP is almost always exhibited in increased plant P concentrations compared to traditional fertilizers (Hill et al., 2013a; Hopkins et al., 2013).

Although there is evidence that the mode of action for both AVAIL and CBP are related to soil chemistry (Hill et al., 2013a, 2013b; Hopkins, 2012; Hopkins et al., 2013), questions remain as to whether or not the impacts of CBP and AVAIL could be related some way with plant physiology—some type of general growth response triggered within the plant cells beyond the impact of P chemistry in soil. Additionally, there are long-chain molecules present in both of these products which might be toxic to plant tissue at high rates. Therefore, the objectives of this study were to compare the impacts of CBP

and AVAIL with impacts of ammonium polyphosphate (APP) at solution pH buffered to 8.0 and 6.0 in hydroponically grown maize (*Zea mays* L.).

## MATERIALS AND METHODS

Four hydroponic experiments in which maize (Syngenta NK N27B-3111; an AgriSure Viptera hybrid) were grown in an environmentally controlled growth chamber located at Brigham Young University in Provo, UT, USA. Phosphorus was supplied at five rates of P as APP, CBP, or AVAIL in nutrient solutions buffered at pH 6 or 8 (Table 1). Maize seeds were germinated by placing them on 4-mm stainless steel screens covered with moist cheesecloth in 9.5-cm deep rectangular plastic trays with 2 L of dilute complete nutrient solution (Nichols et al., 2012; Steinberg, 1953) reaching the bottom of the stainless steel screens. The modified Steinberg (1953) solution contained:  $\mu\text{M}$  concentrations, 635 calcium nitrate [ $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ], 135 magnesium nitrate [ $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ], 115 ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), 43 dipotassium phosphate ( $\text{K}_2\text{HPO}_4$ ), 125 potassium sulfate ( $\text{K}_2\text{SO}_4$ ), 125 potassium chloride (KCl), 120 potassium nitrate ( $\text{KNO}_3$ ), 135 ammonium sulfate [ $(\text{NH}_4)_2\text{SO}_4$ ], 17 boric acid ( $\text{H}_3\text{BO}_3$ ), 5.9 manganese chloride ( $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ), 1.5 zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ), 0.40 copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), 0.26 sodium molybdate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ), and 0.50 iron-ethylenediamine-N,N'-bis(2-hydroxyphenylacetic acid (Fe-EDDHA). Germination and elongation of plants were accomplished over a seven-d period at  $\sim 25^\circ\text{C}$  (three d in darkness and four d in light). To promote elongation, white butcher paper with small slits was placed over the containers during the four d in light. After seven d, seedlings were transferred into polyethylene buckets placed in silver wooden boxes with randomly

selected plants (eight per container) suspended through holes in opaque plastic lids with 14 L of dilute, complete nutrient solution in each bucket (Nichols et al., 2012; Steinberg, 1953) and placed in an environmental growth chamber [ $\mu\text{M}$  concentrations were 977  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 210  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , 180  $\text{NH}_4\text{NO}_3$ , 71  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 133  $\text{K}_2\text{HPO}_4$ , 116  $\text{K}_2\text{SO}_4$ , 115  $\text{KCl}$ , 75  $\text{KNO}_3$ , 41  $(\text{NH}_4)_2\text{SO}_4$ , 10  $\text{H}_3\text{BO}_3$ , 3.6  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.94  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.25  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.16  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , and 1.4 Fe-EDDHA]. The base of the plant stem was wrapped in foam for support, with the foam wrapped with parafilm (Parafilm M Laboratory Film, Alcan Inc., Chicago, IL) to prevent brace roots from growing into the foam. Plants were grown in this solution for seven d prior to transfer into treatments.

The treatment phase of the experiments consisted of four healthy maize plants of uniform size transferred into 14 L of modified Steinberg nutrient solution for each P treatment (Nichols et al., 2012; Steinberg, 1953). Maize plants were suspended using the same container system used for pretreatment [ $\mu\text{M}$  concentrations were 1,430  $\text{Ca}(\text{NO}_3)_2$ , 820  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 250  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 20  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 400  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 5.9  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 1.3  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.3  $\text{NH}_4\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ , 1.2  $\text{H}_3\text{BO}_4$ , 100 HEDTA, 1500 KOH, and 2000 2-Morpholinoethanesulphonic acid (MES hydrate) pH buffer. Solution pH was initially adjusted and then maintained daily with potassium hydroxide (KOH). Nutrient solutions were replenished by adding half of the original concentrations twice during each study. Treatments consisted of P concentrations of 72, 400, 3200, 6400, or 9600  $\mu\text{M}$  P supplied as APP, CBP or AVAIL (Table 1). Additions of each rate and source of P were split evenly seven times over the course of the experiment (every 2.3 d). Nitrogen was balanced across treatment with addition of  $(\text{NH}_4)_2\text{SO}_4$ .

Plants were grown in a 14/10 h light/dark photoperiod and temperatures of 25°C±1° during the light period and of 19°C±1° during the dark period. Nutrient solutions were aerated continuously during all growth phases of the experiment. Plants were observed daily and rated for general health, vigor and nutrient deficiency or toxicity development. Plants were harvested at the end of 18 d in treatment by rinsing roots of the four plants per pot repeatedly in ddH<sub>2</sub>O and separating as shoots and roots, oven dried at 65°C for a minimum of 72 h to uniform dryness, weighed, ground (Wiley mill, 1-mm sieve), digested in nitric-perchloric acid and analyzed for nutrient content by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Thermo Electron Corporation, Franklin, MD, USA) spectroscopy.

The experiments were arranged in randomized complete block designs with three replications of each of the ten treatments (two fertilizer sources at five P concentrations). Statistical analyses were performed using Statistical Analysis System (Version 9.1, SAS, SAS Institute, Cary, NC, USA). The data for yield, nutrient concentration, and shoot, root, and total nutrient uptake were analyzed using analysis of variance (ANOVA) with mean separation using Duncan Waller Multiple Range Test at the 0.05 level of significance ( $P < 0.05$ ).

## **RESULTS AND DISCUSSION**

The four experiments generally produced similar results regardless of fertilizer source or solution pH level. Fertilizer source produced no favorable or adverse impacts on biomass or P concentration in any of the four studies (Tables 2, 3 and 4 and Figures 1 and 2). None of the interactions between fertilizer source and rate were significant (Table

2), although interactions would be considered significant for root P in the AVAIL study at pH 6 and root biomass in the AVAIL study at pH 8 at  $P = 0.0985$  and  $0.0782$ , respectively. However, any possible trends of root biomass at varying levels of P in these studies with AVAIL were not clear or of any practical significance. Additionally, nutrients other than P were also analyzed and found to be at adequate levels in the plant tissues, but no consistent impact was found based on fertilizer source (data not shown).

Not surprisingly, increasing rates of solution P generally resulted in increased shoot and root P concentrations (Tables 2 and 5). This increase in P concentration resulted in an increase in shoot biomass in a mostly linear fashion (Table 5). For roots, biomass peaked at 400-6400  $\mu\text{M}$  and then plateaued or dropped off as P rate increased (Table 5).

Although it is not possible to compare across studies statistically, it is noteworthy that P concentration was consistently lower for maize grown at pH 8 compared to pH 6 (Figure 1). The average maize P concentrations for the studies conducted at low pH (6.0) were 28 and 33% higher than for studies conducted at pH 8 for shoots and roots, respectively. This seemingly resulted in reduced shoot growth (-19%), but relatively higher root growth (45%) trends in these hydroponic studies (Figure 2).

In contrast to soil-based greenhouse and field studies (Hill et al., 2013a, 2013b; Hopkins, 2012; Hopkins et al., 2013; Stark and Hopkins, 2013), neither CBP nor AVAIL promoted additional maize growth response compared to traditional APP fertilizer in hydroponic solutions. The results of these studies conducted in the absence of soil show conclusively that AVAIL and CBP are not any more or less stimulating or toxic than APP fertilizer supplied to maize. Furthermore, these results also suggest that the impacts



previously observed in greenhouse and field studies (Hill et al., 2013a, 2013b; Hopkins, 2012; Hopkins et al., 2013) result from soil chemistry interactions rather than from plant physiological responses.

Plant growth stimulation is among the positive impacts reported for organic acids (Tan, 2003), but no “growth stimulation” effect was observed with CBP or AVAIL in these hydroponic trials. This suggests that the increases in yield and/or crop quality observed with use of CBP and AVAIL compared to APP in soil-based greenhouse and field trials are limited solely to impacts on P nutrition. Given these results and the results of various soil-based studies that show increased concentrations of plant P with use (Hill et al., 2013a, 2013b; Hopkins, 2012; Hopkins et al., 2013), it is likely, as proposed, that these fertilizer products do impact soil chemistry and make P more soluble. Therefore, soil P is more likely to be available in solution for plant uptake, rather than becoming less soluble and, therefore, less plant available. It is noteworthy that no toxic impact was observed when either AVAIL or CBP were placed in direct contact with roots and this would suggest that AVAIL and CBP could be placed at similar rates currently used for APP in the rooting zone. Field trials conducted by Hopkins et al. (2013) support this conclusion as the highest rates used in this study are relatively higher.

Management practices using AVAIL and CBP which often increase PUE are warranted and are increasingly needed. Further investigation is needed to determine the effectiveness of AVAIL and CBP for a variety of crops and soils.

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## TABLES

**Table 1. Experiment Parameters.**

Phosphorus fertilizer sources and pH of hydroponic nutrient solutions for four hydroponic experiments. Fertilizer sources included either Carbond P® (CBP) or AVAIL® compared to ammonium polyphosphate (APP).

Experiment	Fertilizer Sources Compared	Solution pH
CBP pH 6	APP and CBP	6.0
CBP pH 8	APP and CBP	8.0
AVAIL pH 6	APP and AVAIL	6.0
AVAIL pH 8	APP and AVAIL	8.0

**Table 2. Statistical Significance.**

Statistical significance of shoot and root biomass and P concentrations in four maize hydroponic studies comparing ammonium polyphosphate (APP) to either Carbond P® or AVAIL® blended with APP grown at either pH 6 or 8. Values bolded are statistically significant at  $P < 0.05$ , ANOVA.

Source	-----Shoot-----		-----Root-----		-----Shoot-----		-----Root-----	
	P	Biomass	P	Biomass	P	Biomass	P	Biomass
----- Carbond P Studies -----								
	----- pH 6 -----				----- pH 8 -----			
Overall model	<b>&lt;0.0001</b>	<b>0.0081</b>	<b>&lt;0.0001</b>	0.3277	<b>&lt;0.0001</b>	<b>0.0014</b>	<b>&lt;0.0001</b>	<b>0.0036</b>
P level (L)	<b>&lt;0.0001</b>	<b>0.0003</b>	<b>&lt;0.0001</b>	<b>0.0426</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0003</b>
P source (S)	0.2084	0.9508	0.4691	0.7021	0.9531	0.8406	0.3533	0.3058
L x S	0.1900	0.8652	0.7784	0.8995	0.1676	0.9159	0.2828	0.3496
----- AVAIL P Studies -----								
	----- pH 6 -----				----- pH 8 -----			
Overall model	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0438</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
P level (L)	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>0.0027</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
P source (S)	0.1563	0.3943	0.9569	0.7298	0.6946	0.743	0.3076	0.3554
L x S	0.9518	0.3453	0.0985	0.6687	0.9533	0.2723	0.7393	0.0782

**Table 3. Carbond P and Ammonium Polyphosphate.**

Table 3 has been split into Tables 3a and 3b for formatting purposes. Shoot and root dry matter yields and P concentrations for maize grown hydroponically with five levels of solution P supplied as either ammonium polyphosphate (APP) or Carbond P® (CBP). Differences shown for source are averaged across rates and means separated with Duncan Waller separation; the source x rate interactions were not significant in any instance (Table 2;  $P < 0.05$ ).

**Table 3a**

P, $\mu$ M	----- Shoot -----			
	----- biomass, g pot <sup>-1</sup> -----		----- P, % -----	
	APP	CBP	APP	CBP
	-----Experiment CBP pH 6 -----			
72	26 c	27 d	0.14 e	0.13 d
400	61 b	54 c	0.21 d	0.2 c
3200	77 a	81 a	0.58 c	0.85 b
6400	67 ab	78 a	0.85 b	0.91 b
9600	77 a	71 b	1.07 a	1.04 a
mean	61	62	0.57	0.63
	-----Experiment CBP pH 8 -----			
72	13 d	12 d	0.12 d	0.1 e
400	35 c	32 c	0.25 c	0.21 d
3200	58 b	65 b	0.64 a	0.52 c
6400	71 a	64 b	0.62 a	0.65 b
9600	73 a	84 a	0.58 b	0.72 a
mean	50	51	0.44	0.44



**Table 3b**

----- Root -----							
----- biomass, g pot <sup>-1</sup> -----				----- P, % -----			
APP		CBP		APP		CBP	
----- Experiment CBP pH 6 -----							
9	b	10	b	0.12	e	0.15	d
14	a	14	a	0.3	d	0.21	d
11	ab	10	b	1.28	c	1.16	c
11	ab	11	b	1.48	b	1.55	b
9	b	11	b	1.9	a	1.78	a
11		11		1.02		0.97	
----- Experiment CBP pH 6 -----							
6	c	6	d	0.2	d	0.18	d
11	b	10	c	0.32	c	0.27	d
16	a	18	b	0.75	b	0.83	c
17	a	17	b	0.91	b	1.08	b
13	b	21	a	1.47	a	1.46	a
13		14		0.73		0.76	

**Table 4. AVAIL and Ammonium Polyphosphate**

Table 4 has been split into Tables 4a and 4b for formatting purposes. Shoot and root dry matter yields and P concentrations for maize grown hydroponically with five levels of solution P supplied as either ammonium polyphosphate (APP) or AVAIL®. Differences shown for source are averaged across rates and means separated with Duncan Waller separation; the source x rate interactions were not significant in any instance (Table 2; *P* < 0.05).

**Table 4a**

P, $\mu$ M	----- Shoot -----			
	----- biomass, g pot <sup>-1</sup> -----		----- P, % -----	
	APP	AVAIL	APP	AVAIL
	----- Experiment AVAIL pH 6 -----			
72	19 d	19 d	0.15 c	0.12 c
400	54 c	58 c	0.18 c	0.11 c
3200	72 a	71 b	0.79 b	0.69 b
6400	66 b	85 a	0.93 ab	0.91 a
9600	66 b	60 c	0.99 a	0.95 a
mean	55	59	0.61	0.56
	----- Experiment AVAIL pH 8 -----			
72	11 d	12 e	0.13 d	0.14 e
400	22 c	23 d	0.18 d	0.2 d
3200	65 b	49 c	0.51 c	0.48 c
6400	69 b	79 b	0.73 b	0.71 b
9600	84 a	83 a	0.88 a	0.83 a
mean	50	49	0.49	0.47

**Table 4b**

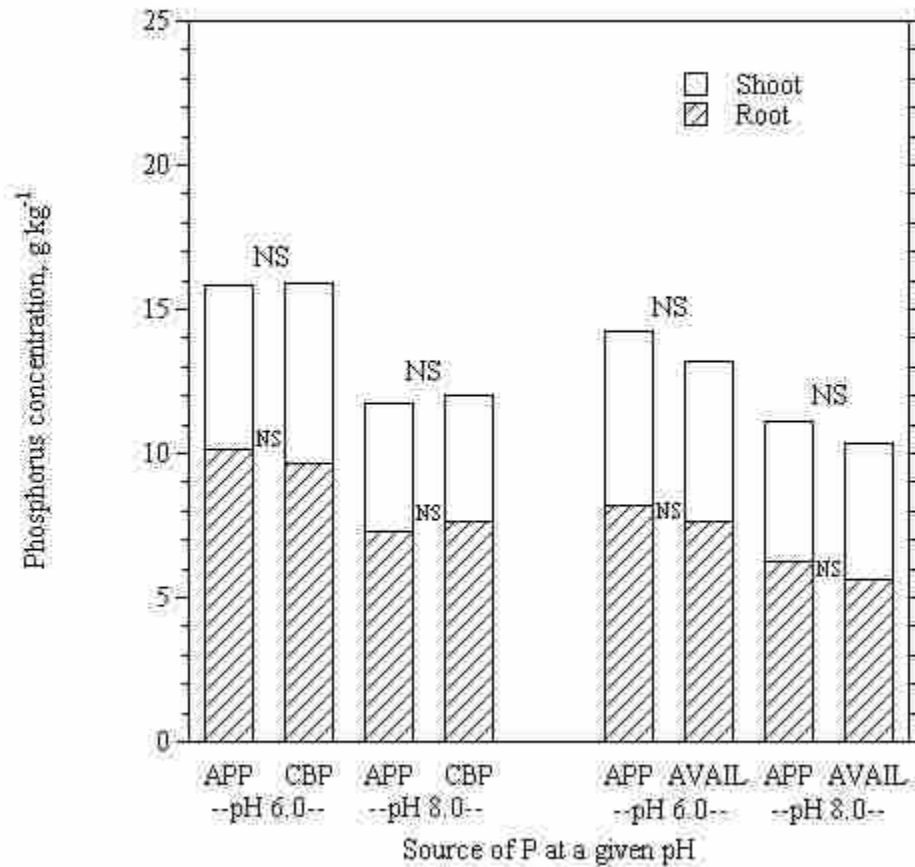
----- Root -----							
----- biomass, g pot <sup>-1</sup> -----				----- P, % -----			
APP		AVAIL		APP		AVAIL	
----- Experiment AVAIL pH 6 -----							
8	c	8	d	0.13	c	0.13	d
17	a	20	a	0.18	c	0.14	d
15	ab	14	bc	0.91	b	0.93	c
13	b	16	b	1.43	a	1.33	b
14	b	12	c	1.32	a	1.46	a
13		14		0.79		0.8	
----- Experiment AVAIL pH 8 -----							
4	c	4	d	0.16	e	0.19	e
7	b	8	c	0.31	d	0.28	d
16	a	11	b	0.67	c	0.45	c
15	a	17	a	0.8	b	0.75	b
17	a	17	a	1.2	a	1.15	a
12		11		0.63		0.56	

**Table 5. Rate Effect.**

Shoot and root dry matter yields and P concentrations for four hydroponic maize experiments conducted at pH 6 or 8 with five levels of solution P concentration applied as either ammonium polyphosphate (APP) Carbond P® (CBP) or AVAIL®; Data are combined across P sources (CBP and APP or AVAIL and APP) to show the rate effect only. Within an experiment, values followed by the same letter are not statistically different ( $P < 0.05$ ).

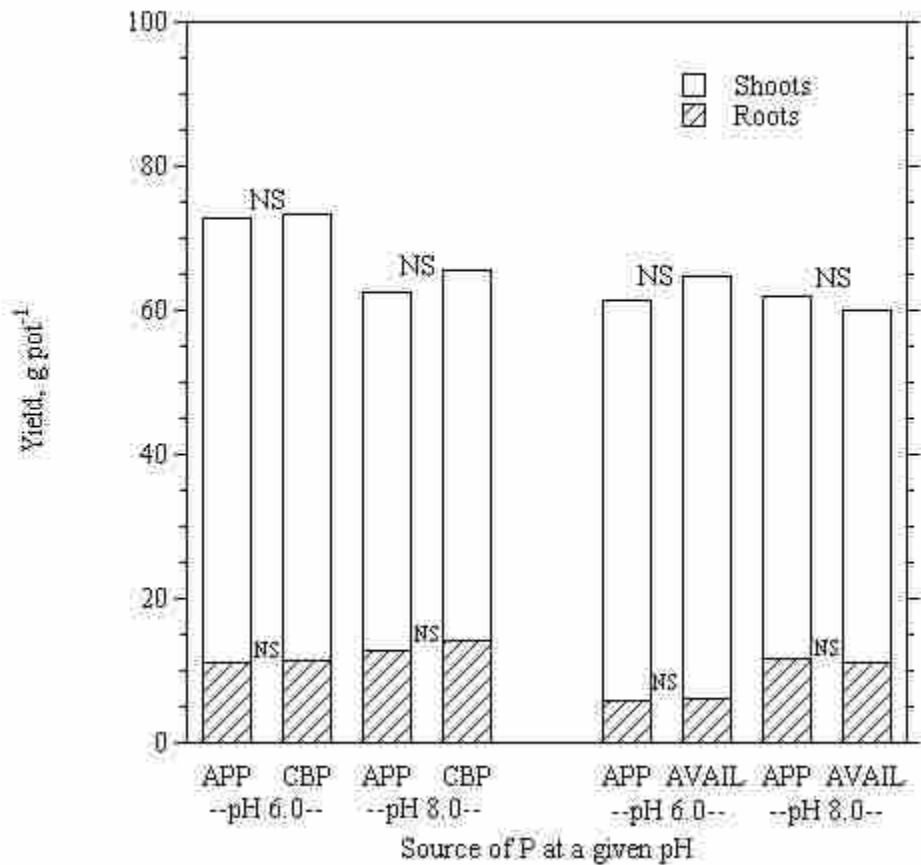
Solution P, $\mu\text{M}$	CBP pH 6	CBP pH 8	AVAIL pH 6	AVAIL pH 8
----- Shoots -----				
----- biomass, g pot <sup>-1</sup> -----				
72	26 b	13 b	19 c	11 d
400	58 a	33 b	56 b	23 c
3200	79 a	62 a	72 a	57 b
6400	73 a	67 a	75 a	74 a
9600	74 a	79 a	63 ab	83 a
----- P, % -----				
72	0.14 d	0.11 c	0.14 c	0.14 d
400	0.21 d	0.23 b	0.15 c	0.19 d
3200	0.72 c	0.58 a	0.74 b	0.50 d
6400	0.88 b	0.64 a	0.92 ab	0.72 b
9600	1.05 a	0.65 a	0.97 a	0.86 a
----- Roots -----				
----- biomass, g pot <sup>-1</sup> -----				
72	10 b	6 c	8 c	4 d
400	14 a	11 b	18 a	7 c
3200	11 b	17 a	15 ab	13 b
6400	11 ab	17 a	14 ab	16 a
9600	10 b	17 a	13 b	17 a
----- P, % -----				
72	0.15 d	0.19 d	0.13 c	0.18 d
400	0.26 d	0.30 d	0.16 c	0.30 d
3200	1.22 c	0.79 c	0.92 b	0.56 c
6400	1.51 b	1.00 b	1.38 a	0.78 b
9600	1.84 a	1.47 a	1.39 a	1.18 a

## FIGURES



**Figure 1. Phosphorus Concentration.**

Phosphorus concentration of shoots and roots for four experiments with maize grown in a controlled environment growth chamber in hydroponic solutions comparing ammonium polyphosphate (APP) to either Carbond P® (CBP) or AVAIL® grown in solutions at either pH 6 or 8 with five rates of solution P. Data shown are averaged across five P rates for each fertilizer source within each experiment. Results comparing APP and CBP or APP and AVAIL are not significantly different (NS) at either pH.



**Figure 2. Shoot and Root Biomass.**

Shoot and root biomass for four experiments with maize grown in a controlled environment growth chamber in hydroponic solutions comparing ammonium polyphosphate (APP) to either Carbond P® (CBP) or AVAIL® grown in solutions at either pH 6 or 8 with five rates of solution P. Data shown are averaged across rates for each fertilizer source within each experiment. Results comparing APP and CBP or APP and AVAIL are not significantly different (NS) at either pH.

## **Chapter 2**

### **Enhanced Phosphorus Fertilizer (Carbond P®) Supplied to Maize in Moderate and High Organic Matter Soils**

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## ABSTRACT

Carbond P (CBP) fertilizer often increases P uptake and crop yields in low P and organic matter soils. A glasshouse study was conducted with maize (*Zea mays* L.) grown in moderate or high organic matter soil with 0, 5, 15, 45, or 135 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup> applied as either ammonium polyphosphate (APP) or CBP. In the high organic matter soil, both CBP and APP fertilization resulted in similar increases in biomass yield and P concentration and uptake. This was also observed in the moderate organic matter soil for maize P concentration, but biomass yield and total P uptake were significantly greater for CBP than APP at the two lowest P rates of fertilization and significantly higher for APP than CBP at the highest P application rate. The presence of high organic matter in the soil seemed to negate the effects of the organic acid bonded P found in CBP

## INTRODUCTION

Phosphorus is an essential nutrient required by plants in every phase of growth and in every plant part (Bennett, 1993). One of the most important roles of P is as a component of adenosine mono/di/triphosphate (AMP/ADP/ATP) used in photosynthesis to convert light to chemical energy. Consequently, adequate P is important for all energy requiring reactions. In addition, P is utilized to modify the activity of various enzymes by phosphorylation and for cell signaling. Furthermore, phosphate esters are structural components of DNA, RNA, and phospholipids.

Providing adequate food, fiber, and fuel for society requires efficient P management (Hopkins et al., 2008). Phosphorus is needed in relatively large quantities for efficient crop production and is applied to most agricultural soils at high rates.



Phosphorus is among the top four mineral nutrients in regard to plant concentration (Bennett, 1993) and is often applied at rates nearly equal to N and K due to poor solubility in soils (Foth and Ellis, 1996; Hopkins et al., 2008). Since P is delivered to roots primarily by diffusion and root growth, P placement near roots and warm soil temperatures favor plant uptake. For this reason, P deficiencies are relatively more common in the early part of the growing season when plant roots are not abundant and soils temperatures are cool (Hopkins et al., 2008).

Phosphorus is often applied at superfluous rates in manure and/or fertilizer to supply P to plants and, in some cases, becomes an environmental concern (Mueller & Dennis, 1996; Sharpley et al., 2003). Phosphorus can be transported to surface water bodies through overland flow or by erosion of sediments, especially if soil or soluble P concentrations in soil are exceptionally high (Mueller & Dennis, 1996). As with N, high concentration of P in surface water bodies is potentially negative. Although N is usually the limiting factor for plant growth in soil-based systems, P is generally the limiting factor in fresh water systems (Mueller & Dennis, 1996; Sharpley et al., 2003). As a result, high concentrations of P in surface water bodies can lead to algae blooms that deplete oxygen and promote death of aquatic organisms. This can have negative ecological and economic impacts, including being unsightly and generating pungent odor (Mueller & Dennis, 1996). Additionally, this loss of P from soil represents a loss in potential nutrient to crops and a waste of grower and mineral resources. Consequently, improved efficiency in the utilization of applied P fertilizer is critical, however elusive.

Increasing the percentage of P from fertilizer that is utilized by plants (P-use efficiency or PUE) is critical for maximizing efficiency of grower and natural resources,

reducing environmental impacts, and reducing consumption of non-renewable P mineral resources. However, improving PUE is challenging due to inherent inefficiencies in the soil-plant system that generally lead to fertilizer P recoveries of 0 to 30% (Randall et al., 1985). Recovery of P from fertilizer is impacted by soil constituents (cations), mineralogy, and pH (Sposito, 2008). For example, solubility of P minerals is dramatically less in strongly alkaline and acidic soils compared to soils of approximately neutral pH. In acidic soil, the negatively charged phosphate bonds with Al, Fe, and Mn and forms poorly soluble mineral precipitates. Similarly, phosphate strongly bonds in alkaline soils with Ca and Mg. Additionally alkaline soils often contain free excess limestone, which further decreases P solubility (Sposito, 2008). In the case of neutral pH soils, the dissolution rate of soil minerals is relatively greater than in strongly acid or basic soils. Clay mineral content also strongly impacts P availability regardless of soil pH.

A number of rate, timing, placement, and source options can be used to manage PUE (Foth and Ellis, 1996; Hill et al., 2013a; Hopkins et al., 2010a, 2010b, 2010c; Hopkins, 2013; Stark and Hopkins, 2013). In addition to these cultural practices that may enhance P uptake and utilization, fertilizer manufacturers seek to engineer materials to enhance PUE (Hopkins et al., 2008). A fertilizer that has been engineered to keep P more plant available is Carbond P® (CBP; 7-24-0; Land View Fertilizer, Rupert, ID, USA).

Organic acids are known to play a major role in soil and plant nutrition. Various studies have shown positive bio-enhancement effects of organic acids. These studies vary in results and organic acid origins but have many similarities. Generally, organic acids have been associated with bio-enhancement which has been attributed to impacts on root growth, elongation, yield, and emergence (Canellas et al., 2002; Eyheraguibel et al.,

2008; Piccolo et al., 1993). Additionally, synthetic organic acids such as humic acids have been proven to be beneficial to plant growth (Seyedbagheri, 2010). Humic and fulvic acids make up 75% of the total organic matter portion in mineral soils (Obreza et al., 1989). These latter acids are found in CBP as well.

These organic acids play a major role in the environmental fate of both organic and inorganic materials (Tan, 2003). In addition to various growth responses as mentioned above, improvement of P nutrition is another documented impact of organic acids (Seyedbagheri, 2010; Hopkins and Stark, 2013; Tan, 2003). Hopkins and Stark (2013) found that ammonium polyphosphate (APP; 10-34-0) mixed with humic acid increased P uptake and potato yield and quality compared to APP alone in moderate organic matter soil (3.6% OM), especially at the lowest rate of P fertilizer applied in those trials. Carbond P has major compositional elements that are classified as organic acids (note that CBP also includes a cocktail of other proprietary additives). One of the theoretical impacts of CBP is chemical bonding of P with these organic acids (Hill et al., 2013a, 2013b).

However, the effects of organic acids are not universal among soils. Hartz and Bottoms (2010) saw that fertilizer combined with humic acid in low organic matter soils (3.6% OM) produced positive effects. However, they concluded that at typical commercial application rates in representative field soils, humic acid is unlikely to significantly improve nutrient uptake or productivity of the vegetable crops studied (romaine lettuce, *Lactuca sativae* L. and tomato, *Lycopersicon esculentum* Mill.). However, Cimrin and Yilmaz (2005) found a significant interaction between humic acid

and P uptake, but yield was not significantly affected. Our studies are searching for a better understanding of the variable impact reported.

The effect of these organic acid P fertilizers has only been evaluated in low organic matter calcareous soils (Hill et al., 2013a; Hopkins et al., 2013). These soils inevitably have a soil solution dominated by  $\text{Ca}^{2+}$  which leads to P and PUE issues. Our studies with organic acids to date have been almost exclusively in calcareous soils, but otherwise this relatively new product, CBP, has not been widely studied.

There is evidence that CBP impacts both soil chemistry and plant growth (Hill et al., 2013a, 2013b, Hopkins et al., 2013; Packer et al., 2013; Summerhays et al., 2013). Summerhays et al. (2013) found in hydroponic conditions that CBP had no direct adverse effect on maize (*Zea mays* L.), even when present at very high concentrations in direct root contact. They also found that when CBP was the P source that there was no impact on biomass yield, P concentration or uptake when compared to traditional ammonium polyphosphate (APP) fertilizer. This research showed conclusively that the impact of CBP in soil based systems is not a bio-stimulant plant response to the P or the organic acids present, but related to traditional theories of soil P chemistry interactions. Recent additional hydroponic studies (Hopkins, 2012, unpublished data), showed that foliar supplied CBP did not impact P concentration in new shoot growth nor roots of maize nor biomass yield compared to non-treated plants. This further supports the idea that impact of CBP is soil chemistry related. Hill et al. (2013a, 2013b) found that maize grown with Carbond P produced significantly more biomass yields and P concentrations across multiple rates and soils than APP. The physiological growth enhancement responses were seen most strongly in medium to highly calcareous soils low in soil test P. Hopkins et al.

(2013) found that field grown alfalfa (*Medicago sativa* L.), maize, and potato (*Solanum tuberosum* L.) had positive responses to CBP and results were more often positive when CBP was applied to soils with low plant available P levels and relatively low fertilizer application rates. Increased availability of P provided as CBP is usually exhibited in increased plant P concentrations compared to traditional fertilizers (Hill et al., 2013a, Hopkins et al., 2013). All of these soil based studies were conducted in calcareous soils low in organic matter and soil test P. The objective of this study was to compare the efficiency of CBP to APP in the presence of moderate or high organic matter soil (that also have low soil test P), and applied at low to very high P rates.

## **MATERIALS AND METHODS**

A glasshouse study was conducted in June 2011 with two soils—one moderate and the other high in organic matter concentration (Table 1). The high organic matter soil (Holdaway silt loam; fine-silty, mixed, superactive, mesic Petrocalcic Calcicquolls) was collected near Lehi, UT, USA. The moderate organic matter soil was constructed using one part high organic matter soil with three parts of sand on a mass basis. The sand originated from a quarry near Provo, UT, USA. The soils were packed in pots (345 and 745 g for the high and moderate organic matter soil, respectively) to achieve a bulk density of approximately 1.55-1.65 g cm<sup>-3</sup> and leaving a 2-3-cm headspace to allow for irrigation water addition. Pots were 25.4 cm high and 6.35 cm diameter at top and tapered to a rounded point at the bottom with drainage holes lined with polypropylene landscape fabric (Weed Block, Easy Gardener, Waco, TX, USA), to prevent soil loss. Phosphorus fertilizer was applied at field-simulated rates of 0, 2.2, 6.6, 20, or 60 kg P ha<sup>-1</sup> as APP or

CBP. Fertilizer rates were calculated based on the simulated field conditions of the fertilizer being applied in a concentrated band with a 6.4 cm diameter core with the plant found in the middle of the core and an assumption of 6 plants m<sup>-1</sup> of row (actual rates were 0.04, 0.12, 0.36, or 1.08 ml APP pot<sup>-1</sup> and 0.06, 0.18, 0.54, or 1.62 ml CBP pot<sup>-1</sup>). Fertilizer was point-injected at 5 cm to the side and 5 cm below the seed immediately prior to planting. Nitrogen was balanced across treatments with urea ammonium nitrate solution at 250 kg N ha<sup>-1</sup>. Other essential nutrients were adequate based on soil test (Table 1).

Three maize seeds (Syngenta NK N27B-3111; an AgriSure Viptera Hybrid) were planted in the center of each pot at a depth of 2.5 cm. Plants were grown in a glasshouse located on the Brigham Young University campus in Provo, Utah, USA, 140°14'37"N, 111°39'02"W, 4570'. Best management practices for maize growth in a glasshouse were observed. Soil in each pot was irrigated to saturation prior to seeding and soils were maintained above ~55% plant available water content throughout the study. No pressure from pests was observed and, therefore, no pesticides were applied. Targeted daytime and nighttime air temperatures were 25°C and 15°C, respectively, although limitations in heating/cooling capacity resulted in ranges of 20-29°C daytime and 11-16°C nighttime. Natural light was supplemented with high-pressure sodium lamps to maintain a minimum 16-hour light cycle.

The numbers of plants per pot were thinned to two and one at 14 and 19 d, respectively, to achieve uniformly healthy plants across the trial. Plants were harvested at 28 d after planting by cutting at 3 cm above the soil surface, bagged, and then oven dried at 65 °C. Drying continued for a minimum of 72 hours to reach uniform dryness. Samples

were then weighed, ground to pass 1-mm sieve, digested in nitric-perchloric acid, and analyzed for nutrient content by Inductively Coupled Plasma Atomic Emission Spectroscopy (Johnson and Ulrich, 1959; ICP-AES, Thermo Electron Corporation, Franklin, Maryland, USA).

Plants were arranged in a randomized complete block design with four replications of each treatment. Additional potted maize plants surrounded the entire study to mitigate previously observed border effects. Statistical analyses were performed using Statistical Analysis System (Version 9.1, SAS, SAS Institute, Cary, NC, USA). The data was analyzed using analysis of variance (ANOVA) with mean separation using Duncan/Waller Multiple Range Test at the  $P < 0.05$  level of significance (Table 3).

## **RESULTS AND DISCUSSION**

Phosphorus deficiency was successfully established as maize leaves in the unfertilized control treatments in both soils showed visual symptoms of P deficiency, specifically purpling and stunted growth. But any amount of P application with either source overcame the visual symptoms.

The broadest analysis produced a significant soil by P rate by P source interaction and thus data for each soil are presented separately. The overall models for each soil were highly significant for the three measured parameters for both soils (Table 2). In general, all measured parameters were impacted by both P rate and source for both soils, although there was a significant interaction between rate and source for biomass and P uptake for maize grown in the moderate organic matter soil (Table 2). There was an incremental increase in biomass with increasing P rate regardless of soil or P source (Tables 2 and 3).

In the high organic matter soil, both fertilizers performed similarly with application of either resulting in increased biomass and P concentration and uptake compared to the control (Tables 2 and 3 and Figures 1a and 2a). When averaged across P rates, both CBP and APP had greater biomass than the control but were not different from each other (Tables 2 and 3). Shoot P concentration and uptake followed similar trends as biomass for maize grown in the high organic matter soil, with significant differences for APP and CBP over the control, but no differences between P sources (Tables 2 and 3).

As with the high organic matter soil, P concentration in maize grown in the moderate organic matter soil increased with P rate regardless of fertilizer source (Tables 2 and 3). When averaged across P rates, maize fertilized with either CBP or APP had greater P concentration than the control but there were no differences between these sources (Tables 2 and 3).

Unlike the high organic matter soil, maize grown in the moderate organic matter soil exhibited significant interactions between P rate and source for biomass and P uptake (Table 2 and Figures 1b and 2b). Shoot biomass was significantly higher for CBP fertilized maize compared to APP at the two lowest P rates, but the effect was reversed at the two highest rates of P (Figure 1b). Predictably, the total P uptake showed an interaction similar to that observed with biomass, although the second to highest P rate showed no difference between sources (Figure 2b).

These data suggest that the high levels of organic acids found in a high organic matter soil can muffle the benefits of Carbond P that have been observed in soils with relatively low organic matter concentration (Hill et al., 2013a; Hopkins et al., 2013). The significant increase in maize biomass and P uptake in the moderate organic matter soil at



the low rates of CBP compared to APP is mostly consistent with previous studies at low soil organic matter (Hill et al., 2013a; Hopkins and Stark, 2013; Hopkins et al., 2013). The reversal of this effect at the highest rate of P supplied as CBP has also been observed previously (Hill et al., 2013a; Hopkins et al., 2013) and is possibly an impact of a higher level of available P with CBP inducing a micronutrient deficiency (Barben et al., 2011; Hopkins et al., 2013; Stark and Hopkins, 2013). Thus, it is likely that the inclusion of organic acids in CBP have little or no impact on P solubility in soils where organic matter levels are high and would likely provide organic acids at high levels. Thus, Carbond P, as a fertilizer, appears to be most effective in supplementing P on soils of low to moderate organic matter concentration, especially when soil test P is also low.

Hopkins et al. (2013) found yield increases with CBP more commonly in low organic matter calcareous soils with low soil test P, although lack of response could not always be associated with organic matter levels. Hill et al. (2013a) found similar responses in maize studies. Both of these studies showed that as the rate of CBP increased, the likelihood of response compared to APP diminished, which was also observed in our study. The fact that the difference between sources disappears at higher rates of P application is logically explained by the concept that once enough P is available to the plant that there is little or no opportunity for further improvement regardless of P source (i.e. a little is good, but more is not necessarily better). In a previous field study with potato, Hopkins and Stark (2013) found that a humic acid blended with APP increased yield and tuber quality, but the effect diminished at higher application rates.

The greater P uptake and biomass associated with CBP at low P rates in the moderate organic matter soil point to improved PUE. This was also observed in previous studies with relatively greater amounts of P ending up in plants, especially at lower P rates (Hill et al., 2013a; Hopkins et al., 2013). Carbond P use has consistently shown an ability to increase P uptake in a variety of species in this and other studies (Hill, et al., 2013a; Hopkins et al., 2013). By providing a P fertilizer bonded with organic acids (CBP), PUE will likely increase. However, this effect did not occur in the high organic matter soil in this study (Fig1 a and 2a).

Our data support the idea that the effects of organic acids are limited. Hartz and Bottoms (2010) saw that fertilizer combined with humic acid in low organic matter soils produced positive effects. However, they also concluded that humic acid is unlikely to significantly improve nutrient uptake or productivity of the vegetable crops studied (romaine lettuce, *Lactuca sativae* L. and tomato, *Lycopersicon esculentum* Mill.) at typical commercial application rates in representative field soils. However, the humic acid applied in their study was a broadcast application while ours was banded near the roots and in combination with P fertilizer. Cimrin and Yilmaz (2005) stated that humic acid and P interactions were significant in their research, although the application of humic acid alone did not have significant effect on yield (weight) with their studies.

The research by Hartz and Bottoms (2010) also supports the finding that soil organic acids have limited effectiveness. What is also clear is that these organic P fertilizer additives are effective in calcareous and alkaline soils. They also confirm our observation that superior results with organic acids are produced at lower rates of P

application (Cimrin and Yilmaz, 2005; Hartz and Bottoms, 2010; Morard et al., 2011; Sharif et al., 2002; Tahir et al., 2011).

Morard et al. (2011) concluded that the application of low rates of 100 to 400 mg L<sup>-1</sup> of SHB® (humic acid-like substances from sawdust) promoted the strongest improvement in plant growth. Sharif et al. (2002) studied the effects of humic acid derived from lignitic coal on maize and saw that the addition of 50 and 100 mg kg<sup>-1</sup> humic acid produced a significant increase of 20 and 23% in shoot weight and 39 and 32% in root weight of plants as compared to an untreated control. However in their study, plant P accumulation was not significantly impacted by the application of different levels of humic acid. Coal-derived synthetic humic acid produced a positive difference with wheat (*Triticum* spp.; Tahir et al., 2011). These researchers saw that the largest increases in height and shoot biomass were found with 60 mg kg<sup>-1</sup> humic acid applications as compared to the control without them. Cimrin and Yilmaz (2005) concluded that the application of 120 kg ha<sup>-1</sup> of P together with 300 kg ha<sup>-1</sup> of humic acid was optimal for the head weight of lettuce. Lu et al. (1998) stated that organic acids can reduce the P adsorption by soils and that the extent of reduction is closely related to the kind of organic acids. Additionally, Lu et al. also states organic acids dissolve large amounts of CaCO<sub>3</sub> in calcareous soil, thereby reducing P adsorption by soils. The dynamic of P is controlled by calcite, which strongly retains P and consequently maintains low P concentration in soil solution (Bertrand et al., 1999).

Our research using a unique new product in which the organic acids and P are bonded supports and confirms these findings. Organic acid supplements to P fertilizers are most effective for plant PUE with low P rate application. These supplemented organic

acids create conditions similar to the presence of organic matter, which minimizes P solubilization from soil cations. Also, this effect is observed in alkaline/calcareous soils, where excess cations precipitate or adsorb labile P. However, high organic matter soil potentially neutralizes the effects of organic acid based fertilizers due an already high abundance of organic acids. Thus, the addition of additional organic acids provided by fertilizers does not magnify P solubilization when already present in ample concentrations in the soil. This is crucial knowledge that CBP and other similar products have a quantifiable limit to their effectiveness.

Lower, more effective rates of P application are vastly important in improving PUE, as well as assisting environmental concerns and grower finances. Additionally, selective treatment with CBP and similar products is important in regards to conserving natural resources. The ability to precisely use P fertilizers, not just in amount used, but also situation specific is a step forward to increasing the longevity of our agriculture and civilization. Continued research on this product (as well as similar products) is needed to refine our understanding of native organic matter interactions with supplemental additions of organic acids and this will allow us to continue making strides towards a sustainable future.

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## TABLES

**Table 1. Soils Properties.**

Properties for two soils used in a P fertilizer glasshouse experiment.

Soil Test Parameter	High Organic Matter Soil	Moderate Organic Matter Soil
Bicarbonate P, $\mu\text{g g}^{-1}$	11.3	8.2
Organic matter, %	15.8	3.6
CEC, $\text{meq } 100\text{g}^{-1}$	102.4	14.9
pH	7.2	7.5
EC, $\text{dS M}^{-1}$	1.7	1.2
$\text{NO}_3\text{-N}$ , $\mu\text{g g}^{-1}$	17	4
Ammonium Acetate K, $\mu\text{g g}^{-1}$	1860	1640
DTPA Zn, $\mu\text{g g}^{-1}$	24.6	8.1
DTPA Fe, $\mu\text{g g}^{-1}$	153	51
DTPA Mn, $\mu\text{g g}^{-1}$	25.1	5.6
DTPA Cu, $\mu\text{g g}^{-1}$	17.0	2.5
Sand, %	37.8	78.8
Clay, %	17.5	6.5
Silt, %	44.7	14.7

**Table 2. Statistically Significance.**

P values associated with shoot biomass, P concentration, and P uptake for two maize glasshouse studies grown at five levels of P (0, 2.2, 6.6, 20, and 60 kg P<sub>2</sub>O<sub>5</sub> ha<sup>-1</sup>) and three sources of P [control, ammonium polyphosphate (APP) or Carbond P® (Carbond P)]. Values bolded are statistically significant at  $P < 0.05$ , ANOVA.

Source	Maize biomass	P concentration	P uptake
-----High OM Study-----			
Overall model	<b>&lt;0.0001</b>	<b>0.0005</b>	<b>&lt;0.0001</b>
P rate (R)	<b>0.0093</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
P source (S)	<b>0.0346</b>	<b>0.0182</b>	<b>0.0180</b>
R x S	0.3652	0.2304	0.3157
-----Moderate OM Study-----			
Overall model	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
rate (R)	<b>0.0021</b>	<b>&lt;0.0001</b>	<b>&lt;0.0001</b>
P source (S)	<b>0.0484</b>	<b>0.0288</b>	<b>0.0491</b>
R x S	<b>0.0110</b>	0.2252	<b>0.0476</b>

**Table 3. Biomass, Phosphorus Concentration and Uptake.**

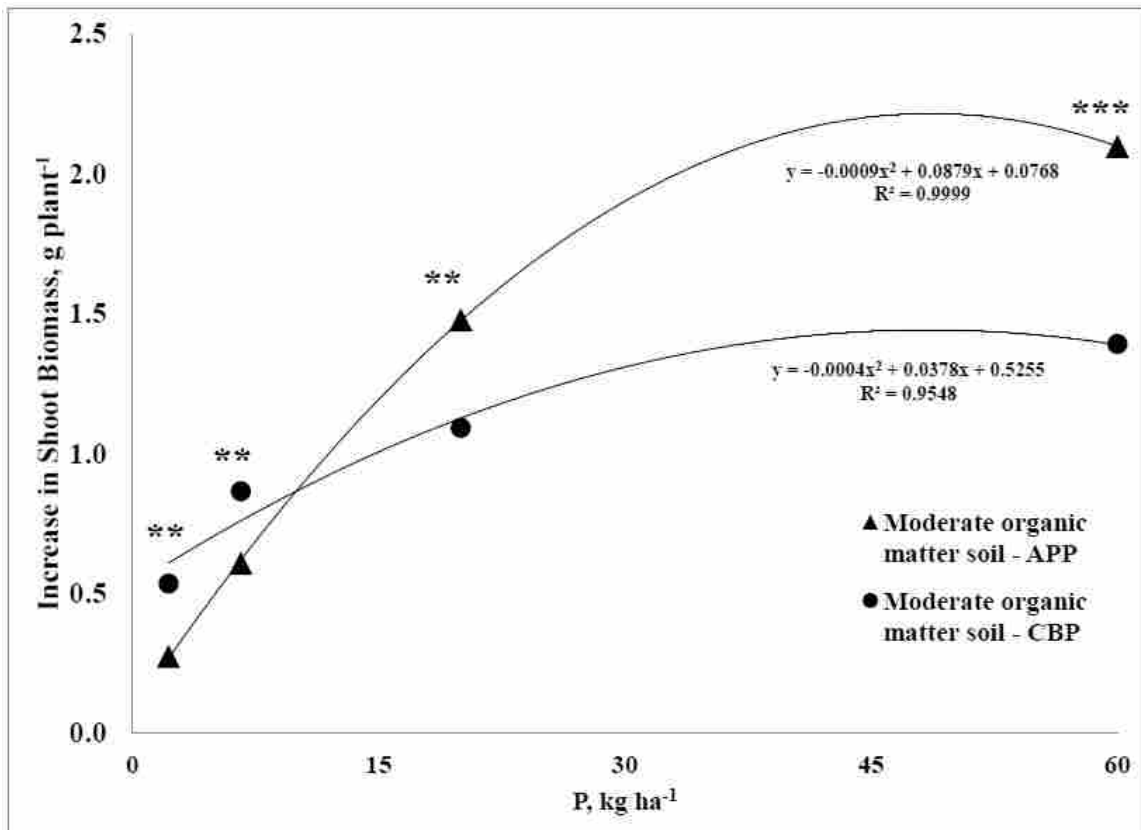
Shoot biomass, P concentration, and P uptake for two maize glasshouse studies comparing ammonium polyphosphate (APP) to Carbond P® (CBP). Values within an experiment for fertilizer source or rate are not different from one another if followed by the same letter ( $P < 0.05$ ).

	Maize Biomass, g pot <sup>-1</sup>	P Concentration, mg kg <sup>-1</sup>	P Uptake, µg pot <sup>-1</sup>
High OM Study			
<u>P Source</u>			
Control	3.60 B	19.0 B	66.0 B
APP	4.60 A	29.3 A	126.8 A
CBP	4.62 A	31.0 A	134.4 A
<u>P, kg ha<sup>-1</sup></u>			
0	3.60 D	19.0 D	66.0 E
2.2	4.02 C	21.6 D	75.2 D
6.6	4.34 C	26.0 C	93.1 C
20	4.84 B	33.0 B	143.7 B
60	5.24 A	40.1 A	210.4 A
Moderate OM Study			
<u>P Source</u>			
Control	2.58 B	19.1 B	37.9 B
APP	3.70 A	31.3 A	105.6 A
CBP	3.55 A	31.9 A	97.9 A
<u>P, kg ha<sup>-1</sup></u>			
0	2.58 E	19.1 D	37.9 D
2.2	2.99 D	21.4 D	45.6 D
6.6	3.32 C	26.8 C	68.6 C
20	3.87 B	33.2 B	105.5 B
60	4.33 A	45.0 A	187.2 A

## FIGURES

**Figure 1. Shoot and Root Biomass.**

Increase in shoot biomass over an unfertilized control for a glasshouse experiment with maize grown in either moderate or high organic matter soil with 2.2, 6.6, 20, or 60 kg P ha<sup>-1</sup> applied as either ammonium polyphosphate (APP) or Carbond P® (CBP). A “\*”, “\*\*”, or “\*\*\*” at a given P rate indicate significance between sources at that rate at  $P < 0.05$ , 0.01, or 0.001, respectively. “NS” indicates not significant at  $P = 0.05$ .



**Figure 1a**

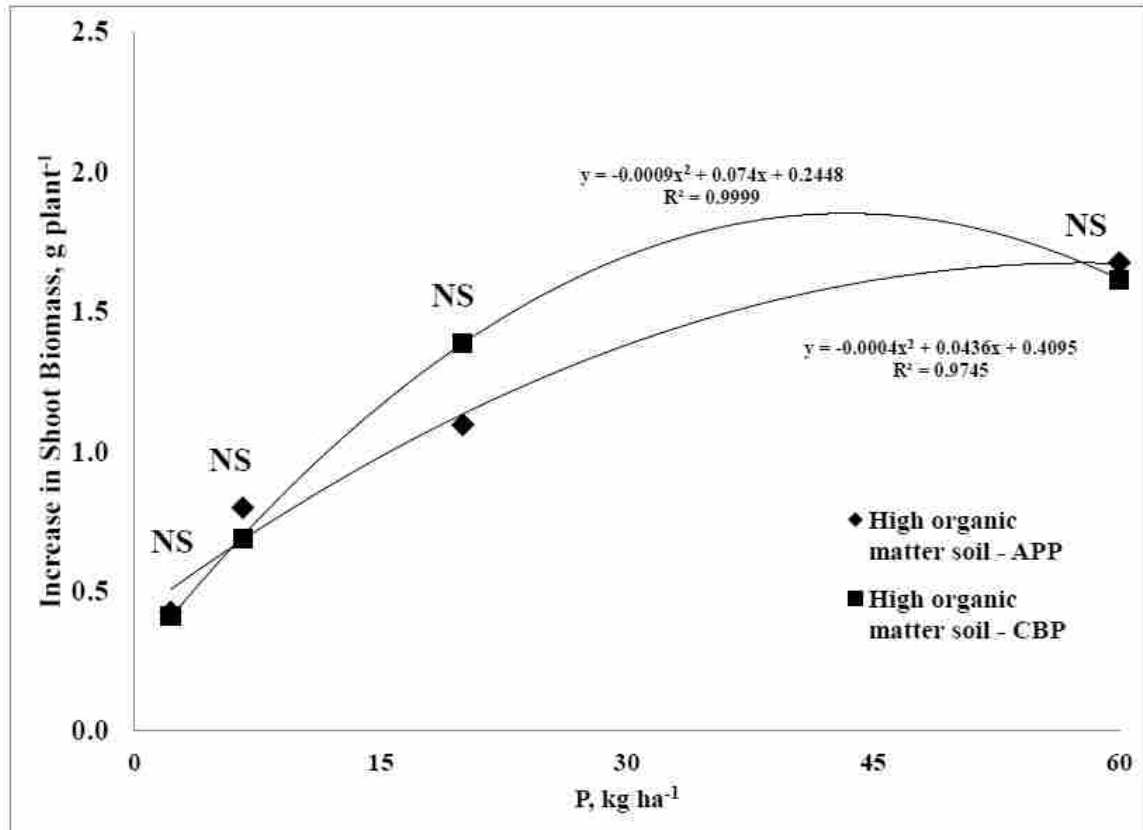


Figure 1b

## Figure 2. Shoot and Root Phosphorus Uptake.

Increase in shoot P uptake over an unfertilized control for a glasshouse experiment with maize grown in either moderate or high organic matter soil with 2.2, 6.6, 20, or 60 kg P ha<sup>-1</sup> applied as either ammonium polyphosphate (APP) or Carbond P® (CBP). A “\*”, “\*\*\*”, or “\*\*\*\*” at a given P rate indicate significance between sources at that rate at  $P < 0.05$ , 0.01, or 0.001, respectively. “NS” indicates not significant at  $P = 0.05$ .

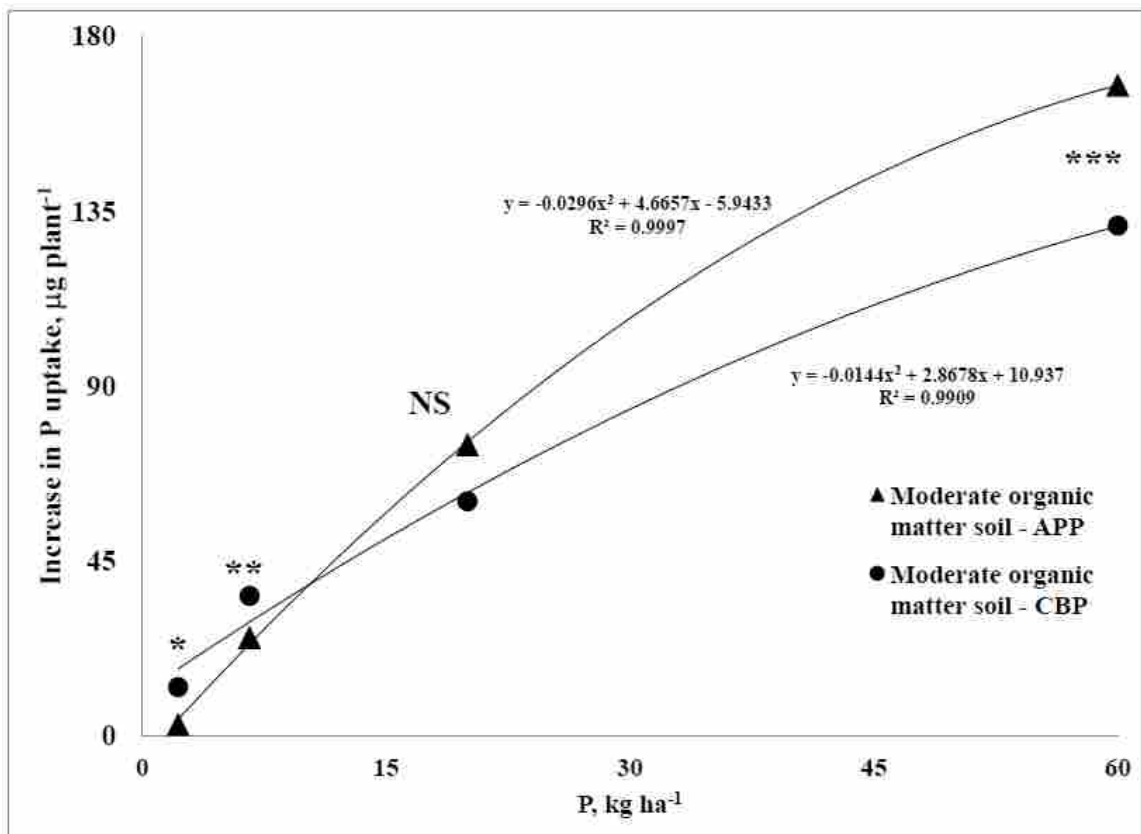


Figure 2a

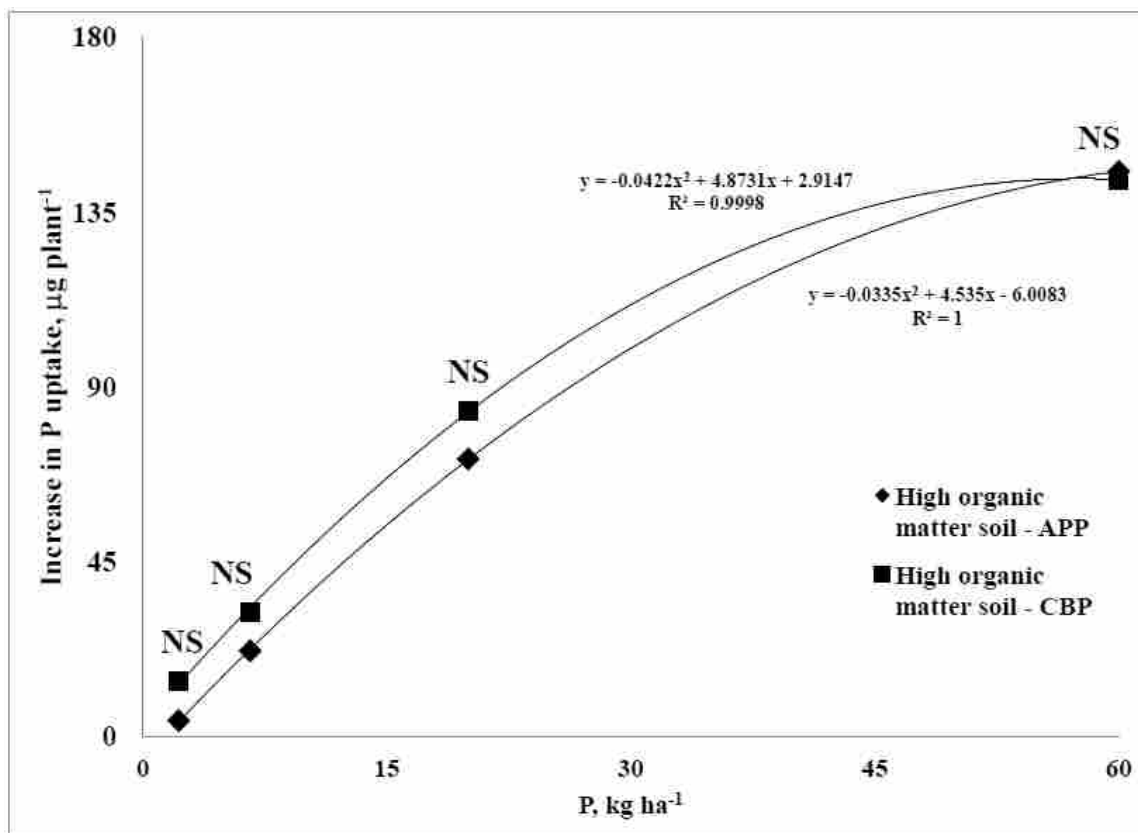


Figure 2b



## **APPENDIX A: PROSPECTUS**

## INTRODUCTION

Phosphorus is an essential nutrient required by plants in every phase of growth and in every plant part (Bennett, 1993). One of the most important roles of P is as a component of adenosine mono/di/triphosphate (AMP/ADP/ATP) used in photosynthesis to convert light to chemical energy and in respiration. Consequently, adequate P is important for all energy requiring reactions. In addition, P is utilized to modify the activity of various enzymes by phosphorylation and for cell signaling. Furthermore, phosphate esters are structural components of DNA, RNA, and phospholipids.

Providing adequate food, fiber, and fuel for society requires efficient P management (Hopkins et al., 2008). Phosphorus is needed in relatively large quantities for efficient crop production and is applied to most agricultural soils at high rates. Phosphorus is among the top four mineral nutrients in regard to plant concentration (Bennett, 1993; Havlin et al., 2005) but is often applied at rates nearly equal to N and K due to poor solubility in soils (Foth and Ellis, 1996; Hopkins et al., 2008). Since P is delivered to roots primarily by diffusion and root growth, P placement near roots and warm soil temperatures favor plant uptake. Unfortunately, P is so poorly soluble that uptake by plant roots can be problematic (Foth and Ellis, 1996; Hopkins et al., 2008). Consequently, P deficiencies are relatively more common in the early part of the growing season when plant roots are not abundant nor developed and soils temperatures are relatively cool (Hopkins et al., 2008).

Growers often applied at superfluous rates of Phosphorus in apply high rates of manure and/or fertilizer to supply P to plants and, in some cases, this rate of application becomes an environmental concern (Mueller & Dennis, 1996; Sharpley et al., 2003).

Phosphorus can be transported to surface water bodies through overland flow or by erosion of sediments, especially if soil or soluble P concentrations in soil are exceptionally high (Mueller & Dennis, 1996). As with N, high concentration of P in surface water bodies is potentially negative. Although N is usually the limiting factor for plant growth in soil-based systems, P is generally the limiting factor in fresh water systems (Mueller & Dennis, 1996; Sharpley et al., 2003). As a result, high concentrations of P in surface water bodies can lead to algae blooms that can deplete the oxygen and promote cause death of aquatic organisms. These hypoxic conditions can be unsightly and have negative ecological and economic impacts, including being unsightly and generating a pungent odor (Mueller & Dennis, 1996). Additionally, this loss of P from soil to water body flow represents a loss in potential fertilizer to crops and a waste of grower and mineral resources. Consequently, improved efficiency in the utilization of applied P fertilizers is critical, however, it remains elusive.

Increasing the percentage of P from fertilizer that is utilized by plants (P-use efficiency or PUE) is critical for maximizing efficiency of grower and natural resources, reducing environmental impacts, and reducing consumption of non-renewable P mineral resources. However, improving PUE is challenging due to inherent inefficiencies in the soil-plant system that generally lead to fertilizer P recoveries of near zero to a maximum of less than 30% (Randall et al., 1985). Recovery of P from fertilizer is impacted by soil constituents (cations), mineralogy, and pH (Sposito, 2008). For example, solubility of P minerals is dramatically less in strongly alkaline and acidic soils compared to soils of approximately slightly acidic to neutral pH. In acidic soil, the negatively charged phosphate bonds with Al, Fe, and Mn and forms poorly soluble mineral precipitates.

Similarly, A similar reaction occurs in alkaline soil, as phosphate strongly bonds in alkaline soils with Ca and Mg. Additionally, alkaline soils often contain free excess calcite (limestone), which further decreases P solubility through adsorption (Sposito, 2008). In the case of neutral pH soils, the dissolution rate of soil minerals is relatively greater than in strongly acid or basic soils. Clay mineral content also strongly impacts P availability regardless of/alkaline soil pH., but the solubility and, therefore, the dissolution rate of soil minerals are relatively greater.

A number of rate, timing, and placement options can be used to manage and improve PUE (Foth and Ellis, 1996; Hill et al., 2013a; Hopkins et al., 2010a, 2010b, 2010c; Hopkins, 2013; Stark and Hopkins, 2013). In addition to these cultural practices that may enhance P uptake and utilization, fertilizer manufacturers have sought to engineer materials to enhance PUE (Hopkins et al., 2008). Two P fertilizer additives that have been theoretically engineered to keep the P more plant available are: AVAIL® (AVAIL, Specialty Fertilizer Products, Leawood, KS, USA) and Carbond P® (CBP; 7-24-0; LandView, Landview Fertilizer, Rupert, ID, USA).

Organic acids are known to play a major role in soil and plant nutrition. Various studies have shown positive bio-enhancement effects of organic acids. These studies vary in results and organic acid origins but have many similarities. Generally, organic acids have been associated with bio-enhancement and have been attributed to impacts on root growth, elongation, yield, and emergence (Canellas et al., 2002; Eyheraguibel et al., 2008; Piccolo et al., 1993). Additionally, synthetic organic acids such as humic acids have been proven to be beneficial to plant growth (Seyedbagheri, 2010). Humic and

fulvic acids make up 75% of the total organic matter portion in mineral soils (Obreza et al., 1989). These latter acids are found in CBP as well.

These organic acids play a major role in the environmental fate of both organic and inorganic materials (Tan, 2003). In addition to various growth responses as mentioned above, improvement of P nutrition is another documented impact of organic acids (Seyedbagheri, 2010; Hopkins and Stark, 2013; Tan, 2003). Hopkins and Stark (2013) found that ammonium polyphosphate (APP; 10-34-0) mixed with humic acid increased P uptake and potato yield and quality compared to APP alone in moderate organic matter soil, especially at the lowest rate of P fertilizer applied in those trials. The proposed mode of action for AVAIL is that its high-charge density sequesters interfering cations, such as calcium or aluminum in the microsite surrounding the fertilizer prill. AVAIL is unique in that it is a water soluble long chain dicarboxylic acid (DCA) copolymer but only slightly mobile from point of contact. According to Dunn and Stevens (2008), this material is specific to adsorption of divalent and trivalent cations and is minimally affected by temperature, pH or ionic strength. Thus, when applied to soils, AVAIL theoretically minimizes the concentration of potentially reactive cations in the immediate vicinity of the P fertilizer. More detail regarding the impacts of AVAIL on production of a variety of crops is reviewed by Hopkins (2012) and Stark and Hopkins (2013). In brief, there is evidence that the AVAIL molecule impacts soil P chemistry and apparently increases solubility but also produces mixed field results. Not surprisingly, positive results with crop yield and quality were reported more often when soil test P levels and fertilizer application rates were relatively low i.e. there is no added benefit to applying AVAIL if plants already have ample P nutrition.

Carbond P is also designed to enhance P nutrition, but has a different mode of action than AVAIL. Carbond P has major compositional elements that are classified as organic acids (note that CBP also includes a cocktail of other proprietary additives). The theoretical impacts of CBP are based on chemical bonding of P with these organic acids (Hill. Organic acids and other fractions of soil play a major role in soil and plant nutrition. Humic substances (HS), especially humic and fulvic acids (HA, FA), make up the majority of soil organic material. These compositional elements play a major role in the environmental fate of both organic and inorganic materials (Tan, 2003). Synthetic HA have been proven to be beneficial to plant growth (Seyedbagheri, 2010). Various studies have shown positive bio-enhancement effects of organic acids. These studies vary in results and organic acid origins, but often have similar findings. Generally, root bio-enhancement has been observed via elongation, yield, and emergence (Canellas et al., 2013a, 2013b).2002; Eyheraguibel et al., 2008; Piccolo et al., 1993). In addition to the claims of various growth responses, improvement of P nutrition is another documented impact of organic acids (Seyedbagheri, 2010; Tan, 2003).

However, the effects of organic acids are not universal among soils. Hartz and Bottoms (2010) saw that fertilizer combined with humic acid in low organic matter soils produced positive effects. However, they concluded that at typical commercial application rates in representative field soils, humic acid is unlikely to significantly improve nutrient uptake or productivity of the vegetable crops studied (romaine lettuce, *Lactuca sativae* L.). As a relatively new product, testing of CBP has not been as widespread as AVAIL, but there is evidence that CBP impacts soil chemistry (Hill et al., 2013a, 2013b; Hopkins et al., 2013). As with AVAIL, positive results were reported more

often when CBP is applied to soil with low plant available P levels and fertilizer application rates of P are also relatively low. Increased availability of P provided as CBP is almost always exhibited in increased plant P concentrations compared to traditional fertilizers (Hill et al., 2013a; Hopkins et al., 2013).

Although there is evidence that the mode of action for both AVAIL and tomato, *Lycopersicon esculentum* Mill.). However, Cimrin and Yilmaz (2005) found a significant interaction between humic acid and P uptake, but yield was not significantly affected. In these studies (specifically with CBP) we are searching for a better understanding of the variable impact reported.

The effect of these organic acid P fertilizers has only been evaluated in low organic matter calcareous soils (Hill et al., 2013a; Hopkins et al., 2013). These soils inevitably have a soil solution dominated by  $\text{Ca}^{2+}$  which leads to P and PUE issues. Our studies with organic acids related to date have been almost exclusively in calcareous soils, but otherwise this relatively new product, CBP, has not been widely studied.

There is evidence that CBP impacts both soil chemistry and plant growth (Hill et al., 2013a, 2013b,; Hopkins, 2012; Hopkins et al., 2013; Packer et al., 2013; Summerhays et al., 2013). Summerhays et al. (2013) found in hydroponic conditions that CBP had no direct adverse effect on maize (*Zea mays* L.), even when present at very high concentrations in direct root contact. They also found that when CBP was the P source that there was no impact on biomass yield, P concentration nor uptake when compared to traditional ammonium polyphosphate (APP) fertilizer. This research showed conclusively that the impact of CBP in soil based systems is not a bio-stimulant plant response to the P or the organic acids present, but related to traditional theories of soil P chemistry

interactions . Recent additional hydroponic studies (Hopkins, 2012, unpublished data), showed that foliar supplied CBP did not impact P concentration in new shoot growth nor roots of maize nor biomass yield compared to non-treated plants. This further supports the idea that impact of CBP is soil chemistry related. Hill et al. (2013a, 2013b) found that maize grown with Carbond P produced significantly more biomass and P concentrations across multiple rates and soils than APP. The physiological growth enhancement responses were seen most strongly in medium to highly calcareous soils low in soil test P. Hopkins et al. (2013) found that field grown alfalfa (*Medicago sativa* L.), maize, potato (*Solanum tuberosum* L.) had positive responses to CBP and results were more often positive when CBP was applied to soils with low plant available P levels and relatively low fertilizer application rates. Increased availability of P provided as CBP is usually exhibited in increased plant P concentrations compared to traditional fertilizers (Hill et al., 2013a, Hopkins et al., 2013). All of these soil based studies were conducted in calcareous soils low in organic matter and soil test P. The objective of this study was to compare the efficiency of CBP to APP in the presence of moderate or high organic matter soil (that also have low soil test P), applied at low to very high P rates.

However, questions remain as to whether or not the impacts of CBP and AVAIL could be related some way with plant physiology—some type of general growth response triggered within the plant cells beyond the impact of P chemistry in soil. Additionally, there are long chain molecules present in both of these products which might be toxic to plant tissue at high rates. Therefore, the objectives of this study were to first compare the use of CBP and AVAIL with use of ammonium polyphosphate (APP) at pH 8.0 and 6.0 in hydroponically grown maize (*Zea mays* L.); Secondly, to compare the efficiency of



CBP to APP in the presence of moderate or high organic matter soil (that also have low soil test P), applied at low to very high P rates.

### **MATERIALS AND METHODS: HYDROPONICS**

Four hydroponic experiments in which maize (Syngenta NK N27B-3111; an AgriSure Viptera hybrid) were grown in an environmentally controlled growth chamber located at Brigham Young University in Provo, UT, USA. Phosphorus was supplied at five rates of P as APP, CBP, or AVAIL in nutrient solutions buffered at pH 6 or 8 (Table 1). Maize seeds were germinated by placing them on 4-mm stainless steel screens covered with moist cheesecloth in 9.5-cm deep rectangular plastic trays with 2 L of dilute complete nutrient germination solution (Nichols et al., 2012; Steinberg, 1953) reaching the bottom of the stainless steel screens. The modified Steinberg (1953) solution contained:  $\mu\text{M}$  concentrations, 635 calcium nitrate [ $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ ], 135 magnesium nitrate [ $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ ], 115 ammonium nitrate ( $\text{NH}_4\text{NO}_3$ ), 43 dipotassium phosphate ( $\text{K}_2\text{HPO}_4$ ), 125 potassium sulfate ( $\text{K}_2\text{SO}_4$ ), 125 potassium chloride (KCl), 120 potassium nitrate ( $\text{KNO}_3$ ), 135 ammonium sulfate [ $(\text{NH}_4)_2\text{SO}_4$ ], 17 boric acid ( $\text{H}_3\text{BO}_3$ ), 5.9 manganese chloride ( $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ ), 1.5 zinc sulfate ( $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ ), 0.40 copper sulfate ( $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ ), 0.26 sodium molybdate ( $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ ), and 0.50 iron-ethylenediamine-N,N'-bis (2-hydroxyphenylacetic acid (Fe-EDDHA). Germination and elongation of plants were accomplished over a seven-d period at  $\sim 25^\circ\text{C}$  (three d in darkness and four d in light). To promote elongation, white butcher paper with small slits was placed over the containers during the four d in light. After seven d, seedlings were transferred into polyethylene buckets placed in silver wooden boxes with randomly

selected plants (eight per container) suspended through holes in opaque plastic lids with 14 L of dilute, complete pre-treatment nutrient solution in each bucket (Nichols et al., 2012; Steinberg, 1953) and placed in an environmental growth chamber [ $\mu\text{M}$  concentrations were 977  $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ , 210  $\text{Mg}(\text{NO}_3)_2 \cdot 6\text{H}_2\text{O}$ , 180  $\text{NH}_4\text{NO}_3$ , 71  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 133  $\text{K}_2\text{HPO}_4$ , 116  $\text{K}_2\text{SO}_4$ , 115  $\text{KCl}$ , 75  $\text{KNO}_3$ , 41  $(\text{NH}_4)_2\text{SO}_4$ , 10  $\text{H}_3\text{BO}_3$ , 3.6  $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ , 0.94  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 0.25  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.16  $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ , and 1.4 Fe-EDDHA]. The base of the plant stem was wrapped in foam for support, with the foam wrapped with parafilm (Parafilm M Laboratory Film, Alcan Inc., Chicago, IL) to prevent brace roots from growing into the foam. Plants were grown in this pretreatment solution for seven d prior to transfer into treatments.

The treatment phase of the experiments consisted of four healthy maize plants of uniform size transferred into 14 L of modified Steinberg nutrient solution for each P treatment (Nichols et al., 2012; Steinberg, 1953). Maize plants were suspended using the same container system used for pretreatment [ $\mu\text{M}$  concentrations were 1,430  $\text{Ca}(\text{NO}_3)_2$ , 820  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , 250  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ , 20  $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$ , 400  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ , 5.9  $\text{MnSO}_4 \cdot \text{H}_2\text{O}$ , 1.3  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 0.3  $\text{NH}_4\text{MoO}_4 \cdot 4\text{H}_2\text{O}$ , 1.2  $\text{H}_3\text{BO}_4$ , 100 HEDTA, 1500 KOH, and 2000 2-Morpholinoethanesulphonic acid (MES hydrate) pH buffer. Solution pH was initially adjusted and then maintained daily with potassium hydroxide (KOH). Nutrient solutions were replenished by adding half of the original concentrations twice during each study. Treatments consisted of P concentrations of 72, 400, 3200, 6400, or 9600  $\mu\text{M}$  P supplied as APP, CBP or AVAIL (Table 1). Additions of each rate and source of P were split evenly seven times over the course of the experiment (every 2.3 d). Nitrogen was balanced across treatment with addition of  $(\text{NH}_4)_2\text{SO}_4$ .

Plants were grown in a 14/10 h light/dark photoperiod and temperatures of 25°C±1° during the light period and of 19°C±1° during the dark period. Nutrient solutions were aerated continuously during all growth phases of the experiment. Plants were observed daily and rated for general health, vigor and nutrient deficiency or toxicity development. Plants were harvested at the end of 18 d in treatment by rinsing roots of the four plants per pot repeatedly in ddH<sub>2</sub>O and separating as shoots and roots, oven dried at 65°C for a minimum of 72 h to uniform dryness, weighed, ground (Wiley mill, 1-mm sieve), digested in nitric-perchloric acid and analyzed for nutrient content by Inductively Coupled Plasma Atomic Emission Spectroscopy (ICP-AES, Thermo Electron Corporation, Franklin, MD, USA) spectroscopy.

The experiments were arranged in randomized complete block designs with three replications of each of the ten treatments (two fertilizer sources at five P concentrations). Statistical analyses were performed using Statistical Analysis System (Version 9.1, SAS, SAS Institute, Cary, NC, USA). The data for yield, nutrient concentration, and shoot, root, and total nutrient uptake were analyzed using analysis of variance (ANOVA) with mean separation using Duncan Waller Multiple Range Test at the 0.05 level of significance ( $P < 0.05$ ).

## **MATERIALS AND METHODS: GLASSHOUSE**

A glasshouse study was conducted in June 2011 with two soils—one moderate and the other high in organic matter concentration (Table 1). The high organic matter soil (Holdaway silt loam; fine-silty, mixed, superactive, mesic Petrocalcic Calciaquolls) was collected near Lehi, UT, USA. The moderate organic matter soil was constructed using

one part high organic matter soil with three parts of sand on a mass basis. The sand originated from a quarry near Provo, UT, USA. The soils were packed in pots (345g and 745g for the high and moderate organic matter soil, respectively) to achieve a bulk density of approximately 1.55-1.65 g cm<sup>-3</sup> and leaving a 2-3-cm headspace to allow for irrigation water addition. Pots had 25.4 cm height and 6.35 cm diameter at top and tapering to a rounded point at the bottom with drainage holes lined with polypropylene landscape fabric (Weed Block, Easy Gardener, Waco, TX, USA), to prevent soil loss. Phosphorus fertilizer was applied at field-simulated rates of 0, 2.2, 6.6, 20, or 60 kg P ha<sup>-1</sup> as APP or CBP. Fertilizer rates were calculated based on the simulated field conditions of the fertilizer being applied in a concentrated band with a 6.4 cm diameter core with the plant found in the middle of the core and an assumption of 6 plants m<sup>-1</sup> of row (actual rates were 0.04, 0.12, 0.36, or 1.08 ml APP pot<sup>-1</sup> and 0.06, 0.18, 0.54, or 1.62 ml CBP pot<sup>-1</sup>). Fertilizer was point-injected at 5 cm to the side and 5 cm below the seed immediately prior to planting. Nitrogen was balanced across treatments with urea ammonium nitrate solution at 250 kg N ha<sup>-1</sup>. Other essential nutrients were adequate based on soil test (Table 1).

Three maize seeds (Syngenta NK N27B-3111; an AgriSure Viptera Hybrid) were planted in the center of each pot at a depth of 2.5 cm. Plants were grown in a glasshouse located on the Brigham Young University campus at Provo, Utah, USA, 140°14'37"N, 111°39'02"W, 4570'. Best management practices for maize growth in a glasshouse were observed. Soil in each pot was irrigated to saturation prior to seeding and soils were maintained above ~55% plant available water content throughout the study. No pest pressure was observed and, therefore, no pesticides were applied. Targeted daytime and

nighttime air temperatures were 25°C and 15°C, respectively, although limitations in heating/cooling capacity resulted in ranges of 20-29°C daytime and 11-16°C nighttime. Natural light was supplemented with high-pressure sodium lamps to maintain a minimum 16-hour light cycle.

The numbers of plants per pot were thinned to two and one at 14 and 19 d, respectively, to achieve uniformly healthy plants across the trial. Plants were harvested at 28 d after planting by cutting at 3 cm above the soil surface, bagged, and then oven dried at 65 °C. Drying continued for a minimum of 72 hours to reach uniform dryness. Samples were then weighed, ground to pass 1-mm sieve, digested in nitric-perchloric acid, and analyzed for nutrient content by Inductively Coupled Plasma Atomic Emission Spectroscopy (Johnson and Ulrich, 1959; ICP-AES, Thermo Electron Corporation, Franklin, Maryland, USA).

Plants were arranged in a randomized complete block design with four replications of each treatment. Additional potted maize plants surrounded the entire study to mitigate previously observed border effects. Statistical analyses were performed using Statistical Analysis System (Version 9.1, SAS, SAS Institute, Cary, NC, USA). The data was analyzed using analysis of variance (ANOVA) with mean separation using Duncan/Waller Multiple Range Test at the  $P < 0.05$  level of significance (Table 3).

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## **APPENDIX B: COMPLETE (HIGH ORGANIC MATTER) SOIL DESCRIPTION**

Soil description from <http://websoilsurvey.nrcs.usda.gov>

### **Utah County, Utah - Central Part**

#### **Hr—Holdaway silt loam**

##### **Map Unit Setting**

- Elevation: 4,400 to 4,500 feet
- Mean annual precipitation: 12 to 16 inches
- Mean annual air temperature: 46 to 48 degrees F
- Frost-free period: 130 to 150 days

##### **Description of Holdaway**

###### **Setting**

- Landform: Lake terraces
- Landform position (three-dimensional): Tread
- Down-slope shape: Linear
- Across-slope shape: Linear
- Parent material: Lacustrine deposits derived from mixed sources

##### **Properties and qualities**

- Slope: 0 to 3 percent
- Depth to restrictive feature: 20 to 40 inches to petrocalcic
- Drainage class: Poorly drained
- Capacity of the most limiting layer to transmit water (Ksat): Very low to moderately high (0.00 to 0.20 in/hr)
  - Depth to water table: About 12 to 24 inches
  - Frequency of flooding: Rare
  - Frequency of ponding: None
  - Calcium carbonate, maximum content: 75 percent

- Maximum salinity: Nonsaline (0.0 to 2.0 mmhos/cm)
- Sodium adsorption ratio, maximum: 15.0
- Available water capacity: Low (about 3.4 inches)

### **Interpretive groups**

- Land capability classification (irrigated): 3w
- Land capability (nonirrigated): 7w
- Ecological site: Wet Fresh Meadow (R028AY020UT)

### **Typical profile**

- 0 to 7 inches: Silt loam
- 7 to 13 inches: Silt loam
- 13 to 20 inches: Silt loam
- 20 to 28 inches: Indurated
- 28 to 32 inches: Silt loam
- 32 to 67 inches: Cemented material

## APPENDIX C: PHOTOS



**Picture 1.** Maize seedlings in pre-treatment in hydroponic conditions



**Picture 2.** Carbond P and APP in a pH6 solution in hydroponics. See Picture 3 for visual rate differences.



**Picture 3.** Carbond P and APP in a pH 8 solution in hydroponics. In the foreground are stunted maize plants the lowest rate applied. To its left, are greatly improved maize plants with the highest application rate. Irregardless to fertilizer source, maize plants exhibited these visual symptoms.



**Picture 4.** AVAIL and APP in a pH6 solution in hydroponics. Low rates produced P deficiencies like this, regardless of fertilizer source.



**Picture 5.** AVAIL and APP in a pH8 solution in hydroponics. Maize leaf serration and venial banding were common, yet random. However, there was never any effect on study parameters.



**Picture 6.** Moderate OM soil on the left, high OM soil on the right (glasshouse study). Notice the difference in height of maize (not statistically analysed).



**Picture 7.** Moderate OM soil (3.6% OM) in the glasshouse study. Pots were thinned down to one maize seedling.





**Picture 8.** High OM soil (15.8% OM) in the glasshouse study. Pots were thinned down to one maize seedling.