# LOW-MOLECULAR-WEIGHT ORGANIC ACIDS AS A PHOSPHORUS NUTRIENT AMENDMENT FOR SOLANUM MELONGENA PRODUCTION IN CALCAREOUS SOILS OF THE CENTRAL TEXAS REGION

by

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**DEDICATION**

To my uncle, Abel Chavez

August 13, 1964-February 25, 2014

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## ABSTRACT

Phosphorus (P) is a vital component for crop production systems and a non-renewable fertilizer resource. Although P applications to farms has been considered a root cause of increased global yields, it has come at the expense of increased phosphate rock (PR) depletion, the main derivative of P fertilizers which is estimated to decline by 2050. On the other hand, soils frequently contain enough native P for production yet it is unavailable due to P fixation. Low-molecular-weight organic acids (LMWOA) are largely responsible for mineralizing P in the soil rhizosphere and making it plant available in soil solution. This study investigated the potential of two LMWOAs, oxalic and citric acid, to mineralize fixed P in bulk soils and the effects on production of eggplant. Two calcareous soils were used, including an alkaline (pH 7.6-7.8) vertisol from the Houston Black series and a slightly acidic (pH 6.5-6.8) mollisol from the Tarpley soil series. Results showed oxalic and citric acid in the high pH calcareous soil mineralized P in amounts equivalent to applied triple superphosphate (TSP) fertilizer ($p > 0.05$) while eggplant yields indicated no significant difference ($p > 0.05$) between LMWOA applications and TSP fertilizer. In the low pH calcareous soil, LMWOA treatments were significantly less effective at mineralizing native soil P and matching eggplant yield ($p < 0.05$) when compared to TSP fertilizer applications.

# CHAPTER I-INTRODUCTION

## Introduction

### *The Role of Phosphorus in Agriculture*

Phosphorus (P) is an essential element for all living organisms and plays a significant role in an array of processes including energy generation, nucleic acid synthesis, photosynthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling, carbohydrate metabolism, and nitrogen (N) fixation (Vance et al 2003). It is an important constituent of DNA and in ATP for energy transfer within cells (Suh and Yee 2011). P is one of the three most important inorganic elements involved in the process of normal growth and metabolism for plants (Ragothama 1999). Less than optimum levels of P can lead to 5%-15% below maximum yields (Shenoy and Kalagudi 2005). Symptoms of P deficiency in plants may include stunted growth, foliage discoloration, delayed maturity, poor flowering, fruit yield, and seed development (Marschner 2002). Recommended rates of P fertilizer are especially high for vegetable production in order to avoid these deficiencies (Lorenz and Vittum 1980). The use of P fertilizers in agricultural production also aims to replace harvest and erosion losses of P from soil (Liu et al. 2008). While agricultural applications of P have played a significant role in providing sufficient harvest to meet global food demands in the past, industrial agriculture has simultaneously altered the P cycle by relying on mined phosphate rock (PR); a non-renewable fertilizer resource (Cordell and White 2011). Before worldwide mining of PR as a common fertilizer source, P was naturally supplied to soils by recycling animal manure, crushed animal

bones, city waste and ash (Van Vuuren et al. 2010). Over the last half of the 20th Century, the Green Revolution abandoned these methods completely for PR-based fertilizers, only to generate the present-day P scarcity concerns (Cordell and White 2011; Cordell et al. 2009; Liu et al. 2008).

Agricultural fertilizer uses on P-deficient soils that are also P-fixing render these applications inefficient (Simpson et al. 2011). When P fertilizer, in salt form [e.g. $Ca(PO_4)_2$], is added to most soils it quickly becomes immobile and, as such, very little of the added P is recovered with water (Plante 2007). On the other hand, most soils frequently have enough native P for crop production (Oburger et al. 2011; Richardson 2001). Mesic region soils with slightly acidic pH (6.5) have the most available P (Plante 2007). Meanwhile, arid region soils may have slightly acid to alkaline (pH) surfaces with $CaCO_3$ accumulation in upper horizons (Dregne 1976). In acidic soils, P forms low solubility substances with aluminum ($Al^{+3}$) and iron ($Fe^{+3}$), while in alkaline soils it binds with calcium ($Ca^{+2}$) and magnesium ($Mg^{+2}$) to form insoluble P compounds (Bucio et al. 2000). Alkaline or calcareous soils are widespread in dry climates and the richness of free $CaCO_3$ tends to fix P as tricalcium phosphate [$Ca_3(PO_4)_2$] (Marschner 2002). The functions of limited precipitation and, hence, limited leaching of $Ca^{+2}$ in these drier regions are directly proportional to increased carbonate layers on the soil surface (Dregne 1976). At the same time, most soils contain low-molecular-weight organic acids (LMWOAs) that may be used by plants or microorganisms for nutrient acquisition (He et al. 2008). LMWOAs are COOH-containing compounds that allow the binding of metal cations in solution and the displacement of anions from the soil matrix (Jones 1998). These LMWOAs form strong complexes with cations like $Al^{+3}$, $Fe^{+3}$, or $Ca^{+2}$ and

displace phosphate groups from binding sites (Ryan et al. 2001). It is in ion exchange processes that P becomes available for nutrient uptake by crops. The idea of acquiring native P from soils using LMWOAs is a method that merits further investigation as many recent studies have shown the potential of LMWOAs to have a correlative effect on P uptake by plants (Ström et al. 2002; Oberger et al. 2011; Vance et al. 2003).

Complicating P availability in soils is the ever-increasing global demand for fertilizers and the limitation of PR as a dependent resource (Lehr 1980). Global P fertilizer production has been estimated to decline after 2050 (Vance 2003). The mining of PR is an energy-intensive process concentrated in only certain parts of the world. According to a U.S.G.S. Mineral Commodity Summery by Jasinski (2012), PR resources occur mainly as sedimentary marine phosphates with the largest deposits found in northern Africa, China, the Middle East and the U.S, while igneous resources are found in Brazil, Canada, Finland, Russia, and South Africa; totaling world resources of PR at ~300 billion metric tons (Gt). In the past, PR has been sold as a cheap bulk commodity (Van Kauwenbergh 2010). In recent history, the price of PR per metric ton (t) has ranged anywhere between \$70 $t^{-1}$ in October 1992 to \$192 $t^{-1}$ in October 2012 with a record spike of \$475 $t^{-1}$ in August 2008, adjusted for inflation (Index Mundi 2012). According to a report by Huang (2009), the price fluctuation of PR in the U.S. over the last decade has involved a combination of factors including U.S. fertilizer production capacity and production in decline, as well as its increasing dependence on global trade. P fertilizer use increased four to five-fold between 1960 and 2000 and is estimated to increase further by 20 million metric tons (Mt) per year by 2030 (Vance 2001). World mine production increased from 181 Mt in 2010 to 191 Mt in 2011 while in 2012 the U.S.

alone mined an estimated 28.4 Mt of marketable product valued at 2.8 billion dollars, adjusted for inflation (Jasinski 2012).

According to Hinsinger et al. (2011), agricultural productivity needs sustainability, both from an ecological and economical perspective. As defined by Raman (2006), sustainable agriculture is one that enhances environmental quality and the resource base on which agriculture depends, while providing for basic human food and fiber needs, is economically viable, and enhances the quality of life for farmers and society as a whole. From this perspective, agriculture needs alternative options in addition to mined PR, which has limited absorption effects and a high potential for nutrient pollution. P fertilizer runoff from agriculture is, to a significant degree, one of the leading causes of eutrophication in aquatic systems (Cordell and White 2011). Impacts of eutrophication range from the decline of aquatic resources for marine communities, degradation of water for human consumption, to the growth of algal blooms that can directly affect marine and human health (Kleinman et al. 2011). The increase of reactive P to the oceans from anthropogenic activities, mostly due to P fertilization, has been estimated at ~9 Mt $yr^{-1}$ (Rockström et al. 2009).

The fact that global P supply is quickly diminishing means that supply and demand will never be at equilibrium, making it even more difficult for subsistence farmers in third world countries to keep up with future prices. The potential social impact of reduced P availability on family farmers and developing nations is enough to continue research for secondary P sources since agriculture is the main source of livelihood for more than half the world's population (Laegreid et al. 1999). Annual global agricultural application of P is estimated to be ~400 Mt $yr^{-1}$ (FAO 2008). Due to

the importance of P to agricultural production and global food security, it is necessary to address inefficiencies in P use and develop farming systems which aim to reduce P fertilizer inputs (Simpson et al. 2011).

*Phosphorus Availability and Acquisition by Plants in Soils*

Inorganic P is naturally present in soils and sourced from its main parent material, while organic forms of P are derived from the biological P cycle, mostly occurring as esters and orthophosphoric acid (Anderson 1980). Despite the sources of P in agricultural soils, many soils around the world are P-deficient with even the most fertile soils seldom exceeding 10 µM (Bieleski 1973). Concentration in plants is also low and ranges from 0.05% to 0.30% of total dry weight (Vance 2001). Consequently, P has the lowest solubility and availability of major plant nutrients compared to other macro- and micronutrients in most soils (Ragothama 1999; Hinsinger 2001).

The distribution and nature of P in soils is primarily determined by pH, where phosphate ions are dissociated from orthophosphate into solution. Specifically, plants acquire P as orthophosphate anions, mainly as $HPO_4^{-2}$and $H_2PO_4^-$, from the soil solution (Richardson et al. 2009). At pH 7.22 the ratio of $HPO_4^{-2-}$ and $H_2PO_4^-$ ions are equal but anything below and $H_2PO_4^-$ becomes the main form of P adsorption (Barber 1980). As opposed to mass flow or root interception, diffusion is by far the main P acquisition method for plants while the concentration gradient is also highly influential in the process (Marschner 2002). Specifically, more than 90% of P acquired by plants reaches the roots via diffusion at a rate between $10^{-7}$ to $10^{-9}$ $cm^2$ $s^{-1}$ (Nord et al. 2011). Still, for most soils

the diffusion rate of P is inadequate to overcome specific plant needs (Richardson et al. 2009).

Low P availability is frequently cited as a major cause of low yields in crop production both for developed and developing countries (Vance et al 2003). In most soils, the concentration of available P in soil solution (2 μM) is exponentially lower than in plant tissues (5-20 mM) and mainly controlled by its interaction with organic or inorganic surfaces in the soil (Ragothama 1999). As stated, soils with high $Ca^{+2}$ content and high pH tend to fix native P and applied P in the form of $Ca_3(PO_4)_2$. Diverse forms of precipitated P for these soil types may include a range of mono- ($CaHPO_4$), di- and tri-Ca phosphates (e.g. $Ca(PO_4)_2$) and hydrates, hydroxyl ($Ca_5(PO_4)_3OH$, and fluorapatites ($Ca_5(PO_4)F$) (Richardson 2001).

In addition to low availability and low solubility problems involved in the matrix of soils, current fertilizer amendments have not yet solved the P dilemma solely through intensive application practices. Phosphate rock (PR) is not plant available when the pH of soil is greater than 5.5-6.0, and even when conditions are optimal plant yields are lower than those gained from soluble phosphates (Singh and Reddy 2011). Thus, PR is unsuitable in alkaline soils so farmers must rely on inorganic P fertilizer like diammonium phosphate [$(NH_4)_2HPO_4$] or triple superphosphate [$Ca(H_2PO_4)_2$], yet these fertilizers also become rapidly transformed into stable minerals such as $Ca_3(PO_4)_2$ which are relatively unavailable to crops (Asadi et al. 2010). To reduce fixation and increase adsorption, P fertilizer is placed as close to the root zone as possible by broadcasting and mixing with the surface plow layer or by banding near the row when the crop is planted (Barber 1980). Even under adequate P fertilization, only 20% or less is removed by the

first year's growth (Vance 2001). Over time, up to 90% of applied P is adsorbed and remains fixed in the soil (Asmar et al. 2000). These applications result in P loading of prime agricultural land that inevitably leads to increased nutrient pollution in stream flows through runoff (Vance et al 2003).

*Phosphorus Acquisition by Plants in the Rhizosphere*

In most agricultural soils P availability is greater in surface or near-surface horizons, where deposition from decay and plant residues persist, and conditions for P mobilization are more conductive due to several factors including organic matter content, microbial activity, and favorable pH (Lynch and Brown 2001). Compounds released from plant roots into the rhizosphere change the chemical and physical properties of soils, while stimulating the growth of various organisms based on the nature of exudates released, location on the root, and soil type (Gregory 2006). For the most part, research has focused on the significance of these interactions within the rhizosphere due to the sophisticated adaptability by plants to increase P availability from the surrounding environment using various mechanisms (Marschner 2011; Khademi et al. 2010; Richardson 2001; Hoffland 1992).

Crowley et al. (2011) define the rhizosphere as the location where microbial action releases and transforms fixed inorganic nutrients into organic forms through solubilization, chelation and oxidation/reduction. It is also here where plants exude LMWOAs to mobilize mineral nutrients directly or indirectly by delivering the energy for microbial action (Marschner 2002). These acids are part of metabolic functions at the cellular level in biochemical pathways including energy production, formation of

precursors for amino-acid biosynthesis and at the whole plant level in modulating environmental adaptation (Bucio et al. 2000). The best known of the plant-produced LMWOAs are citric, succinic, malic, oxalic, and tartaric (Stevenson 1967). It has also been universally accepted that exudation of these LMWOAs may cause a significant mobilization of P in the rhizosphere (Godbold et al. 2002).

The link between LMWOA association and environment is strong for plants. Numerous dicotyledonous species have been found to release organic anions from their roots when P-deficient, which may continue throughout much of the plant's cycle (Ryan et al. 2001). These anions are mainly produced in mitochondria through tricarboxylic acid and glyoxylate cycles while biosynthesis, accumulation, transport and root exudation of LMWOAs is dramatically increased in response to environmental stress (Bucio et al. 2000). Plants are known to adapt several different mechanisms to increase their acquisition, uptake and adsorption efficiency by including root modifications, symbiotic relationships, and rhizospheric modifications in their search for P (Shenoy and Kalagudi 2005). Plants mobilize P by releasing $H^+$, $OH^-$, $CO_2$, LMWOA anions (e.g. citrate, malate, oxalate) or the release of various phosphatase enzymes (Crowley et al. 2011). Römer and Keller (2001) exemplified the significance of LMWOAs with the reaction of spinach to secrete anions (oxalate, malate, citrate) in response to low P supply.

Depending on plant and soil factors, rhizosphere pH may differ from bulk soil pH by up to two units due to ion imbalances and LMWOA secretions (Marschner 2002). As such, pH alterations to the rhizosphere by LMWOA supplements has initiated research from their ability to mobilize P through root exudation processes (Dinkelaker et al. 1989; Hoffland 1992; Gardner 1983). Neumann and Romheld (1999) compared P-deficient

plant roots of wheat (*Triticum aestivum L.*), tomato (*Lycopersicon esculentum L.*), chickpea (*Cicer arietinum*) and white lupin (*Lupinus albus L.*) to reveal a distinct correlation between the release of protons, LMWOAs and rhizosphere acidification, which ultimately determined the ability in these crops to uptake acid soluble $Ca_3(PO_4)_2$ in calcareous soils while maintaining stable pH. According to a review by Jones (1998), it appears that $H^+$ or $K^+$ and LMWOAs released by roots are likely biochemically separate but spatially coordinated events that accompany LMWOAs to maintain charge neutrality. Considering the overall cation-anion balance, this detail suggests that release of the LMWOA anion will, at least momentarily, acidify the rhizosphere (Hinsinger 2001). Gardner et al. (1983) analyzed the pH of leachates from a field study on release of citrate from *L. albus* and found minimal pH differences between plants and controls, suggesting that plant exudates are of neutral or slightly acid pH and that citrate, not citric acid, was secreted. Oburger et al. (2011) confirmed the efficiency of citrate on P solubilization and its effects based on pH-adjusted solution levels (1 M HCl or KOH) relative to natural soil conditions, with results indicating the more acidic citrate solutions helped to solubilize more P in all soils analyzed. According to Gillespie and Pope (1991), increasing P availability through soil acidification mechanisms will decrease buffering capacity and lead to increased P diffusion rates and supply from the soil. Moreover, LMWOA action in solubilizing phosphates may be attributed to a lowering of the pH but more importantly to the formation of stable complexes with such cations as $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, and $Al^{3+}$ that facilitate P uptake for plants (Stevenson 1967).

Under nutrient deficiency, plant root exudates such as sugars, LMWOA anions and amino acids are released primarily in the zone immediately behind root tips and distal

elongation zone (Crowley et al. 2011). The relationship between plant roots and microorganisms may be attributed to root exudates and is one of the most important factors affecting microbial growth in the rhizosphere (Adriano et al. 2005). Root exudation occurs in any soil horizon with root activity due to passive loss of compounds from roots or as active exudation of organic compounds from roots, including LMWOAs (Strobel 2001). The particular LMWOA that accumulates varies depending upon species, age of plant and tissue type (Bucio et al. 2000). According to Strobel (2001) plant species contain different amounts of LMWOAs in the roots, and thus vegetation might influence the soil solution concentrations of LMWOAs, as well as old and damaged root cells that leak with various LMWOAs.

Plants may decrease growth rate in order to conserve P when deficient, increase growth per unit of P uptake, remobilize internal P, modify C metabolism that bypass P-requiring steps, and take alternative respiratory pathways (Vance et al 2003). In response to P deficiency, various plants have developed proteoid roots that can release significant quantities of carboxylates like citrate, as shown by Gerke et al. (1994) in which they determined the release of citric and malic acid from the proteoid roots of *L. albus* at various concentrations (0-55 μmol g soil$^{-1}$). These nerve-like root clusters are located on lateral roots and are able to strongly acidify the rhizosphere soil by releasing significant amounts of LMWOAs (e.g. citric, malic) (Schubert et al. 2005). The abundant development of lateral roots associated with P-deficiency alterations in root architecture is mostly accompanied by increased root hair density and length (Vance et al 2003). As a result, root architecture determines the exploration and exploitation of P resources by

plants and is a valuable component in a competitive environment of higher mineral nutrition (Lynch and Brown 2001).

LMWOAs are involved in plant energy production as intermediates in the trycarboxylic (TCA) cycle (e.g. malate, citrate) while others are primarily present in cells for cation charge balancing or for maintaining osmotic potential (e.g. malate, malonate, oxalate) (Jones 1998). According to a review by Ragothama (1999) and Jones (1998) malic and citric acid are the ruling LMWOAs excreted by roots under P deficiency. In addition, different plant species may exude particular LMWOAs in order to mobilize different types of soil soluble P (e.g. Al-P) by adapting to specific soil conditions (Cao et al., 1997). Ström (1997) revealed that total concentration of LMWOAs (citric, oxalic) in two calcicole (lime tolerant) species were twice the concentrations found in the soil solution of the calcifuge (lime intolerant) species. Malate and citrate exuded by *Brassica napus L.* grown in $Ca_3(PO_4)_2$ soils acidified the rhizosphere or reduced the concentration of free $Ca^+$, which may also be a result of adaption to calcareous soils (Cao et al. 1997; Hoffland 1992).

*Low-molecular-weight Organic Acids in Soils by Means of Application*

Numerous LMWOAs have been investigated in soil P studies, including use of monocarboxylic (lactic, gluconic, acetic, formatic), dicarboxylic (oxalic, tartaric, malic, fumaric, malonic) and tricarboxylic (citric) acids (Richardson 2001). According to Jones (1998), the effectiveness of individual LMWOAs to mobilize soil P depends on the number of carboxyl groups they possess and follows the series of monocarboxylic, dicarboxylic, and tricarboxylic acid, where an increasing negative charge allows for

compounding of metal cations in solution and displacement of anions from the soil, respectively. In calcareous soils, oxalate and citrate have been directly linked to P mobilization through $Ca^{+2}$ complexation and acidification mechanisms using distinct ionic forms of LMWOAs (Khademi et al. 2010). Here, they tested both oxalate (potassium oxalate; oxalic acid) and citrate (potassium citrate; citric acid) to grow wheat and found no differences between P accumulation in plants using distinct ionic forms of LMWOAs (1mM; 10 mM), although both oxalate forms proved better than citrates, while oxalate (oxalic acid) made P more available in calcareous soil (pH 7.88). Despite these results, citrate is able to increase the availability of P by lowering $Ca^{2+}$ concentrations through chelation and creation of soluble salts such as calcium citrate in calcareous soils (Marschner 2002). Jones (1998) explains that LMWOAs like citrate or malate are highly pH-dependent with minimal metal binding at high pH and where oxalate precipitates with $Ca^{2+}$ in soils against the negative charge of LMWOAs, which may quickly be adsorbed into the solid phase of the soil. These explanations indicate that LMWOA decomposition may also be soil dependent, where decreasing anionic binding sites are correlated with increased decomposition rates of LMWOAs according to the mineral phase of the soil (Oburger et al. 2009). The influence of soils on LMWOAs is exemplified by the occurrence of higher oxalate concentrations found in forest soils around the world and their ability to increase Al-P solubility (Fox and Comerford 1992). Wei et al. (2010) also studied P mobilization in forest and tropical soils using malic, oxalic and citric acid concentrations (10 mM $kg^{-1}$) to find an increase of organic P using citric acid. Although few studies have solely used LMWOA concentrations in soil for P solubilization and

uptake by plants for production purposes, current research has revealed that LMWOAs function in maximizing P availability for both temperate and arid soils.

The application of LMWOAs to many soils over the world has been repeatedly tested to prove these solutions significantly increase P mobilization (Wei et al. 2010; Gerke 1994; Khademi et al. 2010; Oburger et al. 2009; Wang et al 2008; Ström et al. 2001). Oburger et al. (2011) used LMWOA concentrations of 500 μM (2.5 mmol $kg^{-1}$) and confirmed a correlation between increased soil P availability and LMWOA additions, among other factors such as P loading and pH. Gerke (1994) investigated the addition of citrate to a Spodosol and Alfisol soil to compare the effects of single and sequential applications on P desorption, resulting in desorption increases up to a factor of twenty at the highest citrate loading levels (50 μmol $g^{-1}$) in both soils compared to controls. Fox and Comerford (1992) tested the influence of oxalate on P and Al solubilizaton in two Spodosols at single and sequential loading rates, with results indicating that P availability increased as the oxalate concentration (0.5-125 mM $kg^{-1}$) and loading rate increased for both single and sequential additions in the Bh horizon of both soils. Similarly, other research has focused on combining the extracting abilities of LMWOA with added P fertilizers (Kpomblekou-A and Tabatabai 2003; Lui et al. 2012; Comerford and Skinner 1989; Ström et al. 2001; Bolan et al. 1994). Liu et al. (2012) tested PR fertilizer along with specific concentrations of LMWOA amendments with results showing P released from PR increased according to increasing concentrations of LMWOAs (0.1 to 0.5 M $kg^{-1}$). Kpomblekou-A and Tabatabai (2003) used citric and oxalic acid concentrations of 10 mM $kg^{-1}$ in a greenhouse experiment with *Zea mays* to show a positive response in plant growth from both LMWOAs with varied rates and PR sources. Bolan et al. (1994) also

used 10 mM $kg^{-1}$ LMWOAs concentrations (acetic, oxalic, citric) along with water-soluble $CaHPO_4$ and PR to examine the solubilization and plant uptake of P in *Lolium rigidium*, resulting in an increased effectiveness of fertilizers through higher yields using oxalic and citric acid.

Determining soil solution concentrations of P availability resulting from LMWOA experiments has been thoroughly investigated and derived from several techniques and procedures. In soil P testing the fraction of P (organic; inorganic) that is designated as available for plant uptake is derived from extraction procedures using dilute acids or bicarbonate solution, while the unattainable fraction is designated as fixed P (Plante 2007). Extractants such as sodium bicarbonate or water reflect the concentration in the soil solution, whereas strong extractants mostly indicate the buffer capacity of the soil to supply nutrients to the soil solution (Marschner 2002). The most common procedure for determining extractable P in soil investigations using LMWOAs (Comerford and Skinner 1989; Gerke 1992; Wei et al. 2010; Bolan et al. 1994; Ström et al. 2001) is that of Murphy and Riley (1962), in which soil extracts are measured using a single solution reagent containing an acidified solution of ammonium molybdate, ascorbic acid and antimony. Comerford and Skinner (1989) state this method primarily extracts inorganic orthophosphates but may also include phosphate from some organic phosphates subject to acid or molybdate hydrolysis at low pH. Determining extractable P by the Olsen extraction method is also used by various `studies (Khademi et al. 2010; Wang et al. 2008), in which sodium bicarbonate ($NaHCO_3$) is employed to decrease the solution concentration of soluble $Ca^{2+}$ by precipitation as $CaCO_3$. According to Olsen et al. (1954), the Olsen P method can remove $Ca_3(PO_4)_2$ and phosphate adsorbed on surfaces

of $CaCO_3$ and is considered the most suitable P test for alkaline or calcareous soils. An Olsen P value of 10 mg $kg^{-1}$ is generally considered suitable for normal plant growth (Sims 2000). Regardless of test method, extractable P has traditionally been defined by soil testing laboratories as the amount of P in soils available for crop uptake and to verify the probability of crop response to added P fertilizer (Pyrzynski and Sharpley 2000).

*The Role of Microorganisms in the Rhizosphere*

Continuous research indicates both plants and microorganisms facilitate P acquisition by increasing solubility of inorganic P through various biochemical processes (Ragothama 1999; Tyler et al.1995; Ström 1997; Zhang et al. 1997; Gahoonia et al. 2000; Zhu et al. 2005). Different LMWOAs have been studied for their abundance in the rhizosphere where root exudates and microorganisms work together to mobilize P by replenishing it with LMWOA anions and forming stable complexes with $Ca^{+2}$ to release P (Wei et al. 2010). Depending on their carboxylic arrangement, LMWOAs function within mono-, di-, and tricarboxylic acid groups (Strobel 2001). These soil solution LMWOAs originate from several biotic sources, including metabolites from decomposition of soil organic matter in upper horizons, and exudates from fungi or plant roots (Strobel 2001). LMWOAs are a versatile source of C in soil and once released into soil solution are quickly broken down by the soil community (Oburger et al. 2009). Kpomblekou-A and Tabatabai (2003) show plant or microbial potential to exude ions or LMWOAs to facilitate P uptake for plants.

Microorganism activity in the rhizosphere can stimulate, restrain, or exist without effect on root growth, depending on the type of microorganism, plant species and

environment (Marschner 2002). Despite the negative or neutral effects of microbial activity, a report by Richardson (2001) suggests that P solubilization by particular microorganisms is a major mechanism for improved plant growth, with production and release of LMWOAs by these microbes to be a key contributing element. LMWOAs may be quickened by release of organic C and $CO_2$ produced by roots and microorganisms (Marschner 2002). P-dissolving microorganisms may include bacteria, fungi, and actinomycetes (Molla et al. 1984). Mycorrhizal fungi can increase plant P uptake by extending hyphae into the surrounding soil volume to take in P and transfer it to the host plant (Marschner et al. 2011). Oxalic acid is produced by many strains of fungi, including brown-rots, white-rots, mycorrhizae, plant pathogens, and *Aspergillus niger* (Magnuson and Lasure 2004). Microorganisms in the rhizosphere are highly significant for decomposing, mineralizing organic material and transforming inorganic nutrients for plant acquisition, while further influencing nutrient availability by solubilization, chelation and oxidation/reduction (Marschner et al. 2011). Numerous studies (Kim et al. 1998; Molla et al. 1984; Cunningham and Kuiack 1992) have confirmed the P-solubilizing abilities of many different strains of microbes present in soil. A report by Singh and Reddy (2011) suggests that inoculation of *P. oxalicum* significantly enhances the fertilizer value of PR especially in alkaline soils where the solubilization of PR alone is not as possible. Vassilev et al. (2006) applied *A. niger* and *Phanerochaete chrysosporium* to agro-industrial biowaste and determined solubilization of P present in PR from these microorganisms. Zayed et al. (2005) also inoculated strains of *A. niger* and *Trichoderma viride* to compost piles enriched with PR and correlated soluble P with these microorganisms. Further effects of PR enrichment with LMWOAs

(citric and malic) and solubilization of P in wheat straw composting resulted in higher solubilization rates of P (Singh and Amberger 1998).

Although microbes are known to release a wide range of LMWOAs and contribute to P solubilization from PR, Jones (1998) notes that LMWOAs added at realistic concentrations (10-100 μM $kg^{-1}$) to mimic root exudation are just as quickly biodegraded by microorganisms in bulk soil with an average half-life of 2-3 hours depending on soil type. Oburger et al. (2009) tested five contrasting soils to show an initial rapid decomposition phase within the first (24) hours of application, measured through $^{14}CO_2$ release by microbes after the addition of LMWOAs. Ström et al. (2001) used *Z. mays* as a model plant in a calcareous soil and the application of LMWOAs (citric, malic and oxalic) to assess the mobilization rate of nutrients in the rhizosphere and bulk soil, with results showing that malate and citrate were quickly biodegraded by microbial action but oxalate was resistant. A follow-up study by Ström et al. (2002) measured LMWOA degradation in response to addition of LMWOA (1 and 10 mM $kg^{-1}$) concentrations and determined a significant degradation rate within (24) hours but a slower degradation for 10mM concentrations of oxalate as compared to citrate. Gerke (1994) discusses in his research that one-week soil incubations, after single addition citrate treatments, may cause some microbial degradation of the LMWOA and may account for the advantage of sequential loading at low citrate treatment levels (10-20 μmol $g^{-1}$). Van Hees et al. (2003) tested LMWOA concentrations (0-1000 μM $g^{-1}$) of citric, oxalic, and acetic acid in three coniferous forest soil profiles and found increased soil sorption of LMWOAs decreased biodegradation rates of LMWOAs, which mostly depended on soil horizon and LMWOA type; in this case oxalic acid most readily

adsorbed. As a result of this evidence, competition between LMWOAs and microorganisms cannot be overstated and further demonstrates the intricacies involved within LMWOA application processes.

**Objectives**

Despite extensive research on P mobilization through use of LMWOAs in the rhizosphere, there is limited evidence showing supplemental applications of LMWOAs without added P fertilizers and the potential impact on crop production. The purpose of this study was to determine the potential of LMWOAs as a soil amendment to increase P availability for vegetable production purposes. The first objective was to determine the ability of two LMWOAs to mobilize P in two distinct soils native to the Central Texas Region. Second, the study aimed to determine the effects of directly applied LMWOAs on P availability for a high P-demanding crop. Eggplant (*Solanum melongena*) was utilized for crop production since it is a dependent P feeder. The effects of LMWOA-released P were measured by the quality and quantity of fruit produced as compared to a conventionally grown control.

**Justification**

The need for a more stable P nutrient acquisition method is important to reduce the continuing depletion of global resources and to feed a quickly increasing population. Global population is expected to increase from 7 billion currently to 9 billion by year 2050 so there is reason for consideration of alternative methods in P fertilization due to increasingly limited global resources and infinite food necessities (Vance 2001).

Phosphorus is one of three essential macronutrients required for plant growth and there are no substitutes for it in agriculture (Jasinski 2012). Although P availability is difficult to manage due to easy combination with soil cations that form low-solubility substances, LMWOAs can mobilize P and these acids are well-known products of plant and microorganism metabolism. Using LMWOA as a way to release bound P complexes directly in the soil matrix is a technique that needs further investigation since most LMWOA studies have only been performed in solution cultures (Jones 1998). In a world with decreasing supplies and increasing demands, this study may provide a sustainable alternative and contribute to the currently existing research on LMWOAs based on their potential to increase P uptake for plants.

## Materials and Methods

### *Soils*

Soils were collected in Hays County, Texas. The A1 horizon of a mollisol soil (15 cm deep) was collected from the edge of the Edwards Plateau (29.938465, -98.010644). The Hays County Soil Survey (USDA 1984) classifies this soil as a montmorillonitic, thermic Lithic Vertic Arguistoll from the Tarpley (TaB) series with reddish brown clay to a depth of about 60 cm with acceptable drainage. These soils have weathered under $CaCO_3$ conditions and the abundance of $Ca^{+2}$ ions promotes P fixation (Carson 2000). Likewise, the Ap horizon of a vertisol soil was harvested (15 cm deep) from the Blackland Prairie region just east of the plateau (29.782137, -97.970763). This soil is listed as a fine, montmorillonitic, thermic Udic Pellusert from the Houston Black (HvB) series with very dark gray, gravelly clay to a depth of about 90 cm with moderate

drainage (USDA 1984). These soils are also enriched with $Ca^{+2}$ and characterized by an abundance of swelling clays intimately bound to highly polymerized humus and by alternating wet and dry phases (Lozet and Mathieu 1991). Both soils were allowed to air dry and then screened for foreign materials (plant biomass, stones, insects, etc.) using a 4 mm sieve before transferring to grow bags for experiment. Prior to experiments, both soils were analyzed for nutrient availability, pH, EC, lime, and CEC.

*Plants*

A black bell eggplant variety, Galine F1, from Johnny's Selected Seed Company was used for its high yielding uniformity. Eggplant is a traditional summer crop that grows best as a transplant and was chosen due to its relatively high P fertilizer demand (200 kg $ha^{-1}$) in commercial production. Seed was sown in the greenhouse on February 20, 2014 in 2 cm cell trays with a peat moss medium. Seedlings were fertilized once a week after true leaf emergence and throughout greenhouse propagation up to a week before transplant. Plants were managed for ten weeks in the greenhouse until optimum temperatures allowed for transplanting to 19 L grow bags similar to an eggplant study conducted by Nafiu et al. (2011). Plants were hardened-off to reduce transplanting shock by exposing to lower temperature, reduced watering and no fertilization as recommended by Maynard and Hochmuth (2007) and where transplanted on April 24, 2014.

*Fertilizer*

All seedlings were fertilized at a continuous rate of once per week in the greenhouse using $KNO_3$ (15-0-15) as a starter solution (188 ppm) and applied by bottom-

watering method to maintain optimum growth before transplanting. After transplanting, each LMWOA group received an assigned treatment (citric 0.1 mM $L^{-1}$, citric 100 $L^{-1}$, oxalic 0.1 mM $L^{-1}$, oxalic 100 mM $L^{-1}$) as an alternative P fertilizer source. Granular fertilizer treatments were determined after soil analysis of each soil, which included fertilizer recommendations of 4.0 lbs. P/1000 $ft.^2$ for HvB controls and 4.2 lbs. P/1000 $ft^2$ for TaB controls using triple superphosphate [$Ca(H_2PO_4)_2$]. Meanwhile, all treatments (HvB and TaB) were equally treated at 1.6 lbs. N/1000 $ft.^2$ with urea [$CO(NH_2)_2$] as a readily available N source. Granular fertilizer treatments were manually applied to grow bags and mixed thoroughly with the top 6-7 cm of bulk soil to simulate a broadcast top-dress application method. Along with soil test recommendations fertilizers were applied according to grade and timing of application. Fertilizer grade for $CO(NH_2)_2$ was 46% N and 46% P for $Ca(H_2PO_4)_2$. Application times were determined based on vegetative growth stage and yield response of eggplant. Due to the pot-contained production method, granular fertilizer applications were configured for each 19 L grow bag based on lab fertilizer recommendations.

*Irrigation*

Rainwater from a collection tank (6.37 pH) was used for irrigation in combination with precipitation. Although rainwater is known to contain trace ionic elements that play an important role in soil chemistry, these properties were not assessed for this study. Instead, the role of water in these experiments was neutralized by exclusive use of rainwater for irrigation purposes as exemplified by in situ soils.

*Low-molecular-weight Organic Acids*

Oxalic acid ($C_2H_2O_4$, 98%, anhydrous, Acros Organics; F.W. 90.04, ρ 1.9 g/mL) and citric acid ($C_6H_8O_7 \cdot H_2O$, 99 %, monohydrate, Fisher Chemical; F.W. 210.14, ρ 1.54 g/mL) were used at two concentrations (0.1 mM $L^{-1}$, 100 mM $L^{-1}$) for production of eggplant. Molar solutions for each LMWOA concentration was measured and diluted according to its formula weight (g). LMWOAs were tested for pH in solution; citric 0.1 mM $L^{-1}$ (3.4 pH), citric 100 mM $L^{-1}$ (1.9 pH), oxalic 0.1 mM $L^{-1}$ (5.5 pH), oxalic 100 mM $L^{-1}$ (1.2 pH).

*Pilot Study*

In order to determine the viability of each LMWOA for field production, a three-week greenhouse pilot study was conducted to measure the effectiveness of various LMWOA concentrations (0.1 mM $L^{-1}$, 1 mM $L^{-1}$, 10 mM $L^{-1}$, 100 mM $L^{-1}$) on each soil. Container (0.5 L) pots individually received an initial soil drench of the selected LMWOA concentration (mM $L^{-1}$); control pots received deionized water. Soil-to-volume applications of LMWOAs were determined by porosity (*n*) of each soil. The first week of the study allowed for LMWOA drench application to percolate to an estimated field capacity by gravity. Two consecutive rainwater drenches were applied to mimic natural precipitation in order to flush excess $Ca^{+2}$ from the soil. The first rainwater application was added on day (8) and the second rainwater application was added on day (12). A second LMWOA application was applied on day (16) of the study, once the soil reached estimated field capacity. A soil sample from each pot was collected and tested for P

nutrient availability (mg $L^{-1}$) everyday (24 hours) to monitor the effectiveness of each LMWOA concentration for each soil type over time.

Results from this study determined LMWOA concentrations in the pot production study and were based on their capacity to show a higher overall increase in P availability (mg $L^{-1}$). For HvB soil, oxalic acid 100 mM $L^{-1}$ demonstrated the most effective treatment response from continuous LMWOA application while citric acid 0.1 mM $L^{-1}$ treatment was most effective in TaB soil. These responses from both soils to various LMWOA treatments prompted the assignment of lowest and highest LMWOA treatment concentrations (mM $L^{-1}$) for field experimentation. Further, outcomes using LMWOA solutions (mM $L^{-1}$) showed effective results even though effects of P availability (mg $L^{-1}$) were most apparent at the onset of application for each soil type. Results indicated treatments were most effective within (24) hours after initial treatment, followed by decreasing to nonexistent P nutrient availability (mg $L^{-1}$) few days after initial treatment, excluding oxalic 100 mM $L^{-1}$ treatment in HvB soil. Subsequent rainwater (2x) applications, meant to leach excess $Ca^{+2}$, appeared to temporarily remobilize P (mg $L^{-1}$) for TaB soil but not for HvB. Additional soil treatments provided no significant improvements for available P (mg $L^{-1}$) except for HvB soil, whose overall response from oxalic 100 mM $L^{-1}$ was more significant than any other treatment. While TaB soil showed no dominant effects between treatments, citric 0.1 mM $L^{-1}$ averaged the highest P response rate (mg $L^{-1}$).

*Pot Production Study*

Eggplant plugs were transplanted to 19 L grow bags on April 24, 2014, nine weeks from the sow date. In order to develop uniform fruit and maximize yield, spacing was arranged 30 cm between each plant in the row and 60 cm between each row. A 1 m stake was tied to the stem after transplant for support. Soil response to LMWOA treatments was measured (mg $kg^{-1}$) at week (6), week (10) and week (14) after the transplant date. Eggplant response consisted of crop yield comparisons (g) between treatments, calibrated with control and compared to U.S. Standard Grades of Eggplant (USDA 1997) for quality. Growth and maturity rates were recorded by counting fruits and flowers throughout cultivation until harvest. First harvest occurred on June 29, 2014, nine weeks from transplant and subsequent harvests on week (11) and (13). Fruit was harvested by hand and cut above the calyx in order to keep the crown intact, followed by immediate weighing. From sow date through senescence, crop monitoring and maintenance was routinely performed for pest, pathogen and weed control. This included manual removal of intrusive weeds. Common insect problems in the field and greenhouse included aphids, flea beetles, leaf miners, spider mites and whitefly that were controlled through continuous foliar applications of Marathon® and Sevin® insecticides as needed. No other symptoms of pests or pathogens were observed throughout the study.

*Soil Analysis*

Soil tests for P were analyzed using a Palintest® Spectrophotometer 7100, a digital-readout colorimeter used to conduct an extensive range of chemical analyses that determines available P (mg $L^{-1}$) using the Olsen P method of extraction. Colorimetric

analysis has been used by scientists (Oburger et al. 2009; Zayed et al. 2005; Wang et al. 2008) to determine P content in various soil mediums. All soil samples taken from the field were sieved (2mm) after air-drying (23 °C) to constant moisture content in the laboratory (Coale 2000). The Palintest® Photometer required that P be extracted from soil using 0.5 M sodium bicarbonate ($NaHCO_3$) at a soil:water ratio of 1:25. The extracted P was then reacted with ammonium molybdate under reducing conditions in acidic solution to form a blue colored solution, where intensity of blue coloration was proportional to the P level (mg $L^{-1}$) in the soil sample. At the end of the harvest period, soil samples from each treatment were also tested with a pH meter using a 1:1 soil-water ratio in order to measure possible pH changes in bulk soil.

*Statistical Analysis*

The pot study was established as a 2x5 full factorial design, including two soils and five treatments. Response variables included P availability (mg $L^{-1}$) and fruit yield (g). MANOVA was used in IBM SPSS 22.0 software to determine mean differences and significance levels set at $p<0.05$. A complete randomized block design was used for plot layout similar to Lewis and Quirck (1967). The block was arranged by random assignment of treatment per row (10 rows) using a table of random numbers from Hoshmand (2006). An a priori analysis using G*Power 3.1 Software was used to determine initial sample size through statistical power, size of difference between treatment mean values detected, significance level of the test used and experimental error as suggested by Collins and Seeney (1999). Input parameters for a priori analysis

included a 0.3 effect size f, 0.05 α error probability and 0.8 power (1-β error probability) with ten groups, including controls. Sample size was set at fourteen plants per treatment.

# CHAPTER II-MANUSCRIPT

## Introduction

### *The Role of Phosphorus in Agriculture*

Phosphorus (P) is an essential element for all living organisms and plays a significant role in an array of processes including energy generation, nucleic acid synthesis, photosynthesis, glycolysis, respiration, membrane synthesis and stability, enzyme activation/inactivation, redox reactions, signaling, carbohydrate metabolism, and nitrogen (N) fixation (Vance et al 2003). It is an important constituent of DNA and in ATP for energy transfer within cells (Suh and Yee 2011). P is one of the three most important inorganic elements involved in the process of normal growth and metabolism for plants (Ragothama 1999). Less than optimum levels of P can lead to 5%-15% below maximum yields (Shenoy and Kalagudi 2005). Symptoms of P deficiency in plants may include stunted growth, foliage discoloration, delayed maturity, poor flowering, fruit yield, and seed development (Marschner 2002). Recommended rates of P fertilizer are especially high for vegetable production in order to avoid these deficiencies (Lorenz and Vittum 1980). The use of P fertilizers in agricultural production also aims to replace harvest and erosion losses of P from soil (Liu et al. 2008). While agricultural applications of P have played a significant role in providing sufficient harvest to meet global food demands in the past, industrial agriculture has simultaneously altered the P cycle by relying on mined phosphate rock (PR); a non-renewable fertilizer resource (Figure 1) (Cordell and White 2011). Before worldwide mining of PR as a common fertilizer source, P was naturally supplied to soils by recycling animal manure, crushed

animal bones, city waste and ash (Van Vuuren et al. 2010). Over the last half of the 20th Century, the Green Revolution abandoned these methods completely for PR-based fertilizers, only to generate the present-day P scarcity concerns (Cordell and White 2011; Cordell et al. 2009; Liu et al. 2008).

***Figure 1*. Overburden removal from PR mining in Florida, U.S. (Osorio 2014).**

Agricultural fertilizer uses on P-deficient soils that are also P-fixing render these applications inefficient (Simpson et al. 2011). When P fertilizer, in salt form [e.g. $Ca(PO_4)_2$], is added to most soils it quickly becomes immobile and, as such, very little of the added P is recovered with water (Plante 2007). On the other hand, most soils frequently have enough native P for crop production (Oburger et al. 2011; Richardson 2001). Mesic region soils with slightly acidic pH (6.5) have the most available P (Plante 2007). Meanwhile, arid region soils may have slightly acid to alkaline (pH) surfaces with $CaCO_3$ accumulation in upper horizons (Dregne 1976). In acidic soils, P forms low

solubility substances with aluminum ($Al^{+3}$) and iron ($Fe^{+3}$), while in alkaline soils it binds with calcium ($Ca^{+2}$) and magnesium ($Mg^{+2}$) to form insoluble P compounds (Bucio et al. 2000). Alkaline or calcareous soils are widespread in dry climates and the richness of free $CaCO_3$ tends to fix P as tricalcium phosphate [$Ca_3(PO_4)_2$] (Marschner 2002). The functions of limited precipitation and, hence, limited leaching of $Ca^{+2}$ in these drier regions are directly proportional to increased carbonate layers on the soil surface (Dregne 1976). At the same time, most soils contain low-molecular-weight organic acids (LMWOAs) that may be used by plants or microorganisms for nutrient acquisition (He et al. 2008). LMWOAs are COOH-containing compounds that allow the binding of metal cations in solution and the displacement of anions from the soil matrix (Jones 1998). These LMWOAs form strong complexes with cations like $Al^{+3}$, $Fe^{+3}$, or $Ca^{+2}$ and displace phosphate groups from binding sites (Ryan et al. 2001). It is in ion exchange processes that P becomes available for nutrient uptake by crops. The idea of acquiring native P from soils using LMWOAs is a method that merits further investigation as many recent studies have shown the potential of LMWOAs to have a correlative effect on P uptake by plants (Ström et al. 2002; Oberger et al. 2011; Vance et al. 2003).

Complicating P availability in soils is the ever-increasing global demand for fertilizers and the limitation of PR as a dependent resource (Lehr 1980). Global P fertilizer production has been estimated to decline after 2050 (Vance 2003). The mining of PR is an energy-intensive process concentrated in only certain parts of the world. According to a U.S.G.S. Mineral Commodity Summery by Jasinski (2012), PR resources occur mainly as sedimentary marine phosphates with the largest deposits found in northern Africa, China, the Middle East and the U.S, while igneous resources are found

in Brazil, Canada, Finland, Russia, and South Africa; totaling world resources of PR at ~300 billion metric tons (Gt). In the past, PR has been sold as a cheap bulk commodity (Van Kauwenbergh 2010). In recent history, the price of PR per metric ton (t) has ranged anywhere between \$70 $t^{-1}$ in October 1992 to \$192 $t^{-1}$ in October 2012 with a record spike of \$475 $t^{-1}$ in August 2008, adjusted for inflation (Index Mundi 2012). According to a report by Huang (2009), the price fluctuation of PR in the U.S. over the last decade has involved a combination of factors including U.S. fertilizer production capacity and production in decline, as well as its increasing dependence on global trade. P fertilizer use increased four to five-fold between 1960 and 2000 and is estimated to increase further by 20 million metric tons (Mt) per year by 2030 (Vance 2001). World mine production increased from 181 Mt in 2010 to 191 Mt in 2011 while in 2012 the U.S. alone mined an estimated 28.4 Mt of marketable product valued at 2.8 billion dollars, adjusted for inflation (Jasinski 2012).

According to Hinsinger et al. (2011), agricultural productivity needs sustainability, both from an ecological and economical perspective. As defined by Raman (2006), sustainable agriculture is one that enhances environmental quality and the resource base on which agriculture depends, while providing for basic human food and fiber needs, is economically viable, and enhances the quality of life for farmers and society as a whole. From this perspective, agriculture needs alternative options in addition to mined PR, which has limited absorption effects and a high potential for nutrient pollution. P fertilizer runoff from agriculture is, to a significant degree, one of the leading causes of eutrophication in aquatic systems (Cordell and White 2011). Impacts of eutrophication range from the decline of aquatic resources for marine

communities, degradation of water for human consumption, to the growth of algal blooms that can directly affect marine and human health (Kleinman et al. 2011). The increase of reactive P to the oceans from anthropogenic activities, mostly due to P fertilization, has been estimated at ~9 Mt $yr^{-1}$ (Rockström et al. 2009).

The fact that global P supply is quickly diminishing means that supply and demand will never be at equilibrium, making it even more difficult for subsistence farmers in third world countries to keep up with future prices. The potential social impact of reduced P availability on family farmers and developing nations is enough to continue research for secondary P sources since agriculture is the main source of livelihood for more than half the world's population (Laegreid et al. 1999). Annual global agricultural application of P is estimated to be ~400 Mt $yr^{-1}$ (FAO 2008). Due to the importance of P to agricultural production and global food security, it is necessary to address inefficiencies in P use and develop farming systems which aim to reduce P fertilizer inputs (Simpson et al. 2011).

*Phosphorus Availability and Acquisition by Plants in Soils*

Inorganic P is naturally present in soils and sourced from its main parent material, while organic forms of P are derived from the biological P cycle, mostly occurring as esters and orthophosphoric acid (Anderson 1980). Despite the sources of P in agricultural soils, many soils around the world are P-deficient with even the most fertile soils seldom exceeding 10 µM (Bieleski 1973). Concentration in plants is also low and ranges from 0.05% to 0.30% of total dry weight (Vance 2001). Consequently, P has the

lowest solubility and availability of major plant nutrients compared to other macro- and micronutrients in most soils (Ragothama 1999; Hinsinger 2001).

The distribution and nature of P in soils is primarily determined by pH, where phosphate ions are dissociated from orthophosphate into solution (Figure 2). Specifically, plants acquire P as orthophosphate anions, mainly as $HPO_4^{-2}$and $H_2PO_4^-$, from the soil solution (Richardson et al. 2009). At pH 7.22 the ratio of $HPO_4^{-2-}$ and $H_2PO_4^-$ ions are equal but anything below and $H_2PO_4^-$ becomes the main form of P adsorption (Barber 1980). As opposed to mass flow or root interception, diffusion is by far the main P acquisition method for plants while the concentration gradient is also highly influential in the process (Marschner 2002). Specifically, more than 90% of P acquired by plants reaches the roots via diffusion at a rate between $10^{-7}$ to $10^{-9}$ $cm^2$ $s^{-1}$ (Nord et al. 2011). Still, for most soils the diffusion rate of P is inadequate to overcome specific plant needs (Richardson et al. 2009).

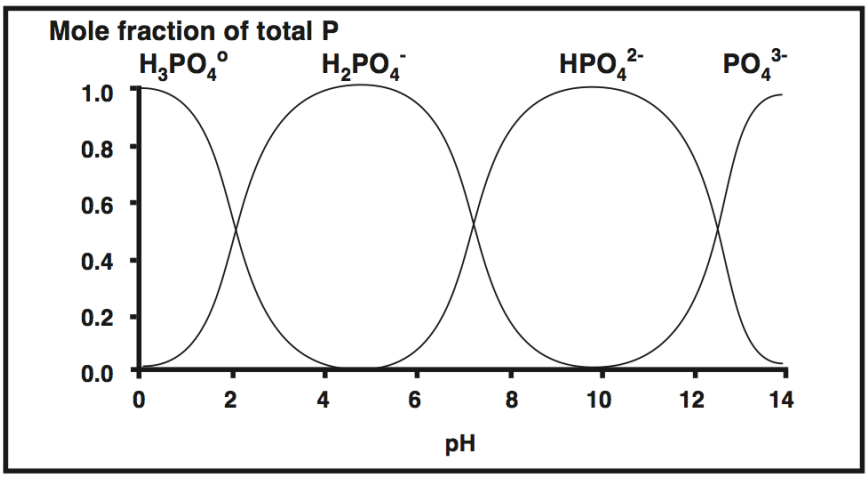


***Figure 2*. Orthophosphate ion availability as a function of pH (Hinsinger 2001).**

Low P availability is frequently cited as a major cause of low yields in crop production both for developed and developing countries (Vance et al 2003). In most soils, the concentration of available P in soil solution (2 μM) is exponentially lower than in plant tissues (5-20 mM) and mainly controlled by its interaction with organic or inorganic surfaces in the soil (Ragothama 1999). As stated, soils with high $Ca^{+2}$ content and high pH tend to fix native P and applied P in the form of $Ca_3(PO_4)_2$. Diverse forms of precipitated P for these soil types may include a range of mono- ($CaHPO_4$), di- and tri-Ca phosphates (e.g. $Ca(PO_4)_2$) and hydrates, hydroxyl ($Ca_5(PO_4)_3OH$, and fluorapatites ($Ca_5(PO_4)F$) (Richardson 2001).

In addition to low availability and low solubility problems involved in the matrix of soils, current fertilizer amendments have not yet solved the P dilemma solely through intensive application practices. Phosphate rock (PR) is not plant available when the pH of soil is greater than 5.5-6.0, and even when conditions are optimal plant yields are lower than those gained from soluble phosphates (Singh and Reddy 2011). Thus, PR is unsuitable in alkaline soils so farmers must rely on inorganic P fertilizer like diammonium phosphate [$(NH_4)_2HPO_4$] or triple superphosphate [$Ca(H_2PO_4)_2$], yet these fertilizers also become rapidly transformed into stable minerals such as $Ca_3(PO_4)_2$ which are relatively unavailable to crops (Asadi et al. 2010). To reduce fixation and increase adsorption, P fertilizer is placed as close to the root zone as possible by broadcasting and mixing with the surface plow layer or by banding near the row when the crop is planted (Barber 1980). Even under adequate P fertilization, only 20% or less is removed by the first year's growth (Vance 2001). Over time, up to 90% of applied P is adsorbed and remains fixed in the soil (Asmar et al. 2000). These applications result in P loading of

prime agricultural land that inevitably leads to increased nutrient pollution in stream flows through runoff (Vance et al 2003).

*Phosphorus Acquisition by Plants in the Rhizosphere*

In most agricultural soils P availability is greater in surface or near-surface horizons, where deposition from decay and plant residues persist, and conditions for P mobilization are more conductive due to several factors including organic matter content, microbial activity, and favorable pH (Lynch and Brown 2001). Compounds released from plant roots into the rhizosphere change the chemical and physical properties of soils, while stimulating the growth of various organisms based on the nature of exudates released, location on the root, and soil type (Gregory 2006). For the most part, research has focused on the significance of these interactions within the rhizosphere due to the sophisticated adaptability by plants to increase P availability from the surrounding environment using various mechanisms (Marschner 2011; Khademi et al. 2010; Richardson 2001; Hoffland 1992) (Table 1; Figure 3).

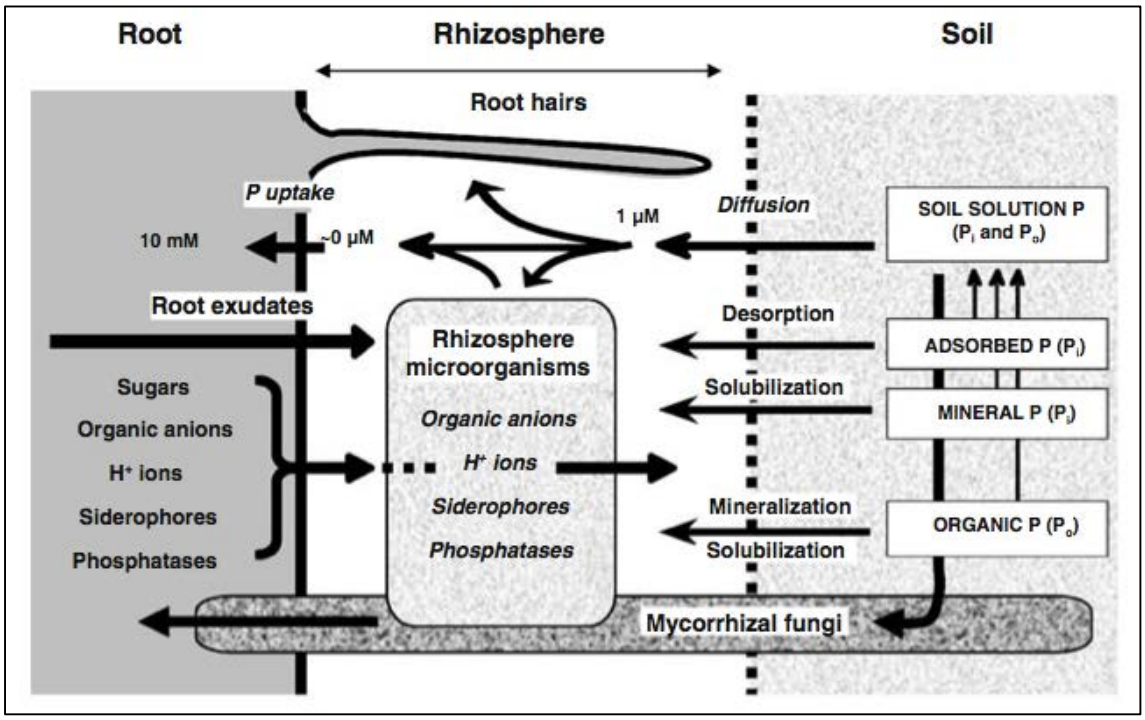


***Figure 3*. Influence of rhizosphere P for plant availability (Richardson 2009).**

***Table 1*. Various responses of plants to P deficiency (Raghothama 1999).**

| | |
|---|---|
| *Morphological* | Increased root: shoot ratio; changes in root morphology; increased root hair population and length; accumulation of anthocyanin pigments; proteoid root formation; increase in mycorrhizal association |
| *Physiological* | Enhanced P uptake; reduced P efflux; increased P use efficiency; P mobilization from vacuole to cytoplasm; increased translocation of P within plants; better P retention in roots; secretion of organic acids; protons and chelators; secretion of phosphatases and RNases; altered respiration; carbon metabolism; photosynthesis; nitrogen fixation; and aromatic enzyme pathways |
| *Biochemical* | Enzyme activation; enhanced production of phosphatases; RNases and organic acids; changes in protein phosphorylation; activation of glycolytic bypass pathway |
| *Molecular* | Gene activation (RNases, phosphatases, phosphate transporters, Ca-ATPase, vegetative storage proteins, β-glucosidase, PEPCase |

Crowley et al. (2011) define the rhizosphere as the location where microbial action releases and transforms fixed inorganic nutrients into organic forms through solubilization, chelation and oxidation/reduction. It is also here where plants exude LMWOAs to mobilize mineral nutrients directly or indirectly by delivering the energy for microbial action (Marschner 2002). These acids are part of metabolic functions at the cellular level in biochemical pathways including energy production, formation of precursors for amino-acid biosynthesis and at the whole plant level in modulating environmental adaptation (Bucio et al. 2000). The best known of the plant-produced LMWOAs are citric, succinic, malic, oxalic, and tartaric (Stevenson 1967). It has also been universally accepted that exudation of these LMWOAs may cause a significant mobilization of P in the rhizosphere (Godbold et al. 2002).

The link between LMWOA association and environment is strong for plants. Numerous dicotyledonous species have been found to release organic anions from their roots when P-deficient, which may continue throughout much of the plant's cycle (Ryan et al. 2001). These anions are mainly produced in mitochondria through tricarboxylic acid and glyoxylate cycles while biosynthesis, accumulation, transport and root exudation of LMWOAs is dramatically increased in response to environmental stress (Bucio et al. 2000). Plants are known to adapt several different mechanisms to increase their acquisition, uptake and adsorption efficiency by including root modifications, symbiotic relationships, and rhizospheric modifications in their search for P (Shenoy and Kalagudi 2005). Plants mobilize P by releasing $H^+$, $OH^-$, $CO_2$, LMWOA anions (e.g. citrate, malate, oxalate) or the release of various phosphatase enzymes (Crowley et al. 2011).

Römer and Keller (2001) exemplified the significance of LMWOAs with the reaction of spinach to secrete anions (oxalate, malate, citrate) in response to low P supply.

Depending on plant and soil factors, rhizosphere pH may differ from bulk soil pH by up to two units due to ion imbalances and LMWOA secretions (Marschner 2002). As such, pH alterations to the rhizosphere by LMWOA supplements has initiated research from their ability to mobilize P through root exudation processes (Dinkelaker et al. 1989; Hoffland 1992; Gardner 1983). Neumann and Romheld (1999) compared P-deficient plant roots of wheat (*Triticum aestivum L.*), tomato (*Lycopersicon esculentum L.*), chickpea (*Cicer arietinum*) and white lupin (*Lupinus albus L.*) to reveal a distinct correlation between the release of protons, LMWOAs and rhizosphere acidification, which ultimately determined the ability in these crops to uptake acid soluble $Ca_3(PO_4)_2$ in calcareous soils while maintaining stable pH. According to a review by Jones (1998), it appears that $H^+$ or $K^+$ and LMWOAs released by roots are likely biochemically separate but spatially coordinated events that accompany LMWOAs to maintain charge neutrality. Considering the overall cation-anion balance, this detail suggests that release of the LMWOA anion will, at least momentarily, acidify the rhizosphere (Hinsinger 2001). Gardner et al. (1983) analyzed the pH of leachates from a field study on release of citrate from *L. albus* and found minimal pH differences between plants and controls, suggesting that plant exudates are of neutral or slightly acid pH and that citrate, not citric acid, was secreted. Oburger et al. (2011) confirmed the efficiency of citrate on P solubilization and its effects based on pH-adjusted solution levels (1 M HCl or KOH) relative to natural soil conditions, with results indicating the more acidic citrate solutions helped to solubilize more P in all soils analyzed. According to Gillespie and Pope (1991), increasing P

availability through soil acidification mechanisms will decrease buffering capacity and lead to increased P diffusion rates and supply from the soil. Moreover, LMWOA action in solubilizing phosphates may be attributed to a lowering of the pH but more importantly to the formation of stable complexes with such cations as $Ca^{2+}$, $Mg^{2+}$, $Fe^{3+}$, and $Al^{3+}$ that facilitate P uptake for plants (Stevenson 1967).

Under nutrient deficiency, plant root exudates such as sugars, LMWOA anions and amino acids are released primarily in the zone immediately behind root tips and distal elongation zone (Crowley et al. 2011). The relationship between plant roots and microorganisms may be attributed to root exudates and is one of the most important factors affecting microbial growth in the rhizosphere (Adriano et al. 2005). Root exudation occurs in any soil horizon with root activity due to passive loss of compounds from roots or as active exudation of organic compounds from roots, including LMWOAs (Strobel 2001). The particular LMWOA that accumulates varies depending upon species, age of plant and tissue type (Bucio et al. 2000). According to Strobel (2001) plant species contain different amounts of LMWOAs in the roots, and thus vegetation might influence the soil solution concentrations of LMWOAs, as well as old and damaged root cells that leak with various LMWOAs.

Plants may decrease growth rate in order to conserve P when deficient, increase growth per unit of P uptake, remobilize internal P, modify C metabolism that bypass P-requiring steps, and take alternative respiratory pathways (Vance et al 2003). In response to P deficiency, various plants have developed proteoid roots that can release significant quantities of carboxylates like citrate, as shown by Gerke et al. (1994) in which they determined the release of citric and malic acid from the proteoid roots of *L. albus* at

various concentrations (0-55 μmol g soil$^{-1}$). These nerve-like root clusters are located on lateral roots and are able to strongly acidify the rhizosphere soil by releasing significant amounts of LMWOAs (e.g. citric, malic) (Schubert et al. 2005). The abundant development of lateral roots associated with P-deficiency alterations in root architecture is mostly accompanied by increased root hair density and length (Vance et al 2003). As a result, root architecture determines the exploration and exploitation of P resources by plants and is a valuable component in a competitive environment of higher mineral nutrition (Lynch and Brown 2001).

LMWOAs are involved in plant energy production as intermediates in the trycarboxylic (TCA) cycle (e.g. malate, citrate) while others are primarily present in cells for cation charge balancing or for maintaining osmotic potential (e.g. malate, malonate, oxalate) (Jones 1998). According to a review by Ragothama (1999) and Jones (1998) malic and citric acid are the ruling LMWOAs excreted by roots under P deficiency. In addition, different plant species may exude particular LMWOAs in order to mobilize different types of soil soluble P (e.g. Al-P) by adapting to specific soil conditions (Cao et al., 1997). Ström (1997) revealed that total concentration of LMWOAs (citric, oxalic) in two calcicole (lime tolerant) species were twice the concentrations found in the soil solution of the calcifuge (lime intolerant) species. Malate and citrate exuded by *Brassica napus L.* grown in $Ca_3(PO_4)_2$ soils acidified the rhizosphere or reduced the concentration of free $Ca^+$, which may also be a result of adaption to calcareous soils (Cao et al. 1997; Hoffland 1992).

*Low-molecular-weight Organic Acids in Soils by Means of Application*

Numerous LMWOAs have been investigated in soil P studies, including use of monocarboxylic (lactic, gluconic, acetic, formatic), dicarboxylic (oxalic, tartaric, malic, fumaric, malonic) and tricarboxylic (citric) acids (Richardson 2001). According to Jones (1998), the effectiveness of individual LMWOAs to mobilize soil P depends on the number of carboxyl groups they possess and follows the series of monocarboxylic, dicarboxylic, and tricarboxylic acid, where an increasing negative charge allows for compounding of metal cations in solution and displacement of anions from the soil, respectively. In calcareous soils, oxalate and citrate have been directly linked to P mobilization through $Ca^{+2}$ complexation and acidification mechanisms using distinct ionic forms of LMWOAs (Khademi et al. 2010). Here, they tested both oxalate (potassium oxalate; oxalic acid) and citrate (potassium citrate; citric acid) to grow wheat and found no differences between P accumulation in plants using distinct ionic forms of LMWOAs (1mM; 10 mM), although both oxalate forms proved better than citrates, while oxalate (oxalic acid) made P more available in calcareous soil (pH 7.88). Despite these results, citrate is able to increase the availability of P by lowering $Ca^{2+}$ concentrations through chelation and creation of soluble salts such as calcium citrate in calcareous soils (Marschner 2002). Jones (1998) explains that LMWOAs like citrate or malate are highly pH-dependent with minimal metal binding at high pH and where oxalate precipitates with $Ca^{2+}$ in soils against the negative charge of LMWOAs, which may quickly be adsorbed into the solid phase of the soil. These explanations indicate that LMWOA decomposition may also be soil dependent, where decreasing anionic binding sites are correlated with increased decomposition rates of LMWOAs according to the mineral phase of the soil

(Oburger et al. 2009). The influence of soils on LMWOAs is exemplified by the occurrence of higher oxalate concentrations found in forest soils around the world and their ability to increase Al-P solubility (Fox and Comerford 1992). Wei et al. (2010) also studied P mobilization in forest and tropical soils using malic, oxalic and citric acid concentrations (10 mM $kg^{-1}$) to find an increase of organic P using citric acid. Although few studies have solely used LMWOA concentrations in soil for P solubilization and uptake by plants for production purposes, current research has revealed that LMWOAs function in maximizing P availability for both temperate and arid soils.

The application of LMWOAs to many soils over the world has been repeatedly tested to prove these solutions significantly increase P mobilization (Wei et al. 2010; Gerke 1994; Khademi et al. 2010; Oburger et al. 2009; Wang et al 2008; Ström et al. 2001). Oburger et al. (2011) used LMWOA concentrations of 500 μM (2.5 mmol $kg^{-1}$) and confirmed a correlation between increased soil P availability and LMWOA additions, among other factors such as P loading and pH. Gerke (1994) investigated the addition of citrate to a Spodosol and Alfisol soil to compare the effects of single and sequential applications on P desorption, resulting in desorption increases up to a factor of twenty at the highest citrate loading levels (50 μmol $g^{-1}$) in both soils compared to controls. Fox and Comerford (1992) tested the influence of oxalate on P and Al solubilizaton in two Spodosols at single and sequential loading rates, with results indicating that P availability increased as the oxalate concentration (0.5-125 mM $kg^{-1}$) and loading rate increased for both single and sequential additions in the Bh horizon of both soils. Similarly, other research has focused on combining the extracting abilities of LMWOA with added P fertilizers (Kpomblekou-A and Tabatabai 2003; Lui et al. 2012; Comerford and Skinner

1989; Ström et al. 2001; Bolan et al. 1994). Liu et al. (2012) tested PR fertilizer along with specific concentrations of LMWOA amendments with results showing P released from PR increased according to increasing concentrations of LMWOAs (0.1 to 0.5 M $kg^{-1}$). Kpomblekou-A and Tabatabai (2003) used citric and oxalic acid concentrations of 10 mM $kg^{-1}$ in a greenhouse experiment with *Zea mays* to show a positive response in plant growth from both LMWOAs with varied rates and PR sources. Bolan et al. (1994) also used 10 mM $kg^{-1}$ LMWOAs concentrations (acetic, oxalic, citric) along with water-soluble $CaHPO_4$ and PR to examine the solubilization and plant uptake of P in *Lolium rigidium*, resulting in an increased effectiveness of fertilizers through higher yields using oxalic and citric acid.

Determining soil solution concentrations of P availability resulting from LMWOA experiments has been thoroughly investigated and derived from several techniques and procedures. In soil P testing the fraction of P (organic; inorganic) that is designated as available for plant uptake is derived from extraction procedures using dilute acids or bicarbonate solution, while the unattainable fraction is designated as fixed P (Plante 2007). Extractants such as sodium bicarbonate or water reflect the concentration in the soil solution, whereas strong extractants mostly indicate the buffer capacity of the soil to supply nutrients to the soil solution (Marschner 2002). The most common procedure for determining extractable P in soil investigations using LMWOAs (Comerford and Skinner 1989; Gerke 1992; Wei et al. 2010; Bolan et al. 1994; Ström et al. 2001) is that of Murphy and Riley (1962), in which soil extracts are measured using a single solution reagent containing an acidified solution of ammonium molybdate, ascorbic acid and antimony. Comerford and Skinner (1989) state this method primarily

extracts inorganic orthophosphates but may also include phosphate from some organic phosphates subject to acid or molybdate hydrolysis at low pH. Determining extractable P by the Olsen extraction method is also used by various `studies (Khademi et al. 2010; Wang et al. 2008), in which sodium bicarbonate ($NaHCO_3$) is employed to decrease the solution concentration of soluble $Ca^{2+}$ by precipitation as $CaCO_3$. According to Olsen et al. (1954), the Olsen P method can remove $Ca_3(PO_4)_2$ and phosphate adsorbed on surfaces of $CaCO_3$ and is considered the most suitable P test for alkaline or calcareous soils. An Olsen P value of 10 mg $kg^{-1}$ is generally considered suitable for normal plant growth (Sims 2000). Regardless of test method, extractable P has traditionally been defined by soil testing laboratories as the amount of P in soils available for crop uptake and to verify the probability of crop response to added P fertilizer (Pyrzynski and Sharpley 2000).

*The Role of Microorganisms in the Rhizosphere*

Continuous research indicates both plants and microorganisms facilitate P acquisition by increasing solubility of inorganic P through various biochemical processes (Ragothama 1999; Tyler et al.1995; Ström 1997; Zhang et al. 1997; Gahoonia et al. 2000; Zhu et al. 2005). Different LMWOAs have been studied for their abundance in the rhizosphere where root exudates and microorganisms work together to mobilize P by replenishing it with LMWOA anions and forming stable complexes with $Ca^{+2}$ to release P (Wei et al. 2010). Depending on their carboxylic arrangement, LMWOAs function within mono-, di-, and tricarboxylic acid groups (Strobel 2001). These soil solution LMWOAs originate from several biotic sources, including metabolites from decomposition of soil organic matter in upper horizons, and exudates from fungi or plant

roots (Strobel 2001). LMWOAs are a versatile source of C in soil and once released into soil solution are quickly broken down by the soil community (Oburger et al. 2009). Kpomblekou-A and Tabatabai (2003) show plant or microbial potential to exude ions or LMWOAs to facilitate P uptake for plants.

Microorganism activity in the rhizosphere can stimulate, restrain, or exist without effect on root growth, depending on the type of microorganism, plant species and environment (Marschner 2002). Despite the negative or neutral effects of microbial activity, a report by Richardson (2001) suggests that P solubilization by particular microorganisms is a major mechanism for improved plant growth, with production and release of LMWOAs by these microbes to be a key contributing element. LMWOAs may be quickened by release of organic C and $CO_2$ produced by roots and microorganisms (Marschner 2002). P-dissolving microorganisms may include bacteria, fungi, and actinomycetes (Molla et al. 1984). Mycorrhizal fungi can increase plant P uptake by extending hyphae into the surrounding soil volume to take in P and transfer it to the host plant (Marschner et al. 2011). Oxalic acid is produced by many strains of fungi, including brown-rots, white-rots, mycorrhizae, plant pathogens, and *Aspergillus niger* (Magnuson and Lasure 2004). Microorganisms in the rhizosphere are highly significant for decomposing, mineralizing organic material and transforming inorganic nutrients for plant acquisition, while further influencing nutrient availability by solubilization, chelation and oxidation/reduction (Marschner et al. 2011). Numerous studies (Kim et al. 1998; Molla et al. 1984; Cunningham and Kuiack 1992) have confirmed the P-solubilizing abilities of many different strains of microbes present in soil. A report by Singh and Reddy (2011) suggests that inoculation of *P. oxalicum*

significantly enhances the fertilizer value of PR especially in alkaline soils where the solubilization of PR alone is not as possible. Vassilev et al. (2006) applied *A. niger* and *Phanerochaete chrysosporium* to agro-industrial biowaste and determined solubilization of P present in PR from these microorganisms. Zayed et al. (2005) also inoculated strains of *A. niger* and *Trichoderma viride* to compost piles enriched with PR and correlated soluble P with these microorganisms. Further effects of PR enrichment with LMWOAs (citric and malic) and solubilization of P in wheat straw composting resulted in higher solubilization rates of P (Singh and Amberger 1998).

Although microbes are known to release a wide range of LMWOAs and contribute to P solubilization from PR, Jones (1998) notes that LMWOAs added at realistic concentrations (10-100 $\mu M\ kg^{-1}$) to mimic root exudation are just as quickly biodegraded by microorganisms in bulk soil with an average half-life of 2-3 hours depending on soil type. Oburger et al. (2009) tested five contrasting soils to show an initial rapid decomposition phase within the first (24) hours of application, measured through $^{14}CO_2$ release by microbes after the addition of LMWOAs. Ström et al. (2001) used *Z. mays* as a model plant in a calcareous soil and the application of LMWOAs (citric, malic and oxalic) to assess the mobilization rate of nutrients in the rhizosphere and bulk soil, with results showing that malate and citrate were quickly biodegraded by microbial action but oxalate was resistant. A follow-up study by Ström et al. (2002) measured LMWOA degradation in response to addition of LMWOA (1 and 10 $mM\ kg^{-1}$) concentrations and determined a significant degradation rate within (24) hours but a slower degradation for 10 mM concentrations of oxalate as compared to citrate. Gerke (1994) discusses in his research that one-week soil incubations, after single addition

citrate treatments, may cause some microbial degradation of the LMWOA and may account for the advantage of sequential loading at low citrate treatment levels (10-20 μmol $g^{-1}$). Van Hees et al. (2003) tested LMWOA concentrations (0-1000 μM $g^{-1}$) of citric, oxalic, and acetic acid in three coniferous forest soil profiles and found increased soil sorption of LMWOAs decreased biodegradation rates of LMWOAs, which mostly depended on soil horizon and LMWOA type; in this case oxalic acid most readily adsorbed. As a result of this evidence, competition between LMWOAs and microorganisms cannot be overstated and further demonstrates the intricacies involved within LMWOA application processes.

**Objectives**

Despite extensive research on P mobilization through use of LMWOAs in the rhizosphere, there is limited evidence showing supplemental applications of LMWOAs without added P fertilizers and the potential impact on crop production. The purpose of this study was to determine the potential of LMWOAs as a soil amendment to increase P availability for vegetable production purposes. The first objective was to determine the ability of two LMWOAs to mobilize P in two distinct soils native to the Central Texas Region. Second, the study aimed to determine the effects of directly applied LMWOAs on P availability for a high P-demanding crop. Eggplant (*Solanum melongena*) was utilized for crop production since it is a dependent P feeder. The effects of LMWOA-released P were measured by the quality and quantity of fruit produced as compared to a conventionally grown control.

## Justification

The need for a more stable P nutrient acquisition method is important to reduce the continuing depletion of global resources and to feed a quickly increasing population. Global population is expected to increase from 7 billion currently to 9 billion by year 2050 so there is reason for consideration of alternative methods in P fertilization due to increasingly limited global resources and infinite food necessities (Vance 2001). Phosphorus is one of three essential macronutrients required for plant growth and there are no substitutes for it in agriculture (Jasinski 2012). Although P availability is difficult to manage due to easy combination with soil cations that form low-solubility substances, LMWOAs can mobilize P and these acids are well-known products of plant and microorganism metabolism. Using LMWOA as a way to release bound P complexes directly in the soil matrix is a technique that needs further investigation since most LMWOA studies have only been performed in solution cultures (Jones 1998). In a world with decreasing supplies and increasing demands, this study may provide a sustainable alternative and contribute to the currently existing research on LMWOAs based on their potential to increase P uptake for plants.

## Materials and Methods

### *Soils*

Soils were collected in Hays County, Texas. The A1 horizon of a mollisol soil (15 cm deep) was collected from the edge of the Edwards Plateau (29.938465, -98.010644). The Hays County Soil Survey (USDA 1984) classifies this soil as a montmorillonitic, thermic Lithic Vertic Arguistoll from the Tarpley (TaB) series with

reddish brown clay to a depth of about 60 cm with acceptable drainage. These soils have weathered under $CaCO_3$ conditions and the abundance of $Ca^{+2}$ ions promotes P fixation (Carson 2000). Likewise, the Ap horizon of a vertisol soil was harvested (15 cm deep) from the Blackland Prairie region just east of the plateau (29.782137, -97.970763). This soil is listed as a fine, montmorillonitic, thermic Udic Pellusert from the Houston Black (HvB) series with very dark gray, gravelly clay to a depth of about 90 cm with moderate drainage (USDA 1984). These soils are also enriched with $Ca^{+2}$ and characterized by an abundance of swelling clays intimately bound to highly polymerized humus and by alternating wet and dry phases (Lozet and Mathieu 1991). Both soils were allowed to air dry and then screened for foreign materials (plant biomass, stones, insects, etc.) using a 4 mm sieve before transferring to grow bags for experiment (Figure 4). Prior to experiments, both soils were analyzed for nutrient availability, pH, EC, lime, and CEC (Figure 5; Figure 6).

***Figure 4.*** **Eggplant transplant bagged for production in TaB soil (Osorio 2014).**

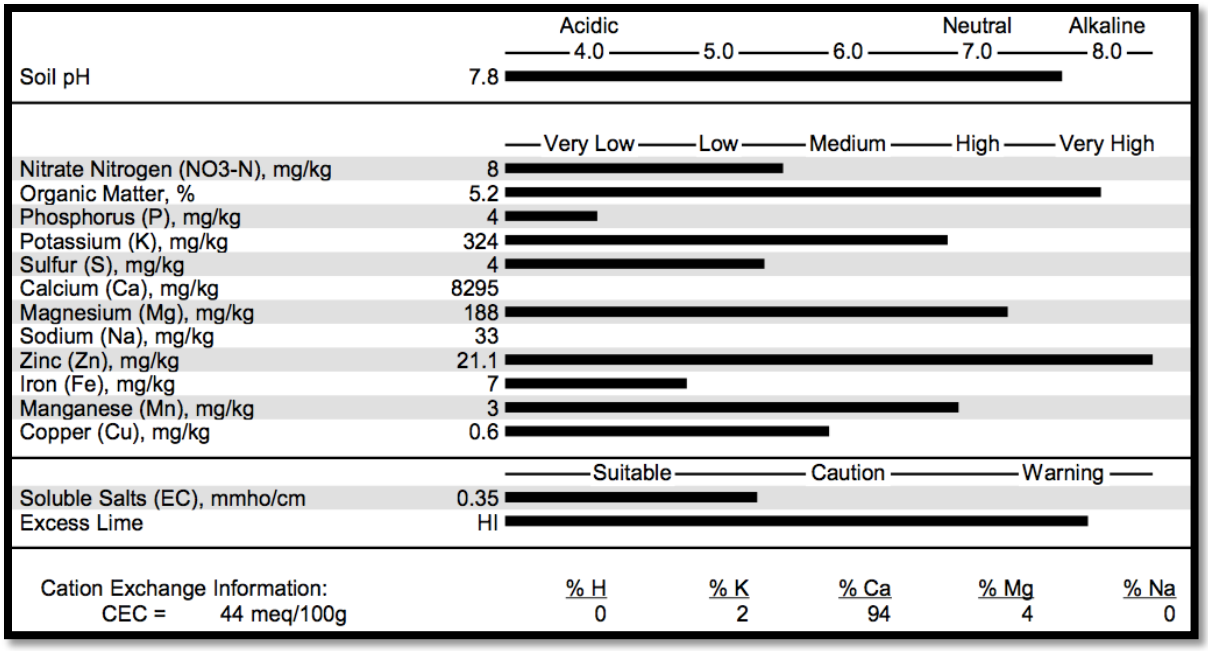


***Figure 5*. Houston Black (HvB) soil analysis (Servi-Tech Laboratories 2014).**

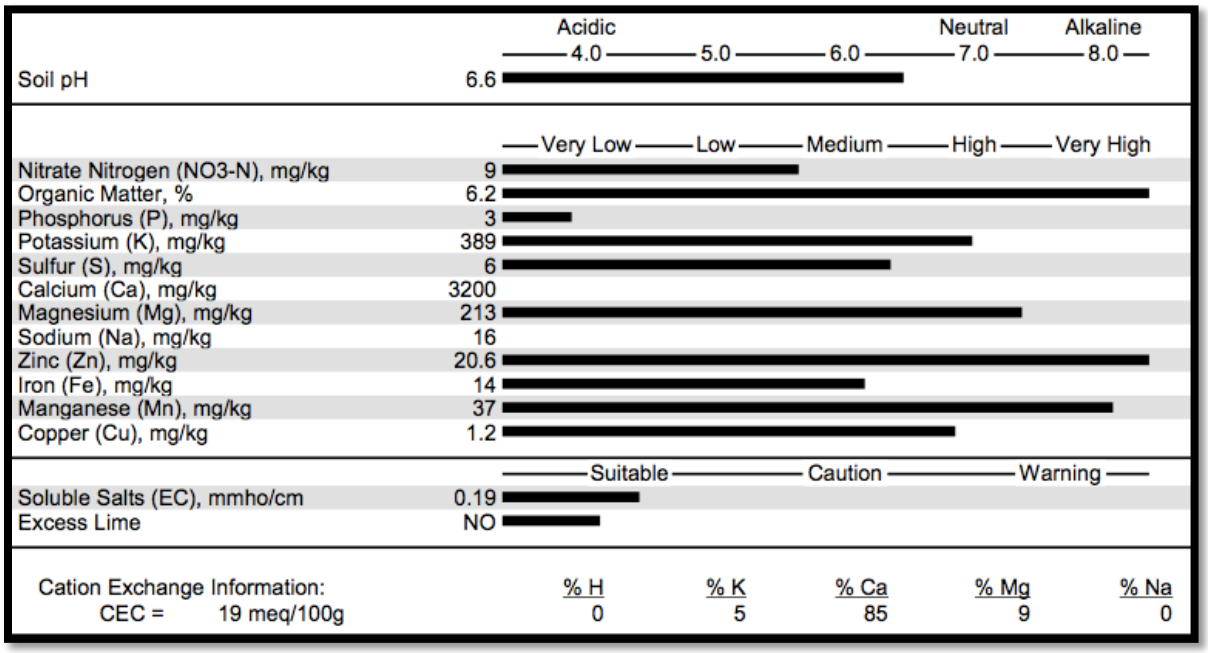


***Figure 6*. Tarpley (TaB) soil analysis (Servi-Tech Laboratories 2014).**

*Plants*

A black bell eggplant variety, Galine F1, from Johnny's Selected Seed Company was used for its high yielding uniformity. Eggplant is a traditional summer crop that grows best as a transplant and was chosen due to its relatively high P fertilizer demand (200 kg $ha^{-1}$) in commercial production. Seed was sown in the greenhouse on February 20, 2014 in 2 cm cell trays with a peat moss medium. Seedlings were fertilized once a week after true leaf emergence and throughout greenhouse propagation up to a week before transplant. Plants were managed for ten weeks in the greenhouse until optimum temperatures allowed for transplanting to 19 L grow bags similar to an eggplant study conducted by Nafiu et al. (2011). Plants were hardened-off to reduce transplanting shock by exposing to lower temperature, reduced watering and no fertilization as recommended by Maynard and Hochmuth (2007) and where transplanted on April 24, 2014.

*Fertilizer*

All seedlings were fertilized at a continuous rate of once per week in the greenhouse using $KNO_3$ (15-0-15) as a starter solution (188 ppm) and applied by bottom-watering method to maintain optimum growth before transplanting. After transplanting, each LMWOA group received an assigned treatment (citric 0.1 mM $L^{-1}$, citric 100 $L^{-1}$, oxalic 0.1 mM $L^{-1}$, oxalic 100 mM $L^{-1}$) as an alternative P fertilizer source. Granular fertilizer treatments were determined after soil analysis of each soil, which included fertilizer recommendations of 4.0 lbs. P/1000 $ft.^2$ for HvB controls and 4.2 lbs. P/1000 $ft^2$ for TaB controls using triple superphosphate [$Ca(H_2PO_4)_2$]. Meanwhile, all treatments (HvB and TaB) were equally treated at 1.6 lbs. N/1000 $ft.^2$ with urea [$CO(NH_2)_2$] as a

readily available N source. Granular fertilizer treatments were manually applied to grow bags and mixed thoroughly with the top 6-7 cm of bulk soil to simulate a broadcast top-dress application method. Along with soil test recommendations fertilizers were applied according to grade and timing of application. Fertilizer grade for $CO(NH_2)_2$ was 46% N and 46% P for $Ca(H_2PO_4)_2$. Application times were determined based on vegetative growth stage and yield response of eggplant. Due to the pot-contained production method, granular fertilizer applications were configured for each 19 L grow bag based on lab fertilizer recommendations as follows:

***HvB and TaB application [$CO(NH_2)_2$]***:

$$\frac{\mathbf{1.6\ N\ lbs.}}{\mathbf{1000\ sq.ft.}} \div \mathbf{46\%\ Urea} = \frac{\mathbf{3.478\ lb.N}}{\mathbf{1000\ sq.ft.}}$$

$$\frac{\mathbf{3.478\ lb.N}}{\mathbf{1000\ sq.ft.}} \times \frac{\mathbf{453.592\ g}}{\mathbf{lb.}} = \frac{\mathbf{1577.593\ g\ N}}{\mathbf{1000\ sq.ft.}} = \frac{\mathbf{1.578\ g\ N}}{\mathbf{1\ sq.ft.}}$$

$$\frac{\mathbf{1.578\ g\ N}}{\mathbf{1\ sq.ft.}} \times \frac{\mathbf{2\ sq.ft.}}{\mathbf{plant}} = \frac{\mathbf{3.156\ g\ N}}{\mathbf{plant}}$$

$$\frac{\mathbf{3.156\ g\ N}}{\mathbf{plant}} \times \mathbf{20\%/0\ weeks} = \frac{\mathbf{0.6312\ g\ N}}{\mathbf{plant}}$$

$$\frac{\mathbf{3.156\ g\ N}}{\mathbf{plant}} \times \mathbf{40\%/3\ weeks} = \frac{\mathbf{1.2624\ g\ N}}{\mathbf{plant}}$$

$$\frac{\mathbf{3.156\ g\ N}}{\mathbf{plant}} \times \mathbf{30\%/6\ weeks} = \frac{\mathbf{0.9468\ g\ N}}{\mathbf{plant}}$$

$$\frac{\mathbf{3.156\ g\ N}}{\mathbf{plant}} \times \mathbf{10\%/9\ weeks} = \frac{\mathbf{0.3156\ g\ N}}{\mathbf{plant}}$$

***HvB application [$Ca(H_2PO_4)_2$]:***

$$\frac{4\ P\ lbs.}{1000\ sq.ft.} \div 46\%\ Superphosphate = \frac{8.696\ lbs.P}{1000\ sq.ft.}$$

$$\frac{8.696\ lbs.P}{1000\ sq.ft.} \times \frac{453.592\ g}{lb.} = \frac{3944.436\ g\ P}{1000\ sq.ft.} = \frac{3.944\ g\ P}{1\ sq.ft.}$$

$$\frac{3.944\ g\ P}{1\ sq.ft.} \times \frac{2\ sq.ft.}{plant} = \frac{7.888\ g\ P}{plant}$$

$$\frac{7.888\ g\ P}{plant} \times\ 25\ \%/0\ weeks = \frac{1.972\ g\ P}{plant}$$

$$\frac{7.888\ g\ P}{plant} \times\ 50\ \%/4\ weeks = \frac{3.944\ g\ P}{plant}$$

$$\frac{7.888\ g\ P}{plant} \times\ 25\ \%/6\ weeks = \frac{1.972\ g\ P}{plant}$$

***TaB application [$Ca(H_2PO_4)_2$]:***

$$\frac{4.2\ P\ lbs.}{1000\ sq.ft.} \div 46\%\ Superphosphate = \frac{9.130\ lbs.P}{1000\ sq.ft.}$$

$$\frac{9.130\ lbs.P}{1000\ sq.ft.} \times \frac{453.592\ g}{lb.} = \frac{4141.295\ g\ P}{1000\ sq.ft.} = \frac{4.141\ g\ P}{1\ sq.ft.}$$

$$\frac{4.141\ g\ P}{1\ sq.ft.} \times \frac{2\ sq.ft.}{plant} = \frac{8.282\ g\ P}{plant}$$

$$\frac{8.282\ g\ P}{plant} \times\ 25\%/0\ weeks = \frac{2.0705\ g\ P}{plant}$$

$$\frac{8.282\ g\ P}{plant} \times\ 50\%/4\ weeks = \frac{4.141\ g\ P}{plant}$$

$$\frac{8.282\ g\ P}{plant} \times\ 25\%/6\ weeks = \frac{2.0705\ g\ P}{plant}$$

*Irrigation*

Rainwater from a collection tank (6.37 pH) was used for irrigation in combination with precipitation. Although rainwater is known to contain trace ionic elements that play an important role in soil chemistry, these properties were not assessed for this study. Instead, the role of water in these experiments was neutralized by exclusive use of rainwater for irrigation purposes as exemplified by in situ soils.

*Low-molecular-weight Organic Acids*

Oxalic acid ($C_2H_2O_4$, 98%, anhydrous, Acros Organics; F.W. 90.04, ρ 1.9 g/mL) and citric acid ($C_6H_8O_7 \cdot H_2O$, 99 %, monohydrate, Fisher Chemical; F.W. 210.14, ρ 1.54 g/mL) were used at two concentrations (0.1 mM $L^{-1}$, 100 mM $L^{-1}$) for production of eggplant. Molar solutions for each LMWOA concentration was measured and diluted according to its formula weight (g) (Figure 7). LMWOAs were tested for pH in solution; citric 0.1 mM $L^{-1}$ (3.4 pH), citric 100 mM $L^{-1}$ (1.9 pH), oxalic 0.1 mM $L^{-1}$ (5.5 pH), oxalic 100 mM $L^{-1}$ (1.2 pH).

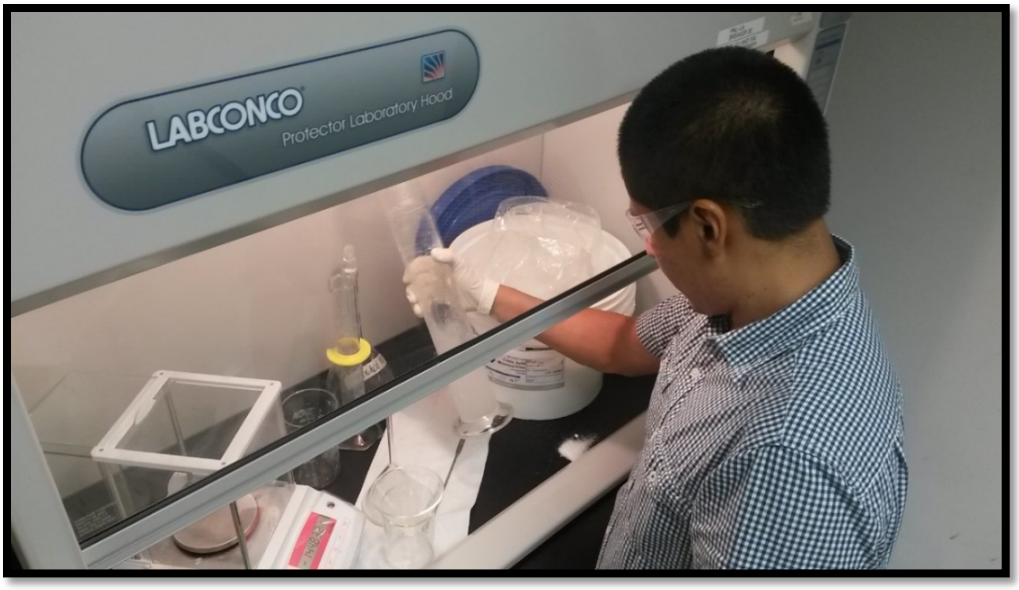


***Figure 7*. Mixing LMWOA solutions for treatment (Osorio 2014).**

*Pilot Study*

In order to determine the viability of each LMWOA for field production, a three-week greenhouse pilot study was conducted to measure the effectiveness of various LMWOA concentrations (0.1 mM $L^{-1}$, 1 mM $L^{-1}$, 10 mM $L^{-1}$, 100 mM $L^{-1}$) on each soil. Container (0.5 L) pots individually received an initial soil drench of the selected LMWOA concentration (mM $L^{-1}$); control pots received deionized water. Soil-to-volume applications of LMWOAs were determined by porosity (*n*) of each soil. The first week of the study allowed for LMWOA drench application to percolate to an estimated field capacity by gravity. Two consecutive rainwater drenches were applied to mimic natural precipitation in order to flush excess $Ca^{+2}$ from the soil. The first rainwater application was added on day (8) and the second rainwater application was added on day (12). A second LMWOA application was applied on day (16) of the study, once the soil reached estimated field capacity. A soil sample from each pot was collected and tested for P

nutrient availability (mg $L^{-1}$) everyday (24 hours) to monitor the effectiveness of each LMWOA concentration for each soil type over time (Figure 8).

***Figure 8*. Testing HvB soil using LMWOAs in greenhouse (Osorio 2014).**

Results from this study determined LMWOA concentrations in the pot production study and were based on their capacity to show a higher overall increase in P availability (mg $L^{-1}$) (Table 2; Table 3). For HvB soil, oxalic acid 100 mM $L^{-1}$ demonstrated the most effective treatment response from continuous LMWOA application while citric acid 0.1 mM $L^{-1}$ treatment was most effective in TaB soil (Table 4). These responses from both soils to various LMWOA treatments prompted the assignment of lowest and highest LMWOA treatment concentrations (mM $L^{-1}$) for field experimentation. Further, outcomes using LMWOA solutions (mM $L^{-1}$) showed effective results even though effects of P availability (mg $L^{-1}$) were most apparent at the onset of application for each

soil type. Results indicated treatments were most effective within (24) hours after initial treatment, followed by decreasing to nonexistent P nutrient availability (mg $L^{-1}$) few days after initial treatment, excluding oxalic 100 mM $L^{-1}$ treatment in HvB soil. Subsequent rainwater (2x) applications, meant to leach excess $Ca^{+2}$, appeared to temporarily remobilize P (mg $L^{-1}$) for TaB soil but not for HvB. Additional soil treatments provided no significant improvements for available P (mg $L^{-1}$) except for HvB soil, whose overall response from oxalic 100 mM $L^{-1}$ was more significant than any other treatment. While TaB soil showed no dominant effects between treatments, citric 0.1 mM $L^{-1}$ averaged the highest P response rate (mg $L^{-1}$).

***Table 2*. Summary of three-week pilot study for HvB soil including consecutive LMWOA treatments and rainwater applications. Phosphate (P) test results (mg L-[1]) based on sample size of 5 plants per treatment.**

| | citric 0.1mM | citric 1mM | citric 10mM | citric 100mM | oxalic 0.1mM | oxalic 1mM | oxalic 10mM | oxalic 100mM | control |
|---|---|---|---|---|---|---|---|---|---|
| 10-Mar | 1st LMWOA application | | | | | | | | |
| 11-Mar | 6 | 6 | 6 | 5 | 4 | 3 | 2 | 5 | 5 |
| 12-Mar | 3 | 4 | 2 | 1 | 1 | 1 | 5 | 5 | 1 |
| 13-Mar | 4 | 0 | 2 | 0 | 3 | 1 | 3 | 4 | 3 |
| 14-Mar | 1 | 5 | 1 | 2 | 2 | 3 | 1 | 4 | 2 |
| 15-Mar | 1 | 0 | 0 | 0 | 0 | 1 | 1 | 2 | 1 |
| 16-Mar | 3 | 0 | 0 | 0 | 1 | 1 | 1 | 3 | 2 |
| 17-Mar | 1 | 1 | 0 | 1 | 2 | 2 | 1 | 3 | 1 |
| 18-Mar | 1st rainwater application | | | | | | | | |
| 19-Mar | 2 | 3 | 1 | 1 | 3 | 3 | 3 | 6 | 2 |
| 20-Mar | 0 | 0 | 1 | 0 | 1 | 3 | 3 | 1 | 1 |
| 21-Mar | 2 | 1 | 0 | 0 | 1 | 1 | 3 | 3 | 3 |
| 22-Mar | 2nd rainwater application | | | | | | | | |
| 23-Mar | 3 | 1 | 1 | 0 | 2 | 0 | 0 | 1 | 0 |
| 24-Mar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 25-Mar | 0 | 1 | 0 | 0 | 1 | 3 | 0 | 0 | 0 |
| 26-Mar | 2nd LMWOA application | | | | | | | | |
| 27-Mar | 1 | 1 | 6 | 0 | 0 | 0 | 0 | 9 | 1 |
| 28-Mar | 1 | 1 | 2 | 1 | 1 | 0 | 0 | 9 | 4 |
| 29-Mar | 3 | 1 | 0 | 0 | 1 | 1 | 1 | 6 | 1 |
| 30-Mar | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 6 | 0 |
| 31-Mar | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 |
| 1-Apr | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 |
| 2-Apr | 1 | 1 | 2 | 0 | 1 | 2 | 1 | 9 | 2 |

***Table 3*. Summary of three-week pilot study for TaB soil including consecutive LMWOA treatments and rainwater applications. Phosphate (P) test results (mg $L^{-1}$) based on sample size of 5 plants per treatment.**

| | citric 0.1mM | citric 1mM | citric 10mM | citric 100mM | oxalic 0.1mM | oxalic 1mM | oxalic 10mM | oxalic 100mM | control |
|---|---|---|---|---|---|---|---|---|---|
| 10-Mar | 1st LMWOA application | | | | | | | | |
| 11-Mar | 10 | 8 | 6 | 5 | 6 | 7 | 12 | 12 | 4 |
| 12-Mar | 2 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 4 |
| 13-Mar | 3 | 4 | 1 | 3 | 3 | 2 | 2 | 3 | 7 |
| 14-Mar | 3 | 2 | 1 | 1 | 3 | 1 | 1 | 0 | 3 |
| 15-Mar | 1 | 2 | 0 | 1 | 2 | 1 | 2 | 1 | 1 |
| 16-Mar | 4 | 5 | 5 | 2 | 5 | 3 | 6 | 2 | 3 |
| 17-Mar | 1 | 3 | 2 | 1 | 2 | 1 | 2 | 1 | 3 |
| 18-Mar | 1st rainwater application | | | | | | | | |
| 19-Mar | 0 | 0 | 0 | 0 | 1 | 1 | 6 | 0 | 2 |
| 20-Mar | 3 | 3 | 4 | 3 | 3 | 3 | 4 | 2 | 4 |
| 21-Mar | 0 | 0 | 0 | 0 | 1 | 0 | 2 | 2 | 2 |
| 22-Mar | 2nd rainwater application | | | | | | | | |
| 23-Mar | 4 | 2 | 2 | 1 | 1 | 1 | 2 | 1 | 1 |
| 24-Mar | 5 | 3 | 4 | 5 | 0 | 2 | 3 | 2 | 1 |
| 25-Mar | 1 | 3 | 1 | 1 | 4 | 3 | 2 | 2 | 1 |
| 26-Mar | 2nd LMWOA application | | | | | | | | |
| 27-Mar | 3 | 1 | 2 | 1 | 3 | 3 | 1 | 1 | 1 |
| 28-Mar | 3 | 2 | 1 | 1 | 0 | 1 | 1 | 1 | 2 |
| 29-Mar | 7 | 2 | 1 | 0 | 2 | 2 | 1 | 1 | 4 |
| 30-Mar | 1 | 2 | 0 | 0 | 2 | 1 | 1 | 0 | 1 |
| 31-Mar | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 |
| 1-Apr | 2 | 3 | 0 | 0 | 0 | 0 | 2 | 1 | 1 |
| 2-Apr | 2 | 3 | 0 | 0 | 2 | 1 | 1 | 1 | 1 |

***Table 4*. Pilot study descriptive statistics for soil phosphate (P) test results (mg $L^{-1}$) in two soils. Greater results were seen from oxalic 100 mM $L^{-1}$ in HvB soil and citric 0.1 mM $L^{-1}$ in TaB soil. Based on sample size of 5 plants per treatment.**

| | citric 0.1mM | citric 1mM | citric 10mM | citric 100mM | oxalic 0.1mM | oxalic 1mM | oxalic 10mM | oxalic 100mM | control |
|---|---|---|---|---|---|---|---|---|---|
| Houston Black | | | | | | | | | |
| Sum | 32 | 26 | 24 | 11 | 24 | 24 | 25 | 85 | 29 |
| Minimum | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Maximum | 6 | 6 | 6 | 5 | 4 | 3 | 5 | 9 | 5 |
| Mean | 1.6 | 1.3 | 1.2 | 0.6 | 1.2 | 1.3 | 1.3 | 4.3 | 1.5 |
| Standard Deviation | 1.6 | 1.7 | 1.8 | 1.2 | 1.1 | 1.1 | 1.4 | 2.8 | 1.4 |
| Tarpley | | | | | | | | | |
| Sum | 56 | 49 | 31 | 25 | 42 | 35 | 52 | 34 | 47 |
| Minimum | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1 |
| Maximum | 10 | 8 | 6 | 5 | 6 | 7 | 12 | 12 | 7 |
| Mean | 2.8 | 2.5 | 2.6 | 1.7 | 2.1 | 1.8 | 2.6 | 1.7 | 2.4 |
| Standard Deviation | 2.4 | 1.8 | 1.8 | 1.5 | 1.6 | 1.5 | 2.7 | 2.5 | 1.6 |

*Pot Production Study*

Eggplant plugs were transplanted to 19 L grow bags on April 24, 2014, nine weeks from the sow date. In order to develop uniform fruit and maximize yield, spacing was arranged 30 cm between each plant in the row and 60 cm between each row. A 1 m stake was tied to the stem after transplant for support. Soil response to LMWOA treatments was measured (mg $kg^{-1}$) at week (6), week (10) and week (14) after the transplant date. Eggplant response consisted of crop yield comparisons (g) between treatments, calibrated with control and compared to U.S. Standard Grades of Eggplant (USDA 1997) for quality. Growth and maturity rates were recorded by counting fruits and flowers throughout cultivation until harvest. First harvest occurred on June 29, 2014,

nine weeks from transplant and subsequent harvests on week (11) and (13). Fruit was harvested by hand and cut above the calyx in order to keep the crown intact, followed by immediate weighing (Figure 9). From sow date through senescence, crop monitoring and maintenance was routinely performed for pest, pathogen and weed control. This included manual removal of intrusive weeds. Common insect problems in the field and greenhouse included aphids, flea beetles, leaf miners, spider mites and whitefly that were controlled through continuous foliar applications of Marathon® and Sevin® insecticides as needed. No other symptoms of pests or pathogens were observed throughout the study.

***Figure 9.*** **Harvesting eggplant after treatments (Osorio 2014).**

*Soil Analysis*

Soil tests for P were analyzed using a Palintest® Spectrophotometer 7100, a digital-readout colorimeter used to conduct an extensive range of chemical analyses that determines available P (mg $L^{-1}$) using the Olsen P method of extraction. Colorimetric analysis has been used by scientists (Oburger et al. 2009; Zayed et al. 2005; Wang et al.

2008) to determine P content in various soil mediums. All soil samples taken from the field were sieved (2mm) after air-drying (23 °C) to constant moisture content in the laboratory (Coale 2000). The Palintest® Photometer required that P be extracted from soil using 0.5 M sodium bicarbonate ($NaHCO_3$) at a soil:water ratio of 1:25. The extracted P was then reacted with ammonium molybdate under reducing conditions in acidic solution to form a blue colored solution, where intensity of blue coloration was proportional to the P level (mg $L^{-1}$) in the soil sample. At the end of the harvest period, soil samples from each treatment were also tested with a pH meter using a 1:1 soil-water ratio in order to measure possible pH changes in bulk soil.

*Statistical Analysis*

The pot study was established as a 2x5 full factorial design, including two soils and five treatments. Response variables included P availability (mg $L^{-1}$) and fruit yield (g). MANOVA was used in IBM SPSS 22.0 software to determine mean differences and significance levels set at $p<0.05$. A complete randomized block design was used for plot layout similar to Lewis and Quirck (1967). The block was arranged by random assignment of treatment per row (10 rows) using a table of random numbers from Hoshmand (2006). An a priori analysis using G*Power 3.1 Software was used to determine initial sample size through statistical power, size of difference between treatment mean values detected, significance level of the test used and experimental error as suggested by Collins and Seeney (1999). Input parameters for a priori analysis included a 0.3 effect size f, 0.05 α error probability and 0.8 power (1-β error probability) with ten groups, including controls. Sample size was set at fourteen plants per treatment.

**Results**

Production of eggplant in the field was conducted from late April to early August 2014. Flower development and production occurred from late May until the end of June. For flowering, HvB soil demonstrated a greater affinity for oxalic acid (0.1 mM $L^{-1}$, 100 mM $L^{-1}$) treatments while TaB soil preferred lower concentrations of LMWOA treatments (citric 0.1 mM $L^{-1}$, oxalic 0.1 mM $L^{-1}$) (Table 5; Table 6; Table 7). Flower production significantly increased five weeks after transplant and decreased for all groups at nine weeks, just before harvest (Figure 10; Figure 12). By the end of bloom stage, a sum total of each treatment showed oxalic 100 $mM^{-1}$ as most productive (140 blooms) for HvB soil and oxalic 0.1 $mM^{-1}$ as most productive (161 blooms) in TaB soil (Figure 11; Figure 13). For fruit yield, all treatments resulted in overall smaller average fruit size compared to USDA Grade Standards for eggplant production (data not presented here). Nevertheless, fruit harvest totals (g) at the end of three harvest periods for HvB soil showed citric acid 100 mM $L^{-1}$ as most effective for yield, while TSP treatment yielded best for TaB soil (Table 8; Table 9). Bar graphs representing individual harvests (I-III) for each treatment in TaB and HvB soils further show differences in yield (g) between soil types and treatments throughout study (Figure 14; Figure 16; Figure 18). Soil P-test sums (mg $L^{-1}$) of each treatment revealed that TSP treatment provided the most P nutrient availability for both soils (Table 10; Table 11). Furthermore, bar graphs of each soil P-test (I-III) in TaB and HvB soils also demonstrated differences in P availability between soil types and treatments (Figure 15; Figure 17; Figure 19).

***Table 5*. Flower production descriptive statistics show plants in HvB soil to produce more flowers using oxalic acid (0.1, 100 mM $L^{-1}$) while for TaB soil lower concentrations of both LMWOAs produced more flowers (0.1 mM $L^{-1}$). Flower count (n) based on sample size of 14 plants per treatment.**

| | citric 0.1mM | citric 100 mM | oxalic 0.1 mM | oxalic 100 mM | TSP |
|---|---|---|---|---|---|
| Houston Black | | | | | |
| Sum | 117 | 125 | 136 | 140 | 126 |
| Minimum | 2 | 0 | 2 | 3 | 1 |
| Maximum | 27 | 24 | 27 | 19 | 22 |
| Mean | 10.6 | 11.4 | 12.4 | 12.7 | 11.5 |
| Standard Deviation | 7.5 | 7.2 | 8.3 | 5.9 | 5.9 |
| Tarpley | | | | | |
| Sum | 157 | 101 | 161 | 57 | 150 |
| Minimum | 2 | 1 | 1 | 0 | 2 |
| Maximum | 43 | 34 | 43 | 21 | 32 |
| Mean | 14.3 | 9.2 | 14.6 | 5.2 | 13.6 |
| Standard Deviation | 12.2 | 10.1 | 11.5 | 5.9 | 9.8 |

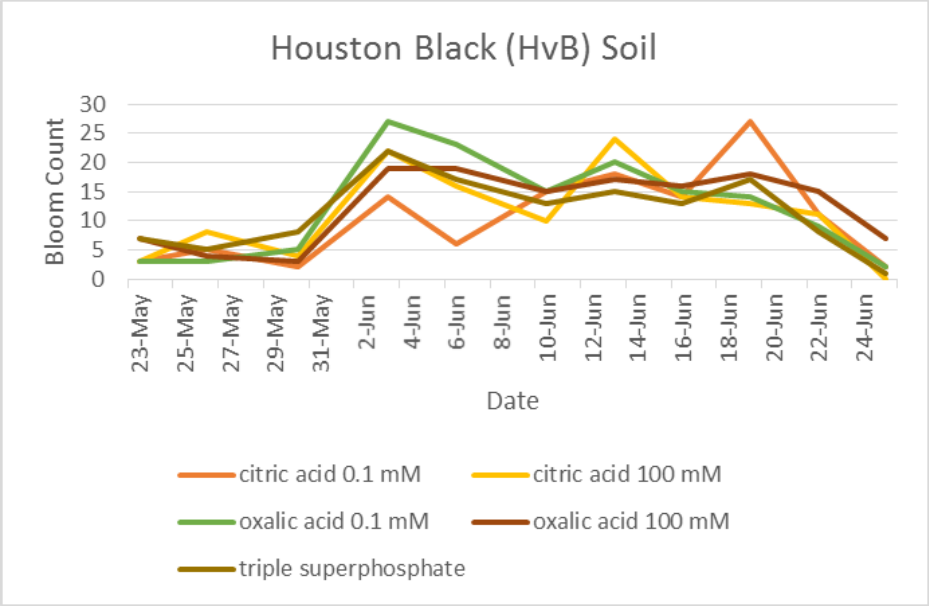


***Figure 10*. Graph of flower development over time for HvB soil and each treatment. Flower count (n) based on sample size of 14 plants per treatment.**

***Table 6*. Summary of flower development over time for HvB soil and each treatment. Flower count (n) based on sample size of 14 plants per treatment.**

| | citric 0.1 mM | citric 100 mM | oxalic 0.1 mM | oxalic 100 mM | TSP |
|---|---|---|---|---|---|
| 23-May | 3 | 3 | 3 | 7 | 7 |
| 26-May | 5 | 8 | 3 | 4 | 5 |
| 30-May | 2 | 4 | 5 | 3 | 8 |
| 3-Jun | 14 | 22 | 27 | 19 | 22 |
| 6-Jun | 6 | 16 | 23 | 19 | 17 |
| 10-Jun | 15 | 10 | 15 | 15 | 13 |
| 13-Jun | 18 | 24 | 20 | 17 | 15 |
| 16-Jun | 14 | 14 | 15 | 16 | 13 |
| 19-Jun | 27 | 13 | 14 | 18 | 17 |
| 22-Jun | 11 | 11 | 9 | 15 | 8 |
| 25-Jun | 2 | 0 | 2 | 7 | 1 |

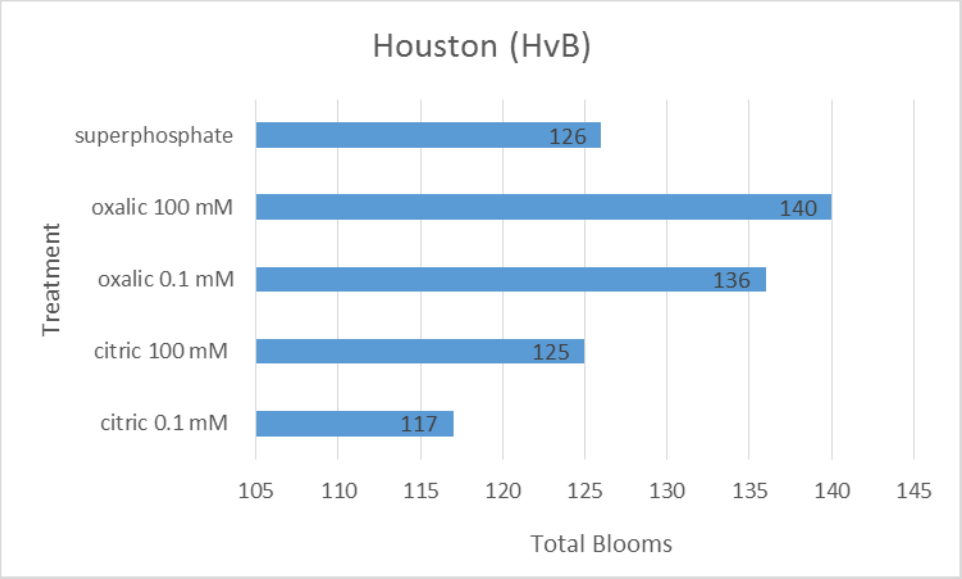


***Figure 11*. Graph of flower development for HVB soil and each treatment showing oxalic 100 $mM^{-1}$ treatment to produce most blooms (n). Based on sample size of 14 plants per treatment.**

***Table 7*. Summary of flower development over time for TaB soil and each treatment. Flower count (n) based on sample size of 14 plants per treatment.**

| | citric 0.1 mM | citric 100 mM | oxalic 0.1 mM | oxalic 100 mM | TSP |
|---|---|---|---|---|---|
| 23-May | 3 | 1 | 3 | 0 | 10 |
| 26-May | 3 | 3 | 1 | 1 | 4 |
| 30-May | 2 | 2 | 2 | 1 | 2 |
| 3-Jun | 9 | 3 | 17 | 6 | 18 |
| 6-Jun | 9 | 11 | 14 | 1 | 15 |
| 10-Jun | 22 | 4 | 14 | 2 | 32 |
| 13-Jun | 14 | 5 | 21 | 3 | 27 |
| 16-Jun | 20 | 23 | 19 | 7 | 9 |
| 19-Jun | 43 | 34 | 43 | 21 | 23 |
| 22-Jun | 27 | 13 | 21 | 11 | 8 |
| 25-Jun | 5 | 2 | 6 | 4 | 2 |

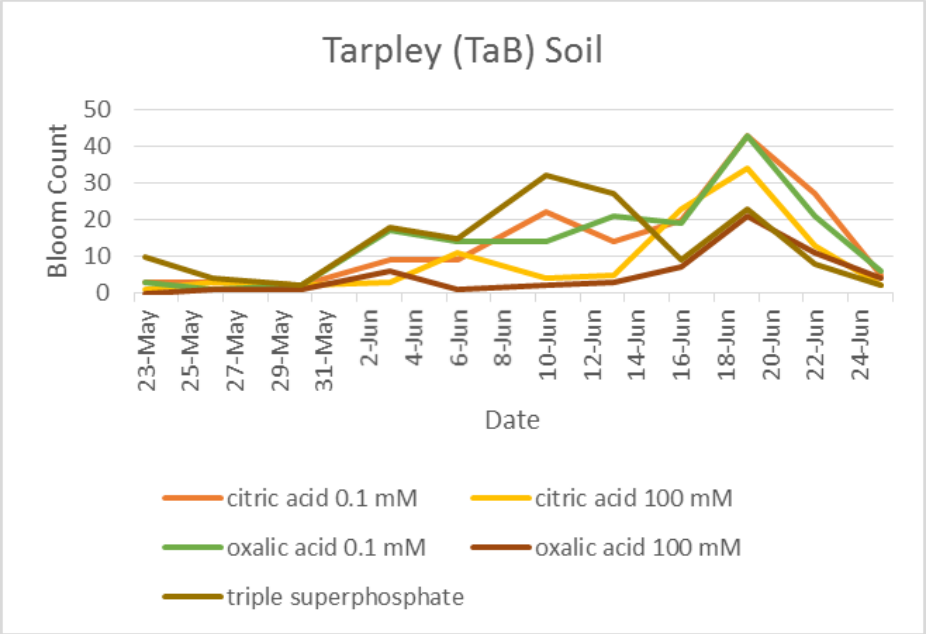


***Figure 12.*** **Graph of flower development over time for TaB soil and each treatment. Flower count (n) based on sample size of 14 plants per treatment.**

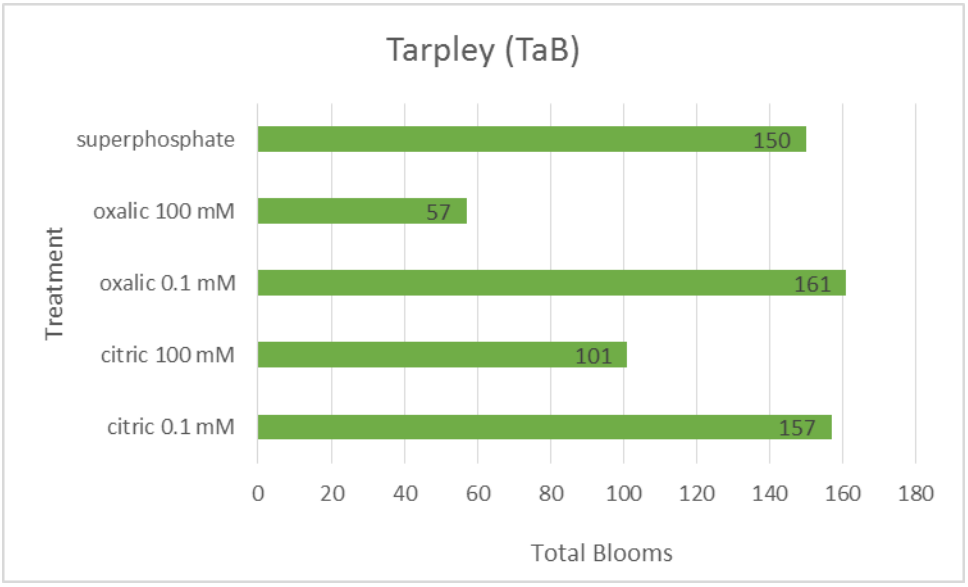


***Figure 13.*** **Graph of flower development for TaB soil and each treatment showing oxalic 0.1 $mM^{-1}$ treatment to produce most blooms (n). Based on sample size of 14 plants per treatment.**

***Table 8*. Yield descriptive statistics for HvB soil over time show citric acid 100 mM $L^{-1}$ to produce highest total yield. Harvest (g) based on sample size of 14 plants per treatment.**

| | citric 0.1mM | citric 100mM | oxalic 0.1mM | oxalic 100mM | TSP |
|---|---|---|---|---|---|
| | Harvest I (g) | | | | |
| Sum | 6098.6 | 6693.7 | 6174.8 | 6820.2 | 6069.1 |
| Minimum | 131.4 | 229.9 | 323.7 | 251 | 175.7 |
| Maximum | 657.6 | 679 | 645.1 | 679.9 | 719.9 |
| Mean | 435.6 | 478.1 | 441.1 | 487.2 | 433.5 |
| Standard Deviation | 150.4 | 132.9 | 105.3 | 134.4 | 133.5 |
| | Harvest II (g) | | | | |
| Sum | 2718.6 | 2774.3 | 2628.9 | 3136.3 | 2818.8 |
| Minimum | 0 | 75.1 | 0 | 0 | 0 |
| Maximum | 358 | 313 | 277.6 | 405.5 | 419.8 |
| Mean | 194.2 | 198.2 | 187.8 | 224.0 | 201.3 |
| Standard Deviation | 121.9 | 77.5 | 76.4 | 114.4 | 137.5 |
| | Harvest III (g) | | | | |
| Sum | 2426.3 | 2131.3 | 1813.5 | 1192.4 | 1637.9 |
| Minimum | 58.3 | 0 | 0 | 0 | 0 |
| Maximum | 323 | 307.5 | 339.9 | 399.1 | 292.7 |
| Mean | 173.3 | 152.2 | 129.5 | 85.2 | 117.0 |
| Standard Deviation | 81.4 | 103.9 | 114.9 | 123.4 | 88.0 |
| | Harvest Total (g) | | | | |
| Sum | 11243.5 | 11599.3 | 10617.2 | 11148.9 | 10525.8 |
| Minimum | 0 | 0 | 0 | 0 | 0 |
| Maximum | 657.6 | 679 | 645.1 | 679.9 | 719.9 |
| Mean | 267.7 | 276.2 | 252.8 | 265.5 | 250.6 |
| Standard Deviation | 168.8 | 179.4 | 168.2 | 207.8 | 180.2 |

***Table 9*. Yield descriptive statistics for TaB soil over time show TSP treatment to produce highest total yield. Harvest (g) based on sample size of 14 plants per treatment.**

| | citric 0.1mM | citric 100mM | oxalic 0.1mM | oxalic 100mM | TSP |
|---|---|---|---|---|---|
| | Harvest I (g) | | | | |
| Sum | 3560.2 | 2749.6 | 3326.1 | 1311.8 | 5822.4 |
| Minimum | 0 | 121.5 | 134.4 | 0 | 135.8 |
| Maximum | 408.6 | 302.5 | 357 | 296.7 | 673.1 |
| Mean | 254.3 | 196.4 | 237.6 | 93.7 | 415.9 |
| Standard Deviation | 115.2 | 54.9 | 81.9 | 116.1 | 150.0 |
| | Harvest II (g) | | | | |
| Sum | 2379.7 | 2217.2 | 2162.5 | 1795.9 | 3692.4 |
| Minimum | 76.7 | 0 | 88.9 | 0 | 0 |
| Maximum | 521.2 | 305.1 | 252.5 | 381.8 | 445.6 |
| Mean | 170.0 | 158.4 | 154.5 | 128.3 | 263.7 |
| Standard Deviation | 112.7 | 82.5 | 57.1 | 127.1 | 111.2 |
| | Harvest III (g) | | | | |
| Sum | 2819.6 | 2248.5 | 3150.3 | 1614.7 | 1627.7 |
| Minimum | 62.1 | 0 | 121.6 | 0 | 0 |
| Maximum | 328.1 | 279.8 | 323.3 | 247 | 314.9 |
| Mean | 201.4 | 160.6 | 225.0 | 115.3 | 116.3 |
| Standard Deviation | 67.6 | 70.0 | 59.2 | 89.3 | 107.1 |
| | Harvest Total (g) | | | | |
| Sum | 8759.5 | 7215.3 | 8638.9 | 4722.4 | 11142.5 |
| Minimum | 0 | 0 | 88.9 | 0 | 0 |
| Maximum | 521.2 | 305.1 | 357 | 381.8 | 673.1 |
| Mean | 208.6 | 171.8 | 205.7 | 112.4 | 265.3 |
| Standard Deviation | 104.5 | 70.6 | 75.1 | 110.1 | 173.3 |

***Table 10*. Descriptive statistics for HvB soil phosphate (P) tests over time show TSP treatment to provide most available P (mg $L^{-1}$). Based on sample size of 14 plants per treatment.**

| | citric 0.1mM | citric 100mM | oxalic 0.1mM | oxalic 100mM | TSP |
|---|---|---|---|---|---|
| | (P) Test I (mg/L) | | | | |
| Sum | 38 | 71 | 63 | 77 | 90 |
| Minimum | 2 | 4 | 2 | 4 | 3 |
| Maximum | 4 | 7 | 6 | 7 | 7 |
| Mean | 2.7 | 5.3 | 4.6 | 5.6 | 6.4 |
| Standard Deviation | 0.9 | 1.0 | 1.1 | 1.1 | 1.2 |
| | (P) Test II (mg/L) | | | | |
| Sum | 61 | 69 | 68 | 70 | 68 |
| Minimum | 4 | 3 | 3 | 3 | 2 |
| Maximum | 5 | 7 | 6 | 7 | 7 |
| Mean | 4.4 | 4.8 | 4.9 | 5.0 | 4.9 |
| Standard Deviation | 0.5 | 1.4 | 0.9 | 1.2 | 1.4 |
| | (P) Test III (mg/L) | | | | |
| Sum | 56 | 52 | 43 | 54 | 73 |
| Minimum | 3 | 2 | 2 | 3 | 3 |
| Maximum | 5 | 5 | 4 | 4 | 7 |
| Mean | 4.0 | 3.7 | 3.2 | 4.0 | 5.2 |
| Standard Deviation | 0.5 | 0.7 | 0.6 | 0.5 | 1.7 |
| | (P) Test Total (mg/L) | | | | |
| Sum | 155 | 192 | 174 | 201 | 231 |
| Minimum | 2 | 2 | 2 | 3 | 2 |
| Maximum | 5 | 7 | 6 | 7 | 7 |
| Mean | 3.7 | 4.6 | 4.1 | 4.8 | 5.5 |
| Standard Deviation | 1 | 1.3 | 1.2 | 1.2 | 1.6 |

***Table 11.*** **Descriptive statistics for TaB soil phosphate (P) tests over time show TSP treatment to provide most available P (mg $L^{-1}$). Based on sample size of 14 plants per treatment.**

| | citric 0.1mM | citric 100mM | oxalic 0.1mM | oxalic 100mM | TSP |
|---|---|---|---|---|---|
| | (P) Test I (mg/L) | | | | |
| Sum | 2 | 0 | 14 | 4 | 36 |
| Minimum | 0 | 0 | 0 | 0 | 0 |
| Maximum | 1 | 0 | 2 | 4 | 7 |
| Mean | 0.2 | 0.0 | 1.1 | 0.3 | 2.6 |
| Standard Deviation | 0.4 | 0.0 | 0.6 | 1.0 | 2.6 |
| | (P) Test II (mg/L) | | | | |
| Sum | 9 | 6 | 9 | 10 | 58 |
| Minimum | 0 | 0 | 0 | 0 | 1 |
| Maximum | 2 | 1 | 1 | 2 | 7 |
| Mean | 0.6 | 0.4 | 0.7 | 0.7 | 4.2 |
| Standard Deviation | 0.7 | 0.5 | 0.6 | 0.6 | 2.4 |
| | (P) Test III (mg/L) | | | | |
| Sum | 1 | 0 | 5 | 3 | 54 |
| Minimum | 0 | 0 | 0 | 0 | 0 |
| Maximum | 1 | 0 | 2 | 2 | 7 |
| Mean | 0.1 | 0.0 | 0.4 | 0.3 | 4.0 |
| Standard Deviation | 0.3 | 0.0 | 0.7 | 0.7 | 2.4 |
| | (P) Test Total (mg/L) | | | | |
| Sum | 12 | 6 | 28 | 17 | 148 |
| Minimum | 0 | 0 | 0 | 0 | 0 |
| Maximum | 2 | 1 | 2 | 4 | 7 |
| Mean | 0.3 | 0.1 | 0.7 | 0.4 | 3.5 |
| Standard Deviation | 0.6 | 0.4 | 0.7 | 0.8 | 2.5 |

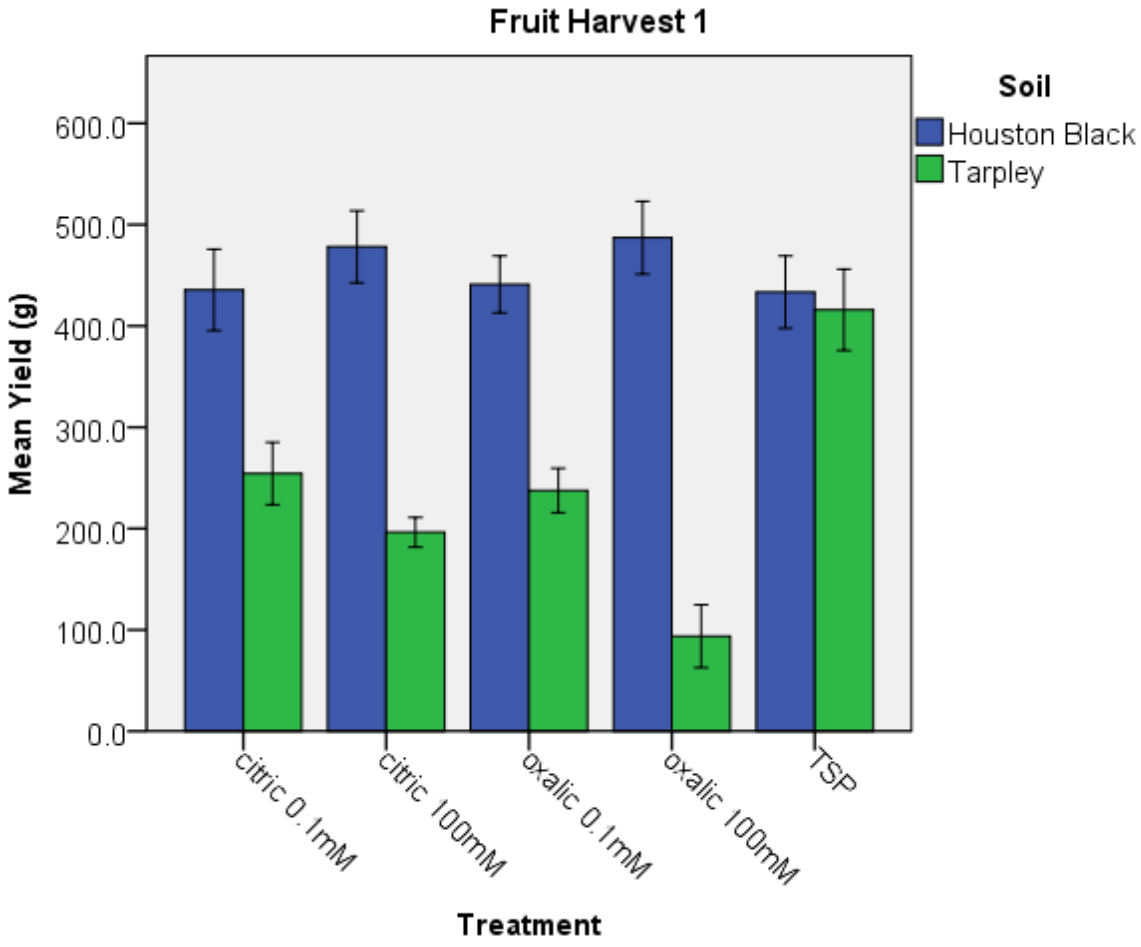


***Figure 14*. Harvest I graph for each treatment in TaB and HvB soils (+/- 1 Standard Error); plotted from table 8 and table 9 data. Mean yield (g) based on sample size of 14 plants per treatment.**

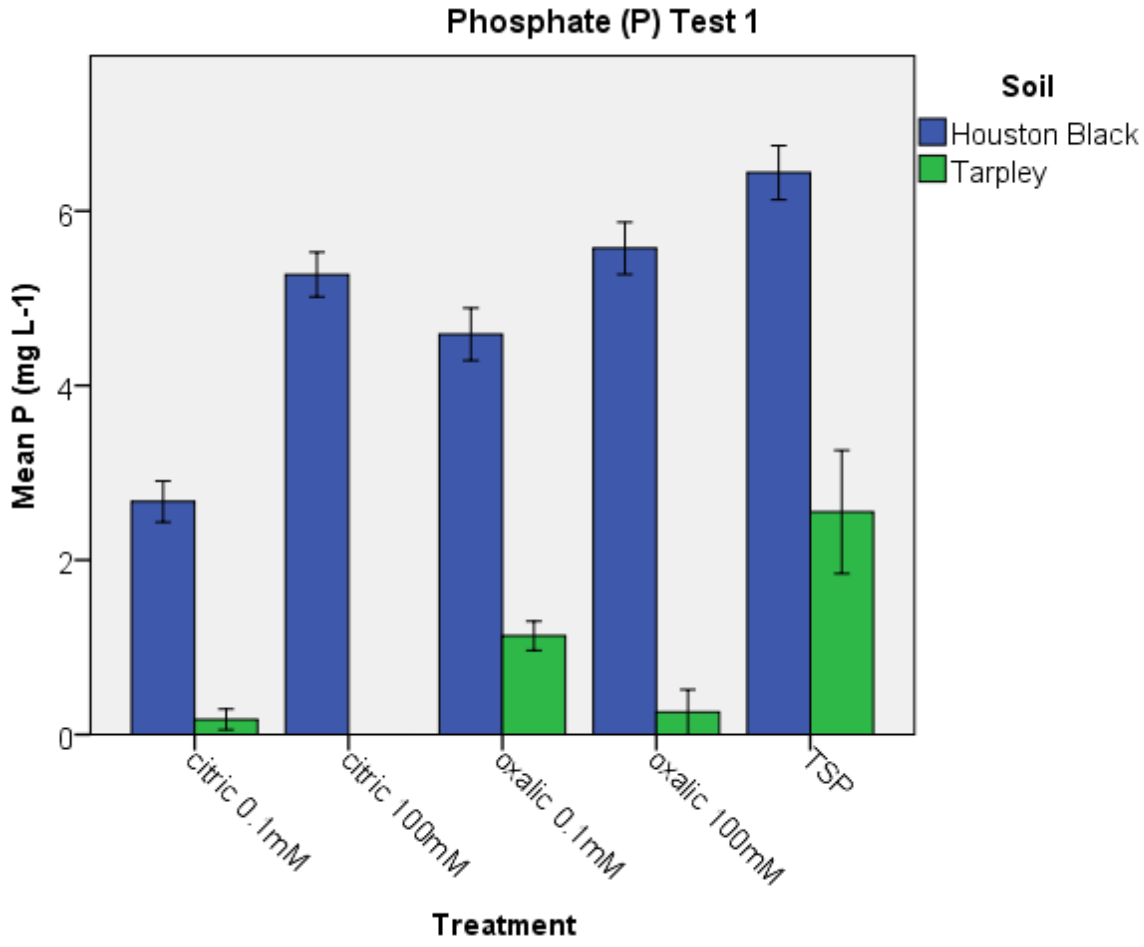


***Figure 15*. Phosphate (P) Test I graph for TaB and HvB soils (+/- 1 Standard Error); plotted from table 10 and table 11 data. Mean P (mg $L^{-1}$) based on sample size of 14 plants per treatment.**

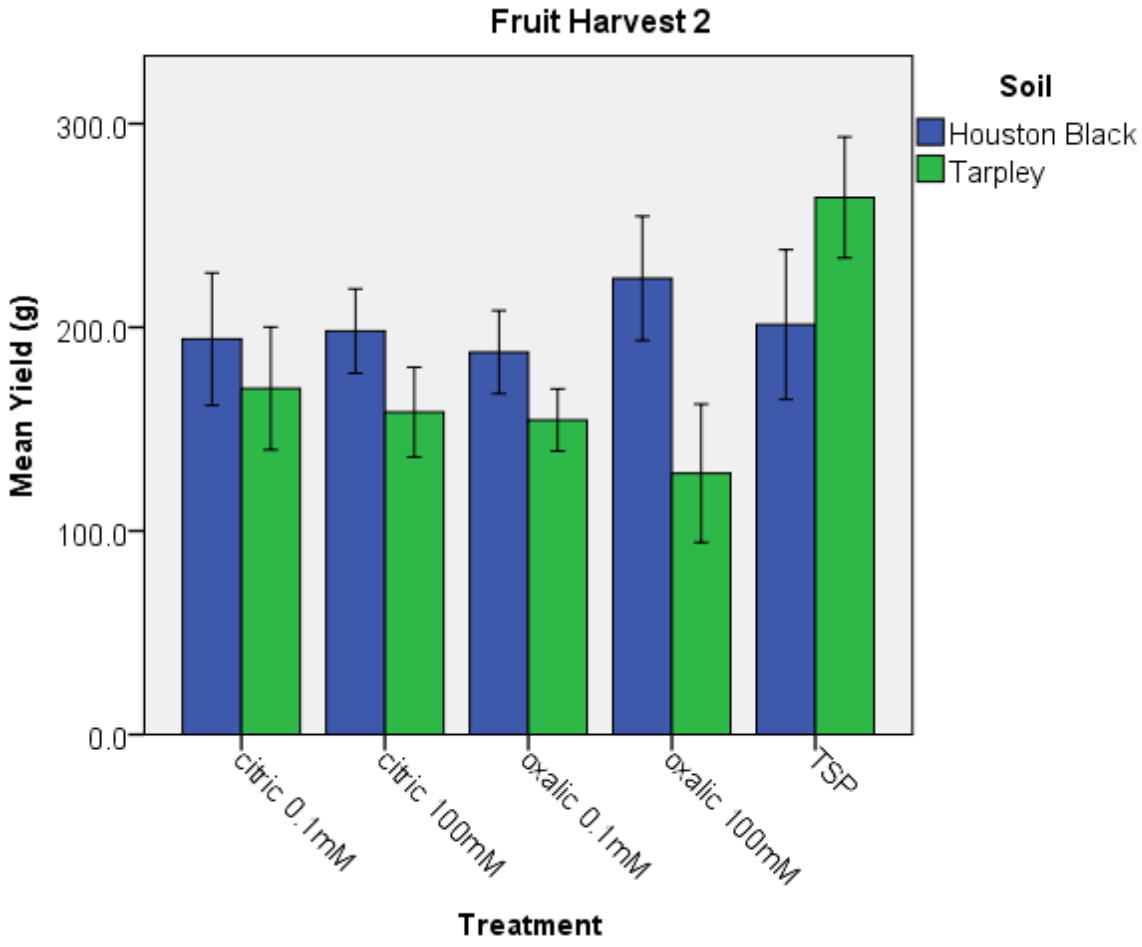


***Figure 16.*** **Harvest II graph for TaB and HvB soils (+/- 1 Standard Error); plotted from table 8 and table 9 data. Mean yield (g) based on sample size of 14 plants per treatment.**

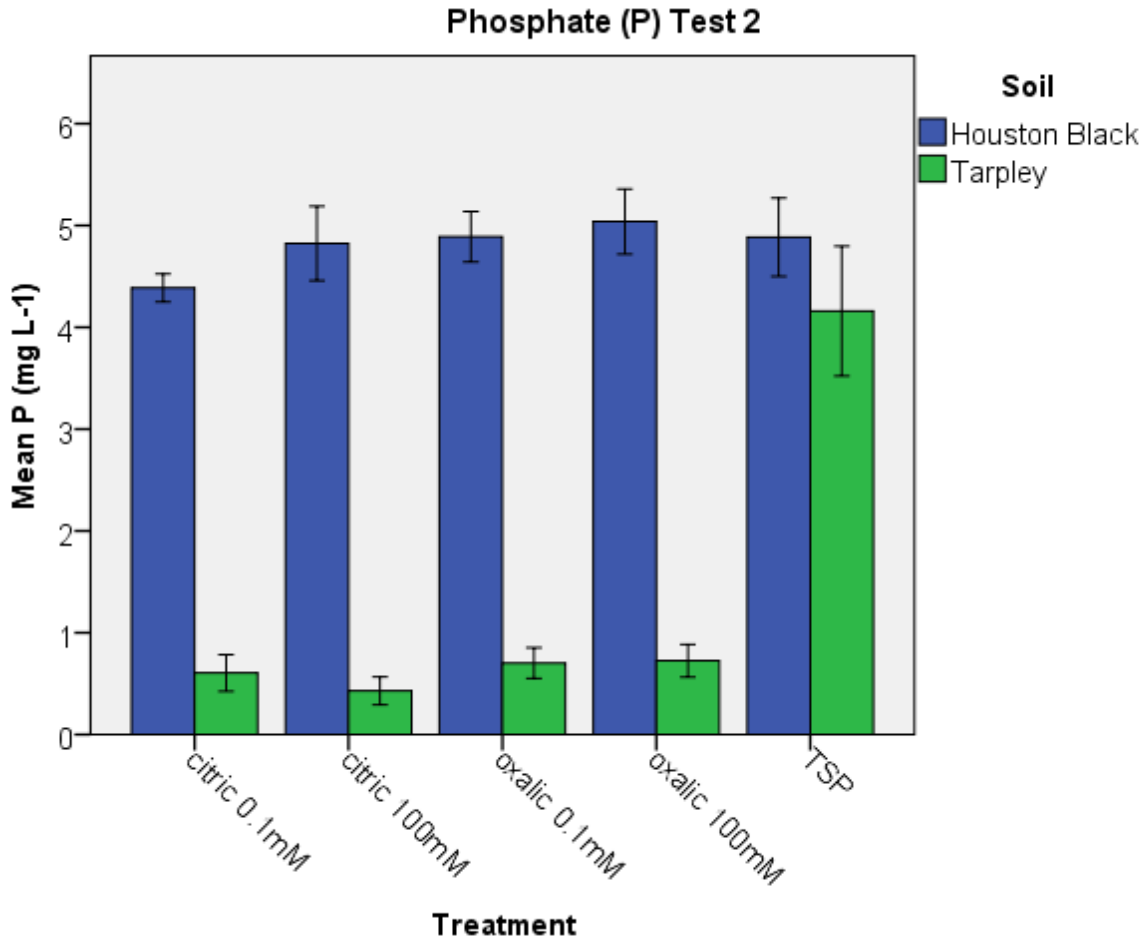


***Figure 17.*** **Phosphate (P) Test II graph for TaB and HvB soils (+/- 1 Standard Error); plotted from table 10 and table 11 data. Mean P (mg $L^{-1}$) based on sample size of 14 plants per treatment.**

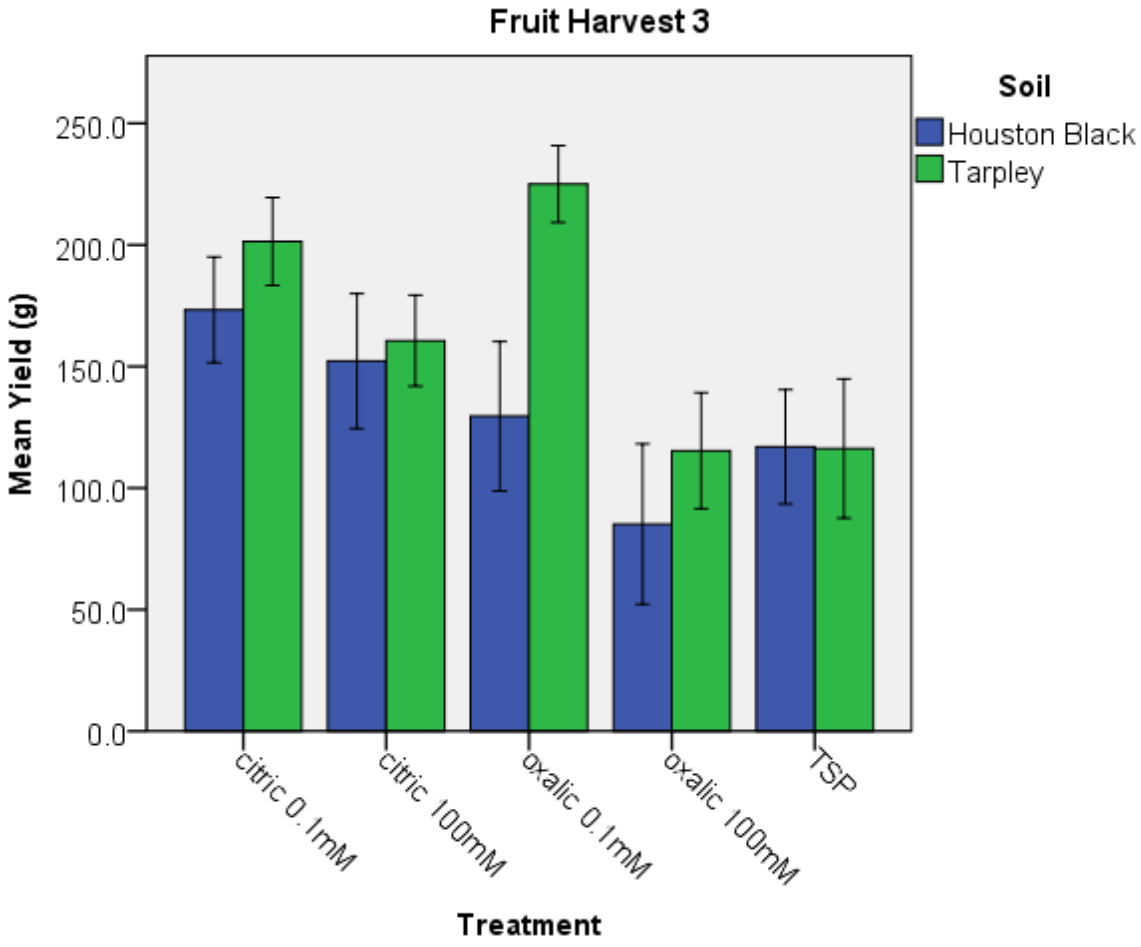


***Figure 18*. Harvest III graph for TaB and HvB soils (+/- 1 Standard Error); plotted from table 8 and table 9 data. Mean yield (g) based on sample size of 14 plants per treatment.**

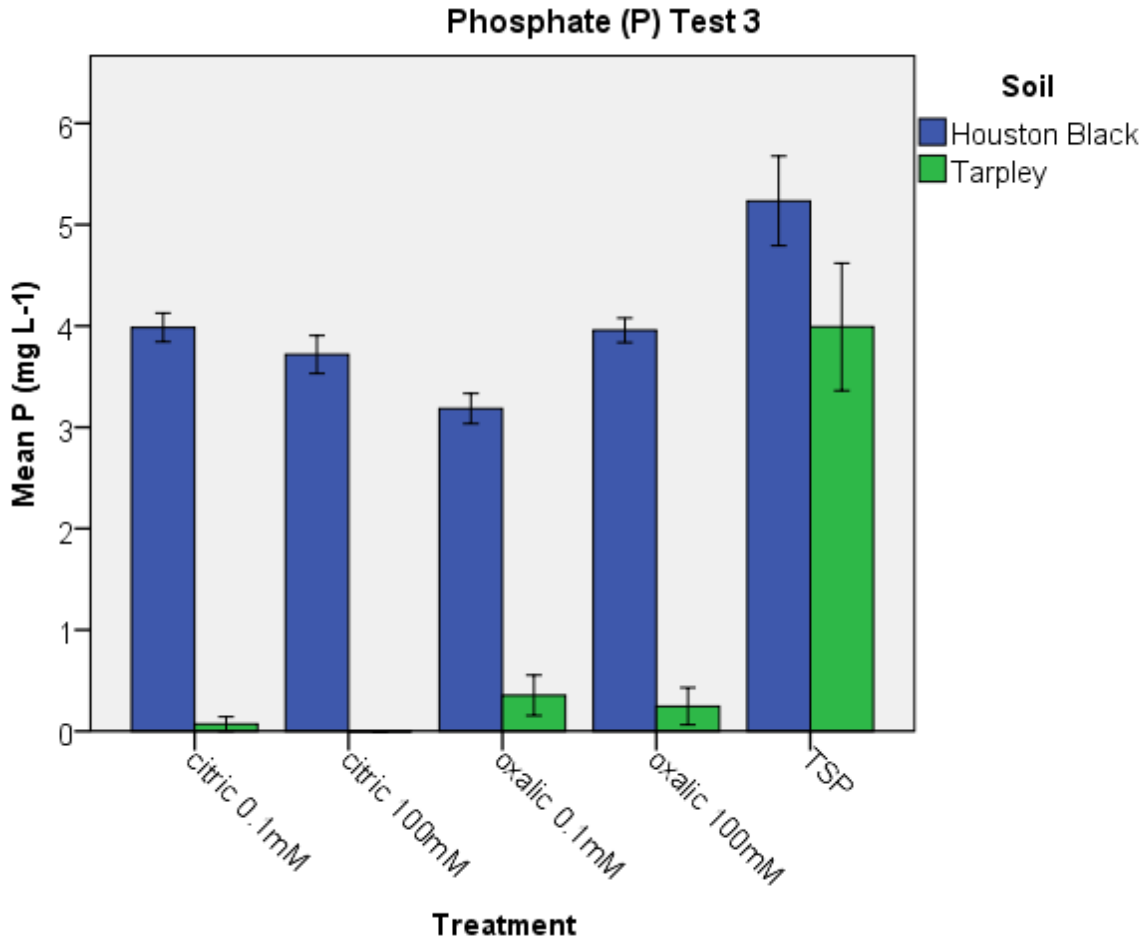


***Figure 19*. Phosphate (P) Test III graph for TaB and HvB soils (+/- 1 Standard Error); plotted from table 10 and table 11 data. Mean P (mg $L^{-1}$) based on sample size of 14 plants per treatment.**

Multivariate ANOVA results for differences in fruit yield (g) indicate harvest [Wilks' $\Lambda$=.323, F(2,129)= 135.34, p=.000], harvest*soil class [Wilks' $\Lambda$=.555, F(2, 129)= 51.80, p=.000], harvest*treatment [Wilks' $\Lambda$=.826, F(8, 258)= 3.244, p=.002], and harvest*soil class*treatment [Wilks' $\Lambda$=.800, F(8, 258)= 3.795, p=.000] were significant (Table 12). Univariate ANOVA results also indicate that main effects of soil class [F(1,130)= 77.101, p=.000], treatment [F(4,130)= 8.049, p=.000] and interaction between soil class*treatment [F(4,130)= 12.573, p=.000] were significant (Table 13). Tukey post-hoc analysis for overall treatments indicate that TSP treatment and LMWOAs (oxalic 0.1 mM $L^{-1}$, citric 0.1 mM $L^{-1}$, citric 100 mM $L^{-1}$) significantly differed from oxalic 100 mM $L^{-1}$ in TaB soil, while there was no significant mean difference between any treatments in HvB soil (Table 16). Pairwise comparisons between HvB soil and individual harvest (g) showed no significant differences for harvest I or harvest II, yet citric acid 0.1 mM $L^{-1}$ significantly differed from oxalic 100 mM $L^{-1}$ treatment for harvest III (Table 18; Table 19; Table 20). Further, pairwise comparisons between TaB soil and individual harvest (g) showed TSP treatment significantly differed from all other treatments and LMWOAs (oxalic 0.1 mM $L^{-1}$, citric 0.1 mM $L^{-1}$, citric 100 mM $L^{-1}$) significantly differed from oxalic 100 mM $L^{-1}$ treatment during Harvest I (Table 21). Pairwise comparisons for Harvest II on TaB soil showed TSP treatment significantly differed from all other treatments (Table 22). Subsequently, pairwise comparisons for Harvest III on TaB soil showed citric 0.1 mM $L^{-1}$ significantly differed from oxalic 100 mM $L^{-1}$ and TSP treatment, while oxalic 0.1 mM $L^{-1}$ significantly differed from oxalic 100 mM $L^{-1}$ and TSP treatment (Table 23).

For P soil testing, data were first transformed to eliminate outliers (untransformed data not presented here). This was accomplished by altering soil test extremities to a maximum value of 50 mg $L^{-1}$ followed by calculating square root values for each soil test value (mg $L^{-1}$). Multivariate ANOVA results indicate that soil test [Wilks' $\Lambda$=.834, F(2,129)= 12.838, p=.000], soil test*soil class [Wilks' $\Lambda$=.846, F(2,129)= 11.753, p=.000], soil test*treatment [Wilks' $\Lambda$=.735, F(8,258)= 5.368, p=.000] and interaction between soil test*soil class*treatment [Wilks' $\Lambda$=.626, F(8,258)= 8.507, p=.000] were significantly different over time (Table 14). Univariate ANOVA results also indicate that main effects of soil class [F(1,130)= 621.73, p=.000], treatment [F(4,130)= 39.688, p=.000] and interaction between soil class*treatment [F(4,130)= 10.398, p=.000] were significantly different (Table 15). Tukey post-hoc analysis for overall treatments indicate that TSP treatment significantly differed from all other treatments in both soils, yet in HvB soil citric 100 mM $L^{-1}$ was significantly different from citric 0.1 mM $L^{-1}$ and oxalic 100 mM $L^{-1}$ was significant against both citric and oxalic (0.1 mM $L^{-1}$) (Table 17). Pairwise comparisons between HvB soil treatments and P-test I (mg $L^{-1}$) indicate that TSP treatment significantly differed from all other treatments except oxalic 100 mM $L^{-1}$, while oxalic acid (100 mM $L^{-1}$) also significantly differed from lower LMWOA concentrations (0.1 mM $L^{-1}$) (Table 24). Meanwhile, P-test II (mg $L^{-1}$) showed no significant difference between treatments (Table 25). P-test III (mg $L^{-1}$) showed TSP treatment as significantly different from other treatments, while citric 0.1 mM $L^{-1}$ and oxalic 100 mM $L^{-1}$ were significantly different from oxalic 0.1 mM $L^{-1}$ (Table 26). Pairwise comparisons between TaB soil treatments and P-test (mg $L^{-1}$) showed TSP treatment was significant from other treatments for P-test I, while oxalic 0.1 mM $L^{-1}$ was

significant from citric acid (0.1 mM $L^{-1}$, 100 mM $L^{-1}$) (Table 27). P-test II and III demonstrated TSP treatment as significantly different from other treatments (Table 28; Table 29).

***Table 12.*** **MANOVA for yield shows a significant relationship between factors in both soil types, *p< 0.05*; based on LMWOAs (citric 0.1, 100 mM $L^{-1}$, oxalic 0.1, 100 mM $L^{-1}$) and TSP treatment. Based on sample size of 14 plants per treatment**.

| Effect | Value | F | Hypothesis df | Error df | Sig. |
|---|---|---|---|---|---|
| Harvest | 0.323 | 135.340 | 2 | 129 | 0.000 |
| Harvest x Soil Class | 0.555 | 51.800 | 2 | 129 | 0.000 |
| Harvest x Treatment | 0.826 | 3.244 | 8 | 258 | 0.002 |
| Harvest x Soil Class x Treatment | 0.800 | 3.795 | 8 | 258 | 0.000 |

***Table 13.*** **ANOVA for yield shows a significant relationship between factors in both soil types, *p< 0.05*; based on LMWOAs (citric 0.1, 100 mM $L^{-1}$, oxalic 0.1, 100 mM $L^{-1}$) and TSP treatment. Based on sample size of 14 plants per treatment.**

| Source | Type III Sum of Sq. | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Intercept | 21766436.040 | 1 | 21766436.040 | 3281.413 | 0.000 |
| Soil Class | 511431.589 | 1 | 511431.589 | 77.101 | 0.000 |
| Treatment | 213563.459 | 4 | 53390.865 | 8.049 | 0.000 |
| Soil Class x Treatment | 333611.317 | 4 | 83402.829 | 12.573 | 0.000 |
| Error | 862322.759 | 130 | 6633.252 | | |

***Table 14.*** **MANOVA for phosphate (P) soil test (mg $L^{-1}$) shows a significant relationship between factors in both soil types, *p< 0.05*; based on LMWOAs (citric 0.1, 100 mM $L^{-1}$, oxalic 0.1, 100 mM $L^{-1}$) and TSP treatment. Based on sample size of 14 plants per treatment.**

| Effect | Value | F | Hypothesis df | Error df | Sig. |
|---|---|---|---|---|---|
| P-test | 0.834 | 12.838 | 2 | 129 | 0.000 |
| P-test x Soil Class | 0.846 | 11.753 | 2 | 129 | 0.000 |
| P-test x Treatment | 0.735 | 5.368 | 8 | 258 | 0.000 |
| P-test x Soil Class x Treatment | 0.626 | 8.507 | 8 | 258 | 0.000 |

***Table 15.*** **ANOVA for phosphate (P) soil test (mg $L^{-1}$) shows a significant relationship between factors in both soil types, *p< 0.05*; based on LMWOAs (citric 0.1, 100 mM $L^{-1}$, oxalic 0.1, 100 mM $L^{-1}$) and TSP treatment. Based on sample size of 14 plants per treatment**.

| Source | Type III Sum of Sq. | df | Mean Square | F | Sig. |
|---|---|---|---|---|---|
| Intercept | 3296.010 | 1 | 3296.010 | 1548.581 | 0.000 |
| Soil Class | 1323.295 | 1 | 1323.295 | 621.73 | 0.000 |
| Treatment | 337.889 | 4 | 84.472 | 39.688 | 0.000 |
| Soil Class x Treatment | 88.527 | 4 | 22.132 | 10.398 | 0.000 |
| Error | 276.693 | 130 | 2.128 | | |

***Table 16.*** **Post-hoc test for total yield (g) showed no significant differences between LMWOA treatments and TSP treatment in HvB soil, while TSP treatment and LMWOAs (oxalic 0.1 mM $L^{-1}$, citric 0.1 mM $L^{-1}$, citric 100 mM $L^{-1}$) significantly differed from oxalic 100 mM $L^{-1}$ in TaB soil, $p< 0.05$. Based on sample size of 14 plants per treatment.**

| Measure | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|
| Houston Black | | | | | |
| citric 0.1 mM | citric 100 mM | -8.471 | 17.773 | 0.634 | -43.633 | 26.690 |
| | oxalic 0.1 mM | 14.912 | 17.773 | 0.403 | -20.249 | 50.073 |
| | oxalic 100 mM | 2.252 | 17.773 | 0.899 | -32.909 | 37.414 |
| | TSP | 17.088 | 17.773 | 0.338 | -18.073 | 52.249 |
| citric 100 mM | citric 0.1 mM | 8.471 | 17.773 | 0.634 | -26.690 | 43.633 |
| | oxalic 0.1 mM | 23.383 | 17.773 | 0.191 | -11.778 | 58.545 |
| | oxalic 100 mM | 10.724 | 17.773 | 0.547 | -24.437 | 45.885 |
| | TSP | 25.560 | 17.773 | 0.153 | -9.602 | 60.721 |
| oxalic 0.1 mM | citric 0.1 mM | -14.912 | 17.773 | 0.403 | -50.073 | 20.249 |
| | citric 100 mM | -23.383 | 17.773 | 0.191 | -58.545 | 11.778 |
| | oxalic 100 mM | -12.660 | 17.773 | 0.478 | -47.821 | 22.502 |
| | TSP | 2.176 | 17.773 | 0.903 | -32.985 | 37.337 |
| oxalic 100 mM | citric 0.1 mM | -2.252 | 17.773 | 0.899 | -37.414 | 32.909 |
| | citric 100 mM | -10.724 | 17.773 | 0.547 | -45.885 | 24.437 |
| | oxalic 0.1 mM | 12.660 | 17.773 | 0.478 | -22.502 | 47.821 |
| | TSP | 14.836 | 17.773 | 0.405 | -20.325 | 49.997 |
| TSP | citric 0.1 mM | -17.088 | 17.773 | 0.338 | -52.249 | 18.073 |
| | citric 100 mM | -25.560 | 17.773 | 0.153 | -60.721 | 9.602 |
| | oxalic 0.1 mM | -2.176 | 17.773 | 0.903 | -37.337 | 32.985 |
| | oxalic 100 mM | -14.836 | 17.773 | 0.405 | -49.997 | 20.325 |
| Tarpley | | | | | |
| citric 0.1 mM | citric 100 mM | 36.767 | 17.773 | 0.041 | 1.605 | 71.928 |
| | oxalic 0.1 mM | 2.871 | 17.773 | 0.872 | -32.290 | 38.033 |
| | oxalic 100 mM | 96.121 | 17.773 | 0.000 | 60.960 | 131.283 |
| | TSP | -56.738 | 17.773 | 0.002 | -91.899 | -21.577 |
| citric 100 mM | citric 0.1 mM | -36.767 | 17.773 | 0.041 | -71.928 | -1.605 |
| | oxalic 0.1 mM | -33.895 | 17.773 | 0.059 | -69.056 | 1.266 |
| | oxalic 100 mM | 59.355 | 17.773 | 0.001 | 24.194 | 94.516 |
| | TSP | -93.505 | 17.773 | 0.000 | -128.666 | -58.344 |
| oxalic 0.1 mM | citric 0.1 mM | -2.871 | 17.773 | 0.872 | -38.033 | 32.290 |
| | citric 100 mM | 33.895 | 17.773 | 0.059 | -1.266 | 69.056 |
| | oxalic 100 mM | 93.250 | 17.773 | 0.000 | 58.089 | 128.411 |
| | TSP | -59.610 | 17.773 | 0.001 | -94.771 | -24.448 |
| oxalic 100 mM | citric 0.1 mM | -96.121 | 17.773 | 0.000 | -131.283 | -60.960 |
| | citric 100 mM | -59.355 | 17.773 | 0.001 | -94.516 | -24.194 |
| | oxalic 0.1 mM | -93.250 | 17.773 | 0.000 | -128.411 | -58.089 |
| | TSP | -152.860 | 17.773 | 0.000 | -188.021 | -117.698 |
| TSP | citric 0.1 mM | 56.738 | 17.773 | 0.002 | 21.577 | 91.899 |
| | citric 100 mM | 93.505 | 17.773 | 0.000 | 58.344 | 128.666 |
| | oxalic 0.1 mM | 59.610 | 17.773 | 0.001 | 24.448 | 94.771 |
| | oxalic 100 mM | 152.860 | 17.773 | 0.000 | 117.698 | 188.021 |

***Table 17.*** **Post-hoc test for total phosphate (P) soil tests (mg $L^{-1}$) indicate TSP treatment significantly differed from all other treatments in both soils, yet in HvB soil citric 100 mM $L^{-1}$ was significantly different from citric 0.1 mM $L^{-1}$ and oxalic 100 mM $L^{-1}$ was significant against both citric and oxalic (0.1 mM $L^{-1}$), *p< 0.05*. Based on sample size of 14 plants per treatment.**

| Measure | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| Houston Black | | | | | | |
| citric 0.1 mM | citric 100 mM | -0.923 | 0.318 | 0.004 | -1.553 | -0.293 |
| | oxalic 0.1 mM | -0.539 | 0.318 | 0.093 | -1.169 | 0.091 |
| | oxalic 100 mM | -1.175 | 0.318 | 0.000 | -1.805 | -0.545 |
| | TSP | -1.838 | 0.318 | 0.000 | -2.467 | -1.208 |
| citric 100 mM | citric 0.1 mM | 0.923 | 0.318 | 0.004 | 0.293 | 1.553 |
| | oxalic 0.1 mM | 0.384 | 0.318 | 0.230 | -0.246 | 1.014 |
| | oxalic 100 mM | -0.252 | 0.318 | 0.431 | -0.881 | 0.378 |
| | TSP | -0.914 | 0.318 | 0.005 | -1.544 | -0.285 |
| oxalic 0.1 mM | citric 0.1 mM | 0.539 | 0.318 | 0.093 | -0.091 | 1.169 |
| | citric 100 mM | -0.384 | 0.318 | 0.230 | -1.014 | 0.246 |
| | oxalic 100 mM | -0.636 | 0.318 | 0.048 | -1.266 | -0.006 |
| | TSP | -1.299 | 0.318 | 0.000 | -1.928 | -0.669 |
| oxalic 100 mM | citric 0.1 mM | 1.175 | 0.318 | 0.000 | 0.545 | 1.805 |
| | citric 100 mM | 0.252 | 0.318 | 0.431 | -0.378 | 0.881 |
| | oxalic 0.1 mM | 0.636 | 0.318 | 0.048 | 0.006 | 1.266 |
| | TSP | -0.663 | 0.318 | 0.039 | -1.293 | -0.033 |
| TSP | citric 0.1 mM | 1.838 | 0.318 | 0.000 | 1.208 | 2.467 |
| | citric 100 mM | 0.914 | 0.318 | 0.005 | 0.285 | 1.544 |
| | oxalic 0.1 mM | 1.299 | 0.318 | 0.000 | 0.669 | 1.928 |
| | oxalic 100 mM | 0.663 | 0.318 | 0.039 | 0.033 | 1.293 |
| Tarpley | | | | | | |
| citric 0.1 mM | citric 100 mM | 0.140 | 0.318 | 0.661 | -0.490 | 0.770 |
| | oxalic 0.1 mM | -0.447 | 0.318 | 0.163 | -1.076 | 0.183 |
| | oxalic 100 mM | -0.127 | 0.318 | 0.691 | -0.757 | 0.503 |
| | TSP | -3.284 | 0.318 | 0.000 | -3.914 | -2.654 |
| citric 100 mM | citric 0.1 mM | -0.140 | 0.318 | 0.661 | -0.770 | 0.490 |
| | oxalic 0.1 mM | -0.587 | 0.318 | 0.068 | -1.216 | 0.043 |
| | oxalic 100 mM | -0.267 | 0.318 | 0.404 | -0.897 | 0.363 |
| | TSP | -3.424 | 0.318 | 0.000 | -4.054 | -2.794 |
| oxalic 0.1 mM | citric 0.1 mM | 0.447 | 0.318 | 0.163 | -0.183 | 1.076 |
| | citric 100 mM | 0.587 | 0.318 | 0.068 | -0.043 | 1.216 |
| | oxalic 100 mM | 0.320 | 0.318 | 0.317 | -0.310 | 0.950 |
| | TSP | -2.838 | 0.318 | 0.000 | -3.468 | -2.208 |
| oxalic 100 mM | citric 0.1 mM | 0.127 | 0.318 | 0.691 | -0.503 | 0.757 |
| | citric 100 mM | 0.267 | 0.318 | 0.404 | -0.363 | 0.897 |
| | oxalic 0.1 mM | -0.320 | 0.318 | 0.317 | -0.950 | 0.310 |
| | TSP | -3.158 | 0.318 | 0.000 | -3.787 | -2.528 |
| TSP | citric 0.1 mM | 3.284 | 0.318 | 0.000 | 2.654 | 3.914 |
| | citric 100 mM | 3.424 | 0.318 | 0.000 | 2.794 | 4.054 |
| | oxalic 0.1 mM | 2.838 | 0.318 | 0.000 | 2.208 | 3.468 |
| | oxalic 100 mM | 3.158 | 0.318 | 0.000 | 2.528 | 3.787 |

***Table 18.*** **Post-hoc test for Harvest I (g) on HvB soil showed no significant differences between treatments, *p> 0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1 mM | citric 100 mM | -42.507 | 45.708 | 0.354 | -132.935 | 47.921 |
| | oxalic 0.1 mM | -5.443 | 45.708 | 0.905 | -95.871 | 84.985 |
| | oxalic 100 mM | -51.543 | 45.708 | 0.262 | -141.971 | 38.885 |
| | TSP | 2.107 | 45.708 | 0.963 | -88.321 | 92.535 |
| citric 100 mM | citric 0.1 mM | 42.507 | 45.708 | 0.354 | -47.921 | 132.935 |
| | oxalic 0.1 mM | 37.064 | 45.708 | 0.419 | -53.364 | 127.492 |
| | oxalic 100 mM | -9.036 | 45.708 | 0.844 | -99.464 | 81.392 |
| | TSP | 44.614 | 45.708 | 0.331 | -45.814 | 135.042 |
| oxalic 0.1 mM | citric 0.1 mM | 5.443 | 45.708 | 0.905 | -84.985 | 95.871 |
| | citric 100 mM | -37.064 | 45.708 | 0.419 | -127.492 | 53.364 |
| | oxalic 100 mM | -46.100 | 45.708 | 0.315 | -136.528 | 44.328 |
| | TSP | 7.550 | 45.708 | 0.869 | -82.878 | 97.978 |
| oxalic 100 mM | citric 0.1 mM | 51.543 | 45.708 | 0.262 | -38.885 | 141.971 |
| | citric 100 mM | 9.036 | 45.708 | 0.844 | -81.392 | 99.464 |
| | oxalic 0.1 mM | 46.100 | 45.708 | 0.315 | -44.328 | 136.528 |
| | TSP | 53.650 | 45.708 | 0.243 | -36.778 | 144.078 |
| TSP | citric 0.1 mM | -2.107 | 45.708 | 0.963 | -92.535 | 88.321 |
| | citric 100 mM | -44.614 | 45.708 | 0.331 | -135.042 | 45.814 |
| | oxalic 0.1 mM | -7.550 | 45.708 | 0.869 | -97.978 | 82.878 |
| | oxalic 100 mM | -53.650 | 45.708 | 0.243 | -144.078 | 36.778 |

***Table 19.*** **Post-hoc test for Harvest II (g) on HvB soil showed no significant differences between treatments, *p> 0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1mM | citric 100mM | -3.979 | 39.638 | 0.920 | -82.397 | 74.440 |
| | oxalic 0.1mM | 6.407 | 39.638 | 0.872 | -72.011 | 84.825 |
| | oxalic 100mM | -29.836 | 39.638 | 0.453 | -108.254 | 48.582 |
| | TSP | -7.157 | 39.638 | 0.857 | -85.575 | 71.261 |
| citric 100mM | citric 0.1mM | 3.979 | 39.638 | 0.920 | -74.440 | 82.397 |
| | oxalic 0.1mM | 10.386 | 39.638 | 0.794 | -68.032 | 88.804 |
| | oxalic 100mM | -25.857 | 39.638 | 0.515 | -104.275 | 52.561 |
| | TSP | -3.179 | 39.638 | 0.936 | -81.597 | 75.240 |
| oxalic 0.1mM | citric 0.1mM | -6.407 | 39.638 | 0.872 | -84.825 | 72.011 |
| | citric 100mM | -10.386 | 39.638 | 0.794 | -88.804 | 68.032 |
| | oxalic 100mM | -36.243 | 39.638 | 0.362 | -114.661 | 42.175 |
| | TSP | -13.564 | 39.638 | 0.733 | -91.982 | 64.854 |
| oxalic 100mM | citric 0.1mM | 29.836 | 39.638 | 0.453 | -48.582 | 108.254 |
| | citric 100mM | 25.857 | 39.638 | 0.515 | -52.561 | 104.275 |
| | oxalic 0.1mM | 36.243 | 39.638 | 0.362 | -42.175 | 114.661 |
| | TSP | 22.679 | 39.638 | 0.568 | -55.740 | 101.097 |
| TSP | citric 0.1mM | 7.157 | 39.638 | 0.857 | -71.261 | 85.575 |
| | citric 100mM | 3.179 | 39.638 | 0.936 | -75.240 | 81.597 |
| | oxalic 0.1mM | 13.564 | 39.638 | 0.733 | -64.854 | 91.982 |
| | oxalic 100mM | -22.679 | 39.638 | 0.568 | -101.097 | 55.740 |

***Table 20.*** **Post-hoc test for Harvest III (g) on HvB soil showed that citric acid 0.1 mM $L^{-1}$ significantly differed from oxalic 100 mM $L^{-1}$ yet there was no significant differences between other treatments, *p>0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1mM | citric 100mM | 21.071 | 35.052 | 0.549 | -48.276 | 90.418 |
| | oxalic 0.1mM | 43.771 | 35.052 | 0.214 | -25.576 | 113.118 |
| | oxalic 100mM | 88.136 | 35.052 | 0.013 | 18.789 | 157.483 |
| | TSP | 56.314 | 35.052 | 0.111 | -13.033 | 125.661 |
| citric 100mM | citric 0.1mM | -21.071 | 35.052 | 0.549 | -90.418 | 48.276 |
| | oxalic 0.1mM | 22.700 | 35.052 | 0.518 | -46.647 | 92.047 |
| | oxalic 100mM | 67.064 | 35.052 | 0.058 | -2.283 | 136.411 |
| | TSP | 35.243 | 35.052 | 0.317 | -34.104 | 104.590 |
| oxalic 0.1mM | citric 0.1mM | -43.771 | 35.052 | 0.214 | -113.118 | 25.576 |
| | citric 100mM | -22.700 | 35.052 | 0.518 | -92.047 | 46.647 |
| | oxalic 100mM | 44.364 | 35.052 | 0.208 | -24.983 | 113.711 |
| | TSP | 12.543 | 35.052 | 0.721 | -56.804 | 81.890 |
| oxalic 100mM | citric 0.1mM | -88.136 | 35.052 | 0.013 | -157.483 | -18.789 |
| | citric 100mM | -67.064 | 35.052 | 0.058 | -136.411 | 2.283 |
| | oxalic 0.1mM | -44.364 | 35.052 | 0.208 | -113.711 | 24.983 |
| | TSP | -31.821 | 35.052 | 0.366 | -101.168 | 37.526 |
| TSP | citric 0.1mM | -56.314 | 35.052 | 0.111 | -125.661 | 13.033 |
| | citric 100mM | -35.243 | 35.052 | 0.317 | -104.590 | 34.104 |
| | oxalic 0.1mM | -12.543 | 35.052 | 0.721 | -81.890 | 56.804 |
| | oxalic 100mM | 31.821 | 35.052 | 0.366 | -37.526 | 101.168 |

***Table 21.*** **Post-hoc test for Harvest I on TaB soil showed TSP treatment significantly differed from all other treatments and LMWOAs (oxalic 0.1 mg $L^{-1}$, citric 0.1 mM $L^{-1}$, citric 100 mM $L^{-1}$) significantly differed from oxalic 100 mM $L^{-1}$ treatment, *p< 0.05*. Based on sample size of 14 plants per treatment.**

| | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|
| citric 0.1 mM | citric 100 mM | 57.900 | 45.708 | 0.208 | -32.528 | 148.328 |
| | oxalic 0.1 mM | 16.721 | 45.708 | 0.715 | -73.706 | 107.149 |
| | oxalic 100 mM | 160.600 | 45.708 | 0.001 | 70.172 | 251.028 |
| | TSP | -161.586 | 45.708 | 0.001 | -252.014 | -71.158 |
| citric 100 mM | citric 0.1 mM | -57.900 | 45.708 | 0.208 | -148.328 | 32.528 |
| | oxalic 0.1 mM | -41.179 | 45.708 | 0.369 | -131.606 | 49.249 |
| | oxalic 100 mM | 102.700 | 45.708 | 0.026 | 12.272 | 193.128 |
| | TSP | -219.486 | 45.708 | 0.000 | -309.914 | -129.058 |
| oxalic 0.1 mM | citric 0.1 mM | -16.721 | 45.708 | 0.715 | -107.149 | 73.706 |
| | citric 100 mM | 41.179 | 45.708 | 0.369 | -49.249 | 131.606 |
| | oxalic 100 mM | 143.879 | 45.708 | 0.002 | 53.451 | 234.306 |
| | TSP | -178.307 | 45.708 | 0.000 | -268.735 | -87.879 |
| oxalic 100 mM | citric 0.1 mM | -160.600 | 45.708 | 0.001 | -251.028 | -70.172 |
| | citric 100 mM | -102.700 | 45.708 | 0.026 | -193.128 | -12.272 |
| | oxalic 0.1 mM | -143.879 | 45.708 | 0.002 | -234.306 | -53.451 |
| | TSP | -322.186 | 45.708 | 0.000 | -412.614 | -231.758 |
| TSP | citric 0.1 mM | 161.586 | 45.708 | 0.001 | 71.158 | 252.014 |
| | citric 100 mM | 219.486 | 45.708 | 0.000 | 129.058 | 309.914 |
| | oxalic 0.1 mM | 178.307 | 45.708 | 0.000 | 87.879 | 268.735 |
| | oxalic 100 mM | 322.186 | 45.708 | 0.000 | 231.758 | 412.614 |

***Table 22.*** **Post-hoc test for Harvest II on TaB soil showed TSP treatment significantly differed from all other treatments, *p< 0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1 mM | citric 100 mM | 11.607 | 39.638 | 0.770 | -66.811 | 90.025 |
| | oxalic 0.1 mM | 15.514 | 39.638 | 0.696 | -62.904 | 93.932 |
| | oxalic 100 mM | 41.700 | 39.638 | 0.295 | -36.718 | 120.118 |
| | TSP | -93.764 | 39.638 | 0.019 | -172.182 | -15.346 |
| citric 100 mM | citric 0.1 mM | -11.607 | 39.638 | 0.770 | -90.025 | 66.811 |
| | oxalic 0.1 mM | 3.907 | 39.638 | 0.922 | -74.511 | 82.325 |
| | oxalic 100 mM | 30.093 | 39.638 | 0.449 | -48.325 | 108.511 |
| | TSP | -105.371 | 39.638 | 0.009 | -183.790 | -26.953 |
| oxalic 0.1 mM | citric 0.1 mM | -15.514 | 39.638 | 0.696 | -93.932 | 62.904 |
| | citric 100 mM | -3.907 | 39.638 | 0.922 | -82.325 | 74.511 |
| | oxalic 100 mM | 26.186 | 39.638 | 0.510 | -52.232 | 104.604 |
| | TSP | -109.279 | 39.638 | 0.007 | -187.697 | -30.860 |
| oxalic 100 mM | citric 0.1 mM | -41.700 | 39.638 | 0.295 | -120.118 | 36.718 |
| | citric 100 mM | -30.093 | 39.638 | 0.449 | -108.511 | 48.325 |
| | oxalic 0.1 mM | -26.186 | 39.638 | 0.510 | -104.604 | 52.232 |
| | TSP | -135.464 | 39.638 | 0.001 | -213.882 | -57.046 |
| TSP | citric 0.1 mM | 93.764 | 39.638 | 0.019 | 15.346 | 172.182 |
| | citric 100 mM | 105.371 | 39.638 | 0.009 | 26.953 | 183.790 |
| | oxalic 0.1 mM | 109.279 | 39.638 | 0.007 | 30.860 | 187.697 |
| | oxalic 100 mM | 135.464 | 39.638 | 0.001 | 57.046 | 213.882 |

***Table 23.*** **Post-hoc test for Harvest III on TaB soil showed citric 0.1 mM $L^{-1}$ significantly differed from oxalic 100 mM $L^{-1}$ and TSP treatment, while oxalic 0.1 mM $L^{-1}$ significantly differed from oxalic 100 mM $L^{-1}$ and TSP treatment, *p< 0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1 mM | citric 100 mM | 40.793 | 35.052 | 0.247 | -28.554 | 110.140 |
| | oxalic 0.1 mM | -23.621 | 35.052 | 0.502 | -92.968 | 45.726 |
| | oxalic 100 mM | 86.064 | 35.052 | 0.015 | 16.717 | 155.411 |
| | TSP | 85.136 | 35.052 | 0.017 | 15.789 | 154.483 |
| citric 100 mM | citric 0.1 mM | -40.793 | 35.052 | 0.247 | -110.140 | 28.554 |
| | oxalic 0.1 mM | -64.414 | 35.052 | 0.068 | -133.761 | 4.933 |
| | oxalic 100 mM | 45.271 | 35.052 | 0.199 | -24.076 | 114.618 |
| | TSP | 44.343 | 35.052 | 0.208 | -25.004 | 113.690 |
| oxalic 0.1 mM | citric 0.1 mM | 23.621 | 35.052 | 0.502 | -45.726 | 92.968 |
| | citric 100 mM | 64.414 | 35.052 | 0.068 | -4.933 | 133.761 |
| | oxalic 100 mM | 109.686 | 35.052 | 0.002 | 40.339 | 179.033 |
| | TSP | 108.757 | 35.052 | 0.002 | 39.410 | 178.104 |
| oxalic 100 mM | citric 0.1 mM | -86.064 | 35.052 | 0.015 | -155.411 | -16.717 |
| | citric 100 mM | -45.271 | 35.052 | 0.199 | -114.618 | 24.076 |
| | oxalic 0.1 mM | -109.686 | 35.052 | 0.002 | -179.033 | -40.339 |
| | TSP | -0.929 | 35.052 | 0.979 | -70.276 | 68.418 |
| TSP | citric 0.1 mM | -85.136 | 35.052 | 0.017 | -154.483 | -15.789 |
| | citric 100 mM | -44.343 | 35.052 | 0.208 | -113.690 | 25.004 |
| | oxalic 0.1 mM | -108.757 | 35.052 | 0.002 | -178.104 | -39.410 |
| | oxalic 100 mM | 0.929 | 35.052 | 0.979 | -68.418 | 70.276 |

***Table 24.*** **Post-hoc test for phosphate (P) Soil Test I (mg $L^{-1}$) on HvB soil showed a significant difference between TSP treatment and LMWOAs except for oxalic 100 mM $L^{-1}$,while oxalic 100 mM $L^{-1}$ significantly differed from lower LMWOA concentrations (0.1 mM $L^{-1}$), *p< 0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1 mM | citric 100 mM | -2.600 | 0.449 | 0.000 | -3.488 | -1.713 |
| | oxalic 0.1 mM | -1.916 | 0.449 | 0.000 | -2.803 | -1.028 |
| | oxalic 100 mM | -2.901 | 0.449 | 0.000 | -3.789 | -2.014 |
| | TSP | -3.768 | 0.449 | 0.000 | -4.655 | -2.880 |
| citric 100 mM | citric 0.1 mM | 2.600 | 0.449 | 0.000 | 1.713 | 3.488 |
| | oxalic 0.1 mM | 0.685 | 0.449 | 0.129 | -0.203 | 1.572 |
| | oxalic 100 mM | -0.301 | 0.449 | 0.504 | -1.188 | 0.587 |
| | TSP | -1.167 | 0.449 | 0.010 | -2.055 | -0.280 |
| oxalic 0.1 mM | citric 0.1 mM | 1.916 | 0.449 | 0.000 | 1.028 | 2.803 |
| | citric 100 mM | -0.685 | 0.449 | 0.129 | -1.572 | 0.203 |
| | oxalic 100 mM | -0.986 | 0.449 | 0.030 | -1.873 | -0.098 |
| | TSP | -1.852 | 0.449 | 0.000 | -2.740 | -0.965 |
| oxalic 100 mM | citric 0.1 mM | 2.901 | 0.449 | 0.000 | 2.014 | 3.789 |
| | citric 100 mM | 0.301 | 0.449 | 0.504 | -0.587 | 1.188 |
| | oxalic 0.1 mM | 0.986 | 0.449 | 0.030 | 0.098 | 1.873 |
| | TSP | -0.867 | 0.449 | 0.056 | -1.754 | 0.021 |
| TSP | citric 0.1 mM | 3.768 | 0.449 | 0.000 | 2.880 | 4.655 |
| | citric 100 mM | 1.167 | 0.449 | 0.010 | 0.280 | 2.055 |
| | oxalic 0.1 mM | 1.852 | 0.449 | 0.000 | 0.965 | 2.740 |
| | oxalic 100 mM | 0.867 | 0.449 | 0.056 | -0.021 | 1.754 |

***Table 25.*** **Post-hoc test for phosphate (P) Soil Test II (mg $L^{-1}$) on HvB soil showed no significant differences between treatments, *p> 0.05*. Based on sample size of 14 plants per treatment.**

| | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|
| citric 0.1mM | citric 100mM | -0.435 | 0.440 | 0.324 | -1.306 | 0.435 |
| | oxalic 0.1mM | -0.502 | 0.440 | 0.256 | -1.373 | 0.369 |
| | oxalic 100mM | -0.650 | 0.440 | 0.142 | -1.521 | 0.220 |
| | TSP | -0.497 | 0.440 | 0.261 | -1.368 | 0.374 |
| citric 100mM | citric 0.1mM | 0.435 | 0.440 | 0.324 | -0.435 | 1.306 |
| | oxalic 0.1mM | -0.067 | 0.440 | 0.880 | -0.937 | 0.804 |
| | oxalic 100mM | -0.215 | 0.440 | 0.626 | -1.086 | 0.656 |
| | TSP | -0.061 | 0.440 | 0.890 | -0.932 | 0.810 |
| oxalic 0.1mM | citric 0.1mM | 0.502 | 0.440 | 0.256 | -0.369 | 1.373 |
| | citric 100mM | 0.067 | 0.440 | 0.880 | -0.804 | 0.937 |
| | oxalic 100mM | -0.148 | 0.440 | 0.737 | -1.019 | 0.723 |
| | TSP | 0.005 | 0.440 | 0.990 | -0.865 | 0.876 |
| oxalic 100mM | citric 0.1mM | 0.650 | 0.440 | 0.142 | -0.220 | 1.521 |
| | citric 100mM | 0.215 | 0.440 | 0.626 | -0.656 | 1.086 |
| | oxalic 0.1mM | 0.148 | 0.440 | 0.737 | -0.723 | 1.019 |
| | TSP | 0.154 | 0.440 | 0.727 | -0.717 | 1.025 |
| TSP | citric 0.1mM | 0.497 | 0.440 | 0.261 | -0.374 | 1.368 |
| | citric 100mM | 0.061 | 0.440 | 0.890 | -0.810 | 0.932 |
| | oxalic 0.1mM | -0.005 | 0.440 | 0.990 | -0.876 | 0.865 |
| | oxalic 100mM | -0.154 | 0.440 | 0.727 | -1.025 | 0.717 |

***Table 26.*** **Post-hoc test for phosphate (P) Soil Test III (mg $L^{-1}$) on HvB soil showed TSP treatment as significantly different from LMWOA treatments, while citric 0.1 mM $L^{-1}$ and oxalic 100 mM $L^{-1}$ were significantly different from oxalic 0.1 mM $L^{-1}$, *p< 0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1mM | citric 100mM | 0.266 | 0.390 | 0.496 | -0.506 | 1.038 |
| | oxalic 0.1mM | 0.801 | 0.390 | 0.042 | 0.029 | 1.572 |
| | oxalic 100mM | 0.027 | 0.390 | 0.945 | -0.745 | 0.799 |
| | TSP | -1.248 | 0.390 | 0.002 | -2.020 | -0.477 |
| citric 100mM | citric 0.1mM | -0.266 | 0.390 | 0.496 | -1.038 | 0.506 |
| | oxalic 0.1mM | 0.534 | 0.390 | 0.173 | -0.237 | 1.306 |
| | oxalic 100mM | -0.239 | 0.390 | 0.541 | -1.011 | 0.533 |
| | TSP | -1.515 | 0.390 | 0.000 | -2.286 | -0.743 |
| oxalic 0.1mM | citric 0.1mM | -0.801 | 0.390 | 0.042 | -1.572 | -0.029 |
| | citric 100mM | -0.534 | 0.390 | 0.173 | -1.306 | 0.237 |
| | oxalic 100mM | -0.773 | 0.390 | 0.050 | -1.545 | -0.002 |
| | TSP | -2.049 | 0.390 | 0.000 | -2.821 | -1.277 |
| oxalic 100mM | citric 0.1mM | -0.027 | 0.390 | 0.945 | -0.799 | 0.745 |
| | citric 100mM | 0.239 | 0.390 | 0.541 | -0.533 | 1.011 |
| | oxalic 0.1mM | 0.773 | 0.390 | 0.050 | 0.002 | 1.545 |
| | TSP | -1.276 | 0.390 | 0.001 | -2.047 | -0.504 |
| TSP | citric 0.1mM | 1.248 | 0.390 | 0.002 | 0.477 | 2.020 |
| | citric 100mM | 1.515 | 0.390 | 0.000 | 0.743 | 2.286 |
| | oxalic 0.1mM | 2.049 | 0.390 | 0.000 | 1.277 | 2.821 |
| | oxalic 100mM | 1.276 | 0.390 | 0.001 | 0.504 | 2.047 |

***Table 27.*** **Post-hoc test for phosphate (P) Soil Test I (mg $L^{-1}$) on TaB soil showed TSP treatment as significantly different from all other treatments, while oxalic 0.1 mM $L^{-1}$ was significant against citric acid (0.1, 100 mM $L^{-1}$), *p< 0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1 mM | citric 100 mM | 0.172 | 0.449 | 0.701 | -0.715 | 1.060 |
| | oxalic 0.1 mM | -0.959 | 0.449 | 0.034 | -1.846 | -0.071 |
| | oxalic 100 mM | -0.085 | 0.449 | 0.850 | -0.973 | 0.802 |
| | TSP | -2.379 | 0.449 | 0.000 | -3.267 | -1.492 |
| citric 100 mM | citric 0.1 mM | -0.172 | 0.449 | 0.701 | -1.060 | 0.715 |
| | oxalic 0.1 mM | -1.131 | 0.449 | 0.013 | -2.019 | -0.244 |
| | oxalic 100 mM | -0.258 | 0.449 | 0.567 | -1.145 | 0.630 |
| | TSP | -2.552 | 0.449 | 0.000 | -3.439 | -1.664 |
| oxalic 0.1 mM | citric 0.1 mM | 0.959 | 0.449 | 0.034 | 0.071 | 1.846 |
| | citric 100 mM | 1.131 | 0.449 | 0.013 | 0.244 | 2.019 |
| | oxalic 100 mM | 0.874 | 0.449 | 0.054 | -0.014 | 1.761 |
| | TSP | -1.421 | 0.449 | 0.002 | -2.308 | -0.533 |
| oxalic 100 mM | citric 0.1 mM | 0.085 | 0.449 | 0.850 | -0.802 | 0.973 |
| | citric 100 mM | 0.258 | 0.449 | 0.567 | -0.630 | 1.145 |
| | oxalic 0.1 mM | -0.874 | 0.449 | 0.054 | -1.761 | 0.014 |
| | TSP | -2.294 | 0.449 | 0.000 | -3.182 | -1.407 |
| TSP | citric 0.1 mM | 2.379 | 0.449 | 0.000 | 1.492 | 3.267 |
| | citric 100 mM | 2.552 | 0.449 | 0.000 | 1.664 | 3.439 |
| | oxalic 0.1 mM | 1.421 | 0.449 | 0.002 | 0.533 | 2.308 |
| | oxalic 100 mM | 2.294 | 0.449 | 0.000 | 1.407 | 3.182 |

***Table 28.*** **Post-hoc test for phosphate (P) Soil Test II (mg $L^{-1}$) on TaB soil showed TSP treatment as significantly different from all other treatments, *p< 0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1 mM | citric 100 mM | 0.176 | 0.440 | 0.690 | -0.695 | 1.047 |
| | oxalic 0.1 mM | -0.097 | 0.440 | 0.825 | -0.968 | 0.773 |
| | oxalic 100 mM | -0.120 | 0.440 | 0.785 | -0.991 | 0.751 |
| | TSP | -3.554 | 0.440 | 0.000 | -4.425 | -2.683 |
| citric 100 mM | citric 0.1 mM | -0.176 | 0.440 | 0.690 | -1.047 | 0.695 |
| | oxalic 0.1 mM | -0.273 | 0.440 | 0.536 | -1.144 | 0.597 |
| | oxalic 100 mM | -0.296 | 0.440 | 0.502 | -1.167 | 0.575 |
| | TSP | -3.730 | 0.440 | 0.000 | -4.601 | -2.859 |
| oxalic 0.1 mM | citric 0.1 mM | 0.097 | 0.440 | 0.825 | -0.773 | 0.968 |
| | citric 100 mM | 0.273 | 0.440 | 0.536 | -0.597 | 1.144 |
| | oxalic 100 mM | -0.023 | 0.440 | 0.959 | -0.894 | 0.848 |
| | TSP | -3.457 | 0.440 | 0.000 | -4.327 | -2.586 |
| oxalic 100 mM | citric 0.1 mM | 0.120 | 0.440 | 0.785 | -0.751 | 0.991 |
| | citric 100 mM | 0.296 | 0.440 | 0.502 | -0.575 | 1.167 |
| | oxalic 0.1 mM | 0.023 | 0.440 | 0.959 | -0.848 | 0.894 |
| | TSP | -3.434 | 0.440 | 0.000 | -4.305 | -2.563 |
| TSP | citric 0.1 mM | 3.554 | 0.440 | 0.000 | 2.683 | 4.425 |
| | citric 100 mM | 3.730 | 0.440 | 0.000 | 2.859 | 4.601 |
| | oxalic 0.1 mM | 3.457 | 0.440 | 0.000 | 2.586 | 4.327 |
| | oxalic 100 mM | 3.434 | 0.440 | 0.000 | 2.563 | 4.305 |

***Table 29.*** **Post-hoc test for phosphate (P) Soil Test III (mg $L^{-1}$) on TaB soil showed TSP treatment as significantly different from all other treatments, *p< 0.05*. Based on sample size of 14 plants per treatment.**

| | | Mean Difference | Standard Error | Sig. | Lower Bound | Upper Bound |
|---|---|---|---|---|---|---|
| citric 0.1 mM | citric 100 mM | 0.071 | 0.390 | 0.855 | -0.700 | 0.843 |
| | oxalic 0.1 mM | -0.283 | 0.390 | 0.469 | -1.055 | 0.488 |
| | oxalic 100 mM | -0.175 | 0.390 | 0.655 | -0.947 | 0.597 |
| | TSP | -3.919 | 0.390 | 0.000 | -4.691 | -3.147 |
| citric 100 mM | citric 0.1 mM | -0.071 | 0.390 | 0.855 | -0.843 | 0.700 |
| | oxalic 0.1 mM | -0.355 | 0.390 | 0.365 | -1.127 | 0.417 |
| | oxalic 100 mM | -0.246 | 0.390 | 0.529 | -1.018 | 0.525 |
| | TSP | -3.991 | 0.390 | 0.000 | -4.763 | -3.219 |
| oxalic 0.1 mM | citric 0.1 mM | 0.283 | 0.390 | 0.469 | -0.488 | 1.055 |
| | citric 100 mM | 0.355 | 0.390 | 0.365 | -0.417 | 1.127 |
| | oxalic 100 mM | 0.108 | 0.390 | 0.781 | -0.663 | 0.880 |
| | TSP | -3.636 | 0.390 | 0.000 | -4.408 | -2.864 |
| oxalic 100 mM | citric 0.1 mM | 0.175 | 0.390 | 0.655 | -0.597 | 0.947 |
| | citric 100 mM | 0.246 | 0.390 | 0.529 | -0.525 | 1.018 |
| | oxalic 0.1 mM | -0.108 | 0.390 | 0.781 | -0.880 | 0.663 |
| | TSP | -3.744 | 0.390 | 0.000 | -4.516 | -2.972 |
| TSP | citric 0.1 mM | 3.919 | 0.390 | 0.000 | 3.147 | 4.691 |
| | citric 100 mM | 3.991 | 0.390 | 0.000 | 3.219 | 4.763 |
| | oxalic 0.1 mM | 3.636 | 0.390 | 0.000 | 2.864 | 4.408 |
| | oxalic 100 mM | 3.744 | 0.390 | 0.000 | 2.972 | 4.516 |

***Table 30*. Summary of post-harvest soil pH for each treatment in TaB and HvB soils. Based on sample size of 14 plants per treatment.**

| | citric 0.1 mM | citric 100 mM | oxalic 0.1 mM | oxalic 100 mM | TSP |
|---|---|---|---|---|---|
| Houston Black | 7.2 | 7.6 | 7.6 | 7.5 | 7.4 |
| | 7.3 | 7.7 | 7.6 | 7.5 | 7.2 |
| | 7.4 | 7.7 | 7.6 | 7.5 | 7.4 |
| | 7.5 | 7.6 | 7.6 | 7.5 | 7.4 |
| | 7.4 | 7.7 | 7.6 | 7.5 | 7.5 |
| | 7.4 | 7.5 | 7.5 | 7.5 | 7.3 |
| | 7.5 | 7.7 | 7.6 | 7.5 | 7.5 |
| | 7.4 | 7.6 | 7.6 | 7.5 | 7.6 |
| | 7.7 | 7.8 | 7.5 | 7.6 | 7.6 |
| | 7.4 | 7.7 | 7.6 | 7.7 | 7.7 |
| | 7.5 | 7.7 | 7.6 | 7.7 | 7.7 |
| | 7.4 | 7.7 | 7.6 | 7.7 | 7.5 |
| | 7.5 | 7.7 | 7.5 | 7.6 | 7.5 |
| | 7.5 | 7.8 | 7.4 | 7.7 | 7.7 |
| Tarpley | 5.0 | 4.4 | 5.5 | 5.1 | 5.4 |
| | 4.7 | 4.7 | 5.9 | 4.9 | 5.3 |
| | 5.0 | 4.0 | 5.6 | 5.0 | 5.0 |
| | 5.1 | 4.2 | 5.6 | 5.1 | 5.1 |
| | 5.5 | 4.4 | 5.4 | 5.1 | 5.1 |
| | 5.4 | 4.5 | 5.5 | 5.4 | 5.3 |
| | 5.0 | 3.9 | 5.5 | 5.0 | 5.1 |
| | 5.2 | 4.6 | 5.3 | 5.0 | 5.0 |
| | 5.1 | 4.6 | 5.5 | 4.8 | 5.0 |
| | 5.3 | 4.1 | 5.5 | 5.5 | 5.1 |
| | 4.8 | 4.2 | 5.2 | 5.1 | 4.9 |
| | 5.0 | 4.6 | 5.8 | 4.9 | 5.3 |
| | 5.1 | 4.5 | 5.3 | 4.7 | 5.4 |
| | 4.9 | 4.5 | 5.2 | 5.0 | 5.6 |

***Table 31*. Post-harvest soil pH descriptive statistics of each treatment for TaB and HvB soils. Based on sample size of 14 plants per treatment.**

| | citric 0.1 mM | citric 100 mM | oxalic 0.1 mM | oxalic 100 mM | TSP |
|---|---|---|---|---|---|
| Houston Black | | | | | |
| Minimum | 7.2 | 7.5 | 7.4 | 7.5 | 7.2 |
| Maximum | 7.7 | 7.8 | 7.6 | 7.7 | 7.7 |
| Mean | 7.4 | 7.7 | 7.6 | 7.6 | 7.5 |
| Standard Deviation | 0.1 | 0.1 | 0.1 | 0.1 | 0.1 |
| Tarpley | | | | | |
| Minimum | 4.7 | 3.9 | 5.2 | 4.7 | 4.9 |
| Maximum | 5.5 | 4.7 | 5.9 | 5.5 | 5.6 |
| Mean | 5.1 | 4.4 | 5.5 | 5.0 | 5.2 |
| Standard Deviation | 0.2 | 0.2 | 0.2 | 0.2 | 0.2 |

**Discussion**

LMWOAs appear to have been as effective for P availability (mg $L^{-1}$) as TSP application in HvB soil but not as effective in TaB soil. In HvB soil, eggplant yields (g) were not significantly different but it is essential to acknowledge the ability of LMWOA treatments (mM $L^{-1}$) to mobilize P (mg $L^{-1}$) comparable with conventional TSP treatment for production purposes. In fact, analysis of eggplant harvest (g) for HvB soil demonstrates that all LMWOA treatments (mM $L^{-1}$) yielded slightly higher than TSP treatment by the end of the study (Table 8). This was likely due to relatively high CEC (44 meq 100 $g^{-1}$) with $Ca^{+2}$, which easily reacted under LMWOA treatments to render soluble P through dissolution and anion exchange of existing $Ca_3(PO_4)_2$ compounds as shown by Jones and Darrah (1994). The ability of all LMWOA treatments to sustain

eggplant production similar to yield using TSP suggests that precipitation of LMWOAs was not an impeding factor or that biodegradation from microorganism activity had an impact on LMWOAs as a P mobilization mechanism. This observation was somewhat different from the cases of Ström et al. (2001, 2002) in which they showed minimal microbial degradation rates for oxalic but not citric acid in calcareous soil at high pH. In comparison, this study demonstrated higher overall yields for citric 100 mM $L^{-1}$ than TSP treatment in HvB soil and may simply be due to differences in soil order used by Ström et al. (2001, 2002, 2005), in which they experimented with a calcareous mollisol (7.58 pH) but not vertisol soil. However, results of citric acid yields comply with a study by Gerke (1992) in which varying concentrations of citric acid (10-50 μmol g $soil^{-1}$) were found to slightly alter pH but that all soils tested showed a significant increase in P concentrations, with effects remaining persistent after 140 days of initial application. In fact, changes in original pH (7.8) for HvB soil were determined to be minimal which indicates its buffering capacity from high or low LMWOA concentrations (Table 30; Table 31). In addition, similarity of yield results between high and low LMWOA concentrations suggests lower LMWOA concentrations (e.g. 0.1 mM $L^{-1}$) may be very effective for production in HvB soils.

Compared to HvB, TaB soil was comparatively lower in CEC (19 meq 100 $g^{-1}$), which may have limited the amount of ion-exchange sites available for P dissolution as compared to HvB soils and significantly contributed to lower fruit yield in LMWOA treatments. TaB soils also contained less $CaCO_3$ content which was relevant from the beginning of experiments due to the visible reaction with applied LMWOAs and resulting effervescence ($CO_2$) in HvB soil but not in TaB soil applications (100 mM $L^{-1}$). With

less calcareous characteristics, poor yield in TaB soil may have been linked to low buffering capacity due to LMWOA applications. According to Marschner (2002), pH of calcareous soils is dependent on the presence of $CaCO_3$, which buffers soils ranging between 7.5-8.5 pH. In this position, TaB soil contained much less $CaCO_3$ than HvB soil according to soil test results (Figure 5; Figure 6) and were much less resistant to change in pH by exhibiting significant deviations from in situ soil pH (6.6) (Table 30; Table 31). Moreover, pH tests of TaB soil dropped to levels as low as 4.4 pH for citric (100 mM L-1) which may also explain lower overall yields compared to lower LMWOA concentrations (0.1 mM $L^{-1}$) and TSP treatment (Table 9). According to Hinsinger (2001), decreasing soil pH may result in a stronger retention and decreased mobility of P due to increased positive charges and larger protonation of Fe- or Al-oxides at low pH. Further, soil acidity may increase or decrease the rate of P diffusion while buffering capacity is inversely related to rate of diffusion by increasing the ratio of $H_2PO_4^-$ to $HPO_4^{2-}$ ions available for plant uptake (Gillespie and Pope 1991). These scenarios may also be coupled with a possible negative reaction of LMWOA treatments leading to excess Fe uptake by plants, due to a combination of readily abundant cations in solution from soil acidification effects during treatment. This probability is reminiscent to the outcomes of Jones and Darrah (1994), in which they recorded the mobilization efficiency of citric acid totaling about a 56% release of Ca plus a 10% release of Fe into solution for several soils. It is possible the LMWOAs in the already low pH soils TaB soils released excess $Fe^+$ at a level that was detrimental to production. For this reason, it may have been acceptable to treat TaB soils with non-protonated forms of LMWOAs (e.g. sodium citrate, sodium oxalate, potassium citrate, potassium oxalate) or adjust solution pH of

existing LMWOAs (e.g. HCl, KOH) before application in order to adjust pH in soils. Other studies have employed these methods in calcareous and acidic soils with mixed results (Khademi et al. 2010; Jones and Darrah 1994; Gerke 1992; Gerke 1994; Lopez-Hernandez et al. 1979; Fox and Comerford 1992). Khademi et al. (2010) found potassium citrate was more rapidly biodegraded than the $H^+$ form of citrate while oxalate forms had little to no effect on P availability in a calcareous soil. For two acid soils (3.8 and 6.0 pH), Lopez-Hernandez et al. (1979) found an increase in P due to citrate (20 mM), malate (15 mM), and oxalate (2.5 mM) mixed with KOH and likely due to the exchange of $OH^-$ ions for $H_2PO_4^-$ in addition to chelating mechanisms.

The difference in LMWOA effects between soils was most obvious through repeated P nutrient soil testing (mg $L^{-1}$). Preceding data comparisons between treatments, it was first necessary to remove extreme outliers from TSP treatments (mg $L^{-1}$) in both soils. This was justified by the high likelihood of soil sampling contamination in the field from TSP fertilizer granules, which may have partially inflated spectrophotometer readings ($\geq$ 50 mg $L^{-1}$). Ultimately, LMWOAs were competitive against TSP treatment in HvB soil during Test II (mg $L^{-1}$) analysis although with seemingly marginal differences (Figure 17). On the other hand, TaB soil showed less than excepted success with LMWOA treatments through significant amounts of unfavorable spectrophotometer readings (0 mg $L^{-1}$) even though a few TSP soil samples were similarly marked by equivalent readings (0 mg $L^{-1}$). This circumstance suggested that LMWOA treatments may not have been at fault for low soil test results (0 mg $L^{-1}$) in TaB soil but merely incapable in providing sufficient P nutrition needs for eggplant production purposes. Consistency of poor results for LMWOA treatments in TaB soil lead to further

speculation that instrumental error may have been partially responsible for providing inaccurate results, which were based on Olsen P extraction (1954) methods. Due to the slightly acid pH of TaB soil, it is likely that Fe-P or Al-P compounds were competitive P sources and, therefore, alternative extraction methods (e.g. Bray 1, Mehlich 1, Mehlich 3) may have provided more representable results of available P (mg $L^{-1}$) in this soil. As opposed to the anion replacement mechanism of the Olsen P method, P removal by these extractants includes the solvent action of acids like HCl, $H_2SO_4$, $HNO_3$, and $CH_3COOH$ that may be used to remove P from the solid phase of soil (Kamprath and Watson 1980). Although appropriate for TaB soil, these soil testing alternatives would likely offer a conflicting position in this study due to the predetermined role of LMWOA treatments in providing the main acidic action mechanism to mobilize P from the soil matrix. Even so, soil test results alone should not be the prime factor determining appropriateness of LMWOAs as a P fertilizer alternative but should be considered with various forms of measurement including yield or further changes in soil or plant reactions. According to Marschner (2002), soil analysis mostly provides an indication for soil capacity to supply nutrients to plants but does not always characterize the mobility of nutrients in the soil, which is partially determined by soil structure, microbial activity and plant factors such as root growth, and changes in the rhizosphere.

Production of eggplant using LMWOAs was highly dependent on soil type and treatment throughout experiments. A relatively smaller average fruit size from all treatments was also likely the result of limited root and shoot development from constraints of the pot production approach. Nevertheless, this study demonstrates the ability of LMWOAs to benefit eggplant production in high pH calcareous soils like HvB

Vertisols. The fact that TaB Mollisols showed quite insignificant results from both yield and soil tests also discloses that LMWOAs (citric acid, oxalic acid) may not serve as suitable alternatives for conventional P fertilizers or vegetable production purposes in less calcareous soils (<7.0 pH). However, the partial success of LMWOAs to compete with TSP fertilizers on a P-demanding crop like eggplant is enough evidence to consider further investigation and real-world applications using LMWOAs for large-scale production purposes where calcareous (>7.0 pH) soils dominate. The potential of LMWOAs as a P fertilizer substitute in calcareous soils is backed by an extensive body of knowledge dedicated to recognize LMWOAs as indispensable components in rhizosphere processes for P acquisition and plant nutrient uptake (Marschner et al. 2011; Jones 1998; Khademi et al. 2010; Oburger 2009; Lopez-Hernandez et al. 1979). The use of LMWOAs integrates natural biological cycles produced by plants and microorganisms to increase P availability in soils. Plants like *L. albus* have been shown to exude citric acid from proteoid root zones in response to surrounding P deficiency in calcareous soils (Dinkelater 1989). Microorganisms like *Penicillin bilaii* have been found to produce oxalic and citric acids that solubilized $CaHPO_4$ in agar cultures (Cunningham and Kuiack 1992). Kim et al. (1998) used *Enterobacter agglomerans* as a phosphate solubilizing bacteria along with an arbuscular fungus (*Glomus etunicatum*) to increase P uptake in *S. lycopersicum*. Together, these cases provide sufficient evidence to continue and further expand research for adopting such improvements as LMWOAs or microbial inoculants as marketable products that are linked to P mobilization capabilities in high pH calcareous soils. LMWOAs like citric, oxalic and gluconic acid are easily prepared through fermentation of glucose or sucrose by *A. niger* and in 1998 the worldwide production of

citric acid alone was 879,000 t (Magnuson and Lasure 2004). Other LMWOAs like acetic acid are produced using bacterial strains of *Acetobacter spp.* (Stevenson 1967). The facility of LMWOA production through plant and microbial metabolism alone is noble but the fact that it is possible to mimic these processes through derivative applications is even more profound to the potential impact it could have for future crop or vegetable production systems. There is additional proof in synergistic applications of LMWOAs with added P fertilizers that may also enhance crop productivity (Vassilev et al. 2006; Bolan et al. 1994), yet these methods bypass the conservation efforts for reduced mining of limited PR resources. Nevertheless, similar approaches to aid P soil solubilization should not be overlooked, as shown by Singh and Amberger (1998) in which LMWOAs were exclusively incorporated into a compost system through inoculation of P-solubilizing *Acetobacter spp*. Even further, Vassilev et al. (2006) suggest the use of microorganisms entrapped in gel or polyurethane foam as forms of inoculants, which may also help equip LMWOAs with alternative application modes in the future.

**Conclusion**

Outcomes of this investigation strengthens the prospect of adopting LMWOAs for crop production purposes in calcareous soils with pH >7.0 by examining the nature of P as a limited nutrient from soil sorption and unique plant uptake factors. LMWOA mechanisms serve as an exemplary model for confronting the multiple P obstacles facing agriculture today through simulation of rhizosphere processes that comprise of root and microbial means for facilitating P uptake in plants. By supplementing soils with pure

LMWOA applications and gaining significant results from this study, it may be fitting to directly employ LMWOA supplements as a potential P fertilizer alternative in order to help diminish PR-based fertilizer applications where soil conditions allow and conventional P fertilizers are inefficient. The problem of providing adequate P nutrition to agricultural soils is not just an application dilemma but also a limitation issue due to the growing concern of PR depletion in the next century. This study tackles that environmental issue with progress based on its department from PR fertilizer inputs for plant nutrient management and by promoting a divergent outlook on existing food production practices. The additional environmental factors associated with P fertilizers in agriculture are immense and it seems antithetical that PR scarcity concerns are accompanied by constant misuse of PR-based fertilizers with resulting problems like continuous eutrophication of water bodies. Although it may currently be cheaper to supply crop nutrients with PR-based fertilizers, the long-term costs associated with PR depletion may not sustain the current practices of agriculture business in the near future. With an ever-increasing global population expected to reach 9 billion by year 2050, agriculture faces many new challenges within the next few decades including the exponential demand for food, fiber, fodder and biofuels with a limited amount of natural resources. For these reasons, it is only appropriate to consider embracing natural rhizosphere cycles by adopting LMWOA mechanisms that facilitate native P uptake in calcareous soils among other regions. As shown here, future experiments should continue focusing on sustaining PR resources through enhanced LMWOA approaches that improve yields for alternative crop and soil production systems.

## CHAPTER III-PERSPECTIVES

This study was based on current research recognizing the ability of LMWOAs to assist P mobilization in the rhizosphere. Based on this research it is safe to conclude that using LMWOA treatments in bulk soils that are calcareous throughout serve a beneficial purpose in regards to vegetable production for eggplant. Nevertheless, the realm of these results may be coupled with additional questions that may also need further attention or adjustment for research purposes. Possible research modifications for this topic that should be considered for future research include the addition of a blank (0.0 g P, 0.0 mM LMWOA) control treatment to avoid the chance of confounding variables that may limit results of LMWOAs as a P nutrient alternative in vegetable production. This modification could possibly enhance results by further suggesting that LMWOAs are as useful as TSP treatment (g) and no fertilizer treatment(s) for field production. In addition, variable combinations of treatment applications in this area of research merit further expansion into other calcareous soil regions in order to test more distinctive forms of conventional P fertilizers and LMWOA treatments specific to crop and soil type influences.

Currently available research, including this study, has identified that success using LMWOAs for P mobilization and plant nutrition uptake in bulk soils is dependent on variables that may not apply consistently across different soil series. Further research methods that may serve as beneficial tools for measuring P mobility related to LMWOAs in production include analyzing soils by means of different P-extracting methods that may provide positive results in relation to soil type. Yet, careful consideration should be

taken into account when dealing with the extracting mechanism utilized by these methods since LMWOA treatment mechanisms are similar to those used in standard soil P testing practices for nutrient extraction. In addition to soil analysis, P sorption isotherms for determining soil P requirement, buffering capacity, diffusion coefficients and diffusion rates of P to plant roots may also provide more accurate explanations in phenomena dealing with LMWOAs in production and practices that may not solely be explained through soil P nutrient testing or yields.

Potential improvements in future studies include more considerable alternatives for use of LMWOA treatments in terms of application protocols. For example, managing stronger LMWOA treatments (100 mM $L^{-1}$) in the field may necessitate additional safety measures to avoid direct treatment contact with plant shoot system that could potentially burn leaf or stem parts and result in plant mortality. Meager yields (g) and mortality of four transplants from oxalic 100 mM $L^{-1}$ treatment were possibly due to partial human error during application process, in which acidic solution may have come into contact with shoot system and thus burning leaf tissue to the point of no recovery. Further, yields may also benefit from alternative application methods involving rates of application that mimic fertilizer injection systems and provide roots with a constant flow of LMWOAs for optimum growth and development. In terms of P nutrient availability from resultant treatments, future studies using LMWOAs may also benefit by including measurements that focus on plant nutrient uptake using root, shoot or fruit tissue as analytic forms of P nutrient uptake.

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