

Phosphorus Requirement and Chemical Fate in Containerized Nursery Crop Production

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Dissertation submitted to the faculty of the Virginia Polytechnic Institute and State University in
partial fulfillment of the requirements for the degree of

Doctor of Philosophy
In
Horticulture

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May 9, 2018
Blacksburg, VA

Keywords: orthophosphate, total dissolved phosphorus, particulate phosphorus, pine bark, dolomite, dolomitic limestone, micronutrients, container, nursery, nutrient uptake efficiency, fertilizer, *Hydrangea*, *Ilex*, *Rhododendron*, fractionation

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ABSTRACT

Environmental contamination issues related to phosphorus (P) in surface waters substantiates the need to identify minimally-sufficient P fertilization amounts for production of containerized nursery crops and better understand the effect of routine amendments (i.e., dolomite [DL] and micronutrient fertilizer [MF]) added to pine bark substrates on chemical fate of P fertilizer. Four studies were conducted to accomplish two overarching objectives: 1) determine the minimum P fertilization amount and corresponding pore-water P concentration needed to achieve maximal growth of common containerized nursery crops and 2) determine the effect of DL and MF amendments in pine bark on P retention during irrigation and P fractions in substrate pore-water. In a fertigation, greenhouse study, calculated lowest P-fertilizer concentration that sustained maximal growth in *Hydrangea paniculata* 'Limelight' (panicle hydrangea) and *Rhododendron* 'Karen' (azalea) was 4.7 and 2.9 mg·L⁻¹, respectively, and shoot growth *Ilex crenata* 'Helleri' (holly) was the same when fertilized with 0.5 to 6.0 mg·L⁻¹ P. Pore-water P concentrations corresponding with treatments that sustained maximal growth of panicle hydrangea, azalea and holly were as low as 0.6, 2.2 and 0.08 mg·L⁻¹ P, respectively. In a separate study, utilizing low-P controlled-release fertilizers (CRFs), shoot growth of *Hydrangea macrophylla* 'P11HM-11' (bigleaf hydrangea) produced in two ecoregions was maximal when fertilized with as little as 0.3 g CRF-P per 3.8-L container, a 50% P reduction from the industry-standard CRF. Holly required 0.2 or 0.4 g CRF-P depending on ecoregion. Mean pore-water P concentrations that corresponded with highest SDW were 0.8 and 1.2 mg·L⁻¹ for hydrangea and holly, respectively. When irrigating fallow pine bark columns containing CRF for 48 d,

amending pine bark with DL and MF reduced orthophosphate-P (OP-P) leachate concentrations by $\approx 70\%$, most of which was retained within the substrate. In a greenhouse study, containerized *Lagerstroemia* 'Natchez' (crape myrtle) were grown for 91 d in pine bark containing CRF. In pine bark amended with DL and MF, pore-water OP-P and total P concentrations, measured approximately weekly, were reduced by, on average, 64% and 58%, respectively. Total dry weight values of plants grown with DL plus MF or MF-only were 40% higher than those grown with no amendments; however, tissue P amounts and relative P uptake efficiency were the same among plants in these three treatments. Therefore, sorption of OP-P by DL and MF reduced water-extractable OP-P but did not limit P uptake by plants.

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ABSTRACT

Phosphorus (P) pollution of surface waters is a global issue that has led to widespread fish kills, drinking water contamination and disruption of aquatic ecosystems. Nutrient runoff from agricultural sites is among the leading contributors to P loads in impaired waters. Optimizing P fertilization for containerized nursery crop production is particularly challenging since the primary soilless substrates used to grow containerized crops retain P poorly. Consequently, much of the applied P leaches from containers during irrigation. Reducing amounts of applied P fertilizer and amending substrates (e.g., pine bark) with P-sorbing materials are two methods previously shown to reduce P leaching and increase the proportion of applied P that is absorbed by containerized plants. Four studies were conducted to accomplish two overarching objectives: 1) determine the minimum P fertilization amount necessary for maximal growth of common containerized nursery crops and 2) determine the effect of dolomite (DL) and micronutrient fertilizer (MF) amendments in pine bark on P retention during irrigation. Our findings indicated that P fertilization requirements of woody ornamental crops is species-dependent. When using liquid fertilizer, Japanese holly and evergreen azalea achieved maximal growth when P fertilizer concentrations were reduced by 90% and 40%, respectively, compared to current recommendations. In contrast, the current minimum fertilizer recommendation of 5 ppm P was optimal for panicle hydrangea. In a subsequent study in which containerized woody ornamentals were grown using low-P controlled-release fertilizers (CRFs), bigleaf hydrangea reached maximal growth when given CRFs containing 50% less P than amounts in conventional CRFs. Considering hydrangea and azalea are among the top woody ornamental shrubs produced

in the US, using fertilizers with minimally sufficient P amounts for these species could greatly reduce P runoff from nursery sites. Results of two studies conducted to achieve the second aforementioned objective indicated that amending CRF-fertilized pine bark with DL and MF can reduce water-extractable total P concentrations by > 50%. Despite lower levels of plant-available P in the substrate, P uptake by crape myrtle was unaffected by the amendments. The DL was primarily responsible for P retention in pine bark; however, the addition of MF was needed for maximal growth and P uptake of crape myrtle. According to this research, amending pine bark with DL and MF could be considered a best management practice for reducing P leaching from containerized crops.

Dedication

I dedicate these pages to my wife, Kaitlin. This work would not have been possible without her steadfast love, support and encouragement during my pursuit of this degree.

Acknowledgements

This dissertation would not have materialized without the support provided by my wife, family, friends, advisory committee and various colleagues. Firstly, I am indebted to my wife, Kaitlin, for the innumerable sacrifices she made during my pursuit of this degree. Her encouragement and understanding has been incredible, and words cannot express my appreciation for her standing by my side at every step of this journey. I would like to express my sincerest gratitude to my family: Mom, Dad, Michael, Joby and Annie. They have provided tremendous support and advice throughout my entire academic career and introduced me to the nursery industry. A special thanks is also warranted to Donna, Page and Olivia Kellam for welcoming me into their home countless times during my ventures to the Hampton Roads AREC and offering to assist in any way possible.

I would like to express my deepest appreciation to my co-advisors, Drs. Jim Owen and Alex Niemiera for their unfaltering faith in me, honesty, accessibility and friendship throughout my time under their advisement. Their selfless dedication to the success of their graduate student advisees is exemplary. Specifically, I would like to thank Dr. Owen for his leadership in the research component of my degree, and Dr. Niemiera for his mentorship in teaching.

I am sincerely grateful to Dr. James Altland for his invaluable advice in statistical analysis and technical writing as well as his thorough, yet prompt, reviews of manuscripts. I am greatly appreciative of Dr. Matt Eick, for imparting his expertise of phosphorus chemistry and patiently providing guidance in using Visual MINTEQ. Thank you to Dr. Brian Jackson for his irreplaceable teaching insight and for challenging me to gain a deeper understanding of the various substrate extraction methods.

Staff members in the department of horticulture at both the main campus and Hampton Roads AREC locations have been central to the success of my research. Words cannot fully express my appreciation for Velva Groover's friendship, reliability and selflessness. Velva was a lifeline on many occasions in the lab, field or greenhouse. The Hampton Road AREC team, specifically Julie Brindley and Anna Birnbaum, were so generous with their time, resources and facilities. They were an incredible team with whom to work, and I greatly appreciate the energy put in to supporting me. John James and Jeff Burr were also vital to the success of my studies conducted at the UHC and greenhouses. Doug Sturtz played a key role in my sample analysis using the ICP-OES, patiently communicating any issues and findings throughout the process. A special thanks to Dr. Sarah White and her team for analyzing all of my DOC samples. I am so grateful for all of the above individuals and groups for their time, expertise and support throughout all of my research.

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Chapter 1

Introduction

Nutrient enrichment and subsequent eutrophication of receiving waters has profound effects on aquatic resources. Proliferation of primary producers, including toxic algae and cyanobacteria species, induced by elevated nutrient levels in aquatic ecosystems has resulted in species-biodiversity loss, contamination of drinking water and widespread fish kills (Carpenter et al., 1998). For eutrophication to ensue, critical concentrations of both nitrogen (N) and phosphorus (P) must be present; however, P is generally regarded as the limiting nutrient for accelerated growth of photosynthesizing organisms (e.g., phytoplankton, algae, cyanobacteria, plants) in fresh water ecosystems (Correll, 1998; Khan and Mohammad, 2014; Schindler et al., 2008). Surface water P loads are controlled by two sources: point sources (e.g., sewage, industrial discharge) and nonpoint (i.e., diffuse) sources (e.g. urban and agriculture runoff). Since passing of the Clean Water Act in 1972, P loads delivered by point-sources have largely been curtailed in the U.S. (Bennett et al., 2001). In contrast to point sources, diffuse sources of nutrient inputs are often difficult to identify and, hence, regulate. Accordingly, diffuse P inputs, particularly those from agricultural runoff, remain the primary source of P overenrichment and subsequent eutrophication in U.S. surface waters (Boesch et al., 2001; Carpenter et al., 1998; Michalak et al., 2015; USEPA, 2018).

In the U.S., containerized woody plants contribute 63% of all nursery stock sales (USDA, 2014), and the predominate substrate utilized in Southeastern U.S. for container-grown nursery crops is pine bark (*Pinus taeda*; Bilderback et al., 2013; Lu et al., 2006). Pine bark has limited ability to sorb P (Marconi and Nelson, 1984; Paradelo et al., 2017; Yeager and Wright, 1982); consequently, soluble P fertilizers rapidly leach from pine bark-filled containers during irrigation

(Cole and Dole, 1997; Godoy and Cole, 2000; Yeager and Barrett, 1984, 1985a, 1985b). This issue of P leaching is exacerbated since container-grown crops are typically irrigated daily during the growing season and P fertilization amounts commonly exceed plant requirements for maximal growth (Risvey et al., 2007).

Various best management practices (BMPs) have been developed to reduce nutrient runoff from containerized nursery sites (Bilderback et al., 2013). To reduce nutrient leaching, BMPs recommend applying the minimum amount of fertilizer that achieves the desired growth and quality. Currently, BMPs suggest 5 to 15 mg·L⁻¹ P be maintained in pour-through-extracted substrate pore-water for containerized crop production. However, multiple studies have cited that maximal growth of containerized woody nursery crops was achieved when pore-water P concentrations were consistently < 5 mg·L⁻¹. To optimize P fertilization, research is needed to determine the minimum pore-water P concentration needed for maximal growth of common woody nursery crops.

In addition to decreasing P fertilization, amending pine bark with P-sorbing materials has also been shown to reduce P leaching. Multiple researchers have shown that various calcined clay products can reduce P leaching from container substrates (Owen et al., 2007; Ogutu et al., 2009; Ruter, 2004). However, adoption of P-sorbing clay products by the U.S. nursery industry has been slow (Bilderback, personal communication), which may be partially attributed to their relatively high cost of shipping and purchase (Ogutu et al., 2009) as well as the fact that growers are unaccustomed to these unconventional amendments. In contrast to calcined clay amendments, dolomitic limestone (DL) and micronutrient fertilizer (MF) are routinely mixed into container substrates prior to potting. Both DL and components in MF can interact with P,

subsequently reducing P solubility. However, these interactions have not been thoroughly investigated in pore-water of pine bark substrate.

Four studies were conducted to accomplish two overarching objectives: 1) to determine the minimum P fertilization amount and corresponding pore-water P concentration needed to achieve maximal growth of multiple economically-important containerized nursery crops and 2) determine the effect of DL and MF amendments in pine bark on P retention during irrigation and P fractions in water-extracted substrate pore-water.

Literature Review

Phosphorus uptake efficiency (PUE)

The use of polymer- or resin-coated controlled-release fertilizers (CRFs), a best management practice (Bilderback et al., 2013) that reduces P leaching relative to soluble fertilizers (Broschat, 1995; Diara, 2014), has been widely adopted by the U.S. nursery industry according to survey studies (Dennis et al., 2010; Fain et al., 2000; Mack et al., 2017). However, P uptake efficiency (PUE, percent of applied P taken up by plant roots) of container-grown nursery crops has been reported to remain between 7% and 62% (McGinnis et al., 2009; Owen et al., 2008; Tyler et al., 1996; Warren et al., 1995; Warren et al. 2001) when using CRFs.

Phosphorus uptake efficiency of containerized crops can be improved without affecting plant growth by reducing P fertilization. In two studies utilizing liquid-fertilization, Ristvey et al. (2004, 2007) observed increased PUE of containerized *Rhododendron* 'Karen' grown in a pine bark-peat substrate from $\approx 15\%$ to $\approx 45\%$ by decreasing P from 25 to 5 mg per week when applied N was non-limiting for growth (i.e., 100 to 250 mg·L⁻¹ N). In both studies, shoot growth was unaffected by P fertilizer level. When using a CRF, Owen et al. (2008) found that reducing P from 1.0 to 0.5 g per container improved PUE of *Cotoneaster dammeri* 'Skogholm' by 11% without affecting shoot growth when grown in a pine bark substrate amended with 11% builder's sand (by volume).

Low-phosphorus fertilization

Fertilizer recommendations for container-grown crops are often based on nutrient levels found in substrate pore-water extracted via the pour-through method (Wright, 1986). Currently, BMPs suggest 5 to 15 mg·L⁻¹ P be maintained in pour-through-extracted substrate pore-water

(Bilderback et al., 2013). This recommendation is, in part, based on the study by Yeager and Wright (1982) who grew *Ilex crenata* ‘Helleri’ in 100% pine bark fertigated with P concentrations ranging from 0.0 to 30.0 mg·L⁻¹ P. The authors found that shoot growth increased as applied P and concomitant pore-water P was increased from 0 to 5 mg·L⁻¹, but ≥ 5 mg·L⁻¹ P did not increase shoot growth. However, plant growth response to P fertilizer concentrations in the range of 0.0 to 5.0 mg·L⁻¹ P was not investigated.

Despite BMP recommendations, evidence in scientific literature suggests that < 5 mg·L⁻¹ P in substrate pore-water is sufficient for growing salable woody plants in soilless substrates. In a substrate consisting of 2 perlite : 1 peat (by volume), Havis and Baker (1985) observed maximal growth of *Rhododendron* ‘Victor’ when liquid-fertilizing with 2.5 mg·L⁻¹ P. Similarly, Matysiak (2015) observed that pour-through P concentrations for CRF-fertilized *Euonymus japonicus* ‘Ovatus Aureus’ and *Rhododendron* ‘Geisha Orange’ associated with highest quality ratings and shoot fresh weight had ≤ 2.6 mg·L⁻¹ P in pour-through leachate when grown in a sphagnum peat substrate. Million et al. (2007) observed no change in plant size index [i.e., (plant height + plant width) \div 2] of CRF-fertilized *Viburnum odoratissimum* grown in 2 aged pine bark : 1 sphagnum peat moss : 1 coarse sand (by volume) when doubling the irrigation rate reduced pour-through-extracted P concentration ranges from 5 to 11 mg·L⁻¹ down to 1 to 4 mg·L⁻¹. When growing CRF-fertilized *Cotoneaster dammeri* ‘Skogholm’ in 8 pine bark : 1 sand (by volume), Groves et al. (1998) observed no apparent plant growth reduction even though pour-through extracted P levels were consistently lower than 5 mg·L⁻¹. Few studies have specifically assessed the lowest necessary P content in CRFs and resulting pore-water P concentration for maximal growth of container-grown nursery crops. Leonard et al. (2007) grew *Codiaeum variegatum* var. *pictum* ‘Mammy’ and *Chrysobalanus icaco* potted in a pine bark-peat substrate and fertilized with CRF

containing 18% N, 10% K and 0%, 0.4%, 1.3%, or 2.6% P. In both taxa, growth index values were highest in plants fertilized with CRF granules containing at least 0.4% P in which pour-through leachate concentrations were 0.3 to 0.7 mg·L⁻¹.

Dolomite-phosphorus interactions

Pine bark has an initial pH range of 4.0 to 4.3, yet optimal plant nutrient availability in organic substrates occurs when pH is 5.0 to 5.5 (Bunt, 1988). Consequently, growers routinely amend pine bark with a liming agent to increase pH and supply potentially growth limiting base cations. Dolomitic limestone [DL; CaMg(CO₃)₂] is a common liming agent incorporated into substrates for nursery crop production that supplies plants with both calcium (Ca) and magnesium (Mg). However, the possible effects of DL on P solubility in pine bark substrate has not been assessed. Multiple studies have reported DL sorption of orthophosphate (OP) in aqueous solution (Karaca et al., 2004; Karaca et al., 2006; Mangwandi et al., 2014; Xu et al., 2014; Yuan et al., 2014; Yuan et al., 2015). For example, in batch sorption experiments, Yuan et al. (2014, 2015) found that 10 g DL removed up to 98% P from 1 L solution containing 50 mg·L⁻¹ P. Karaca et al. (2006) reported rapid sorption of OP by DL in solution, with equilibrium occurring within 15 to 30 min depending on initial OP concentration. Several mechanisms have been proposed to explain OP removal by DL, including chemisorption (Karaca et al., 2004; Yuan et al., 2015), intraparticle diffusion (Karaca et al., 2004), physical adsorption (Karaca et al., 2006), outer-sphere complexation and surface precipitation (Mangwandi et al., 2014). Xu et al. (2014) utilized attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, X-ray absorption near edge structure (XANES) spectroscopy, and diffuse layer modeling to investigate P speciation on calcite and DL. When solution pH values were 5.0 to 7.0 and

orthophosphate-P (OP-P) concentrations were 2 or 10 mg·L⁻¹, which are common conditions in pore-water of DL-amended pine bark substrate, the authors determined that sorbed OP on the surface of DL was primarily in the form of Mg and Ca surface complexes and Ca-P secondary minerals.

Evidence of OP sorption by liming agents has also been observed in organic container-substrates. Amending peat-based substrates with varying rates of Ca(OH)₂ was shown to reduce P leaching from containers by 20% (Haynes, 1982) to 88% (Havis and Baker, 1985) compared to substrates without Ca(OH)₂. Two studies by Argo and Biernbaum (1996a and 1996b) reported that the amount of soluble OP in a peat-based substrate amended with various forms of DL was inversely related to the substrate pH. For example, Argo and Biernbaum (1996a) measured OP-P concentrations in saturated media extracts (Warncke, 1986) after growing *Impatiens walleriana* fertilized with 20 mg·L⁻¹ P for 16 weeks in a Sphagnum peat-based substrate containing 1.5 kg·m⁻³ microfine dolomitic hydrated lime [Ca(OH)₂·MgO] or 8.4 kg·m⁻³ dolomitic carbonate lime (CaCO₃·MgCO₃). The authors found that extract OP-P concentrations for both lime treatments were ≈ 21 and 3 mg·L⁻¹ for substrates with pH values of ≈ 5.0 and 8.1, respectively, and speculated that the reduction in soluble OP-P was due to precipitation of P as CaHPO₄ or CaH₅O₆P. However, reports on the effect of liming agents, specifically DL, on OP leaching from pine bark substrate are limited. One week after potting *Buddleia davidii* in a pine bark-based substrate, Altland et al. (2015) observed a linear decrease in pour-through-extracted pore-water P as DL amendment treatment incrementally increased from 0.0 to 14.3 kg·m⁻³. However, DL had no effect on pore-water P in substrate extracts collected at 4 or 16 weeks after potting.

Iron-phosphorus interactions

In addition to a liming agent, growers of containerized crops routinely amend organic substrates with a granular fertilizer containing micronutrients (MFs). Iron, which is a primary constituent of many MFs, is often supplied in chelated form or as FeSO_4 . Ferrous sulfate has been shown to reduce OP solubility due to Fe-PO_4 precipitation (Moore and Miller, 1994; Rich, 2005; Tasistro and Kissel, 2006) and OP sorption to humic substances via Fe bridges (Gerke and Hermann, 1992; Weir and Soper, 1963). Iron-phosphate precipitation is dependent on pH and Fe:P ratio. Hsu (1976) determined that decreasing the P:Fe molar ratio resulted in greater P removal from solution, and when the P:Fe molar ratio was 0.2 to 0.5, the optimal pH for precipitation was 4.7 to 7.1. At a pH of 4.5, Fytianos et al. (1998) found that ferrous iron removed 63% of P in aqueous samples when added at a 1P:1Fe molar ratio. Rich (2005) concluded that ferrous and ferric sulfate effectively removed OP from natural waters when buffered pH was between 7.0 and 8.0 and determined the mechanism of OP removal was formation of the insoluble precipitates, $\text{Fe}_2(\text{HPO}_4)_3$, $\text{Fe}(\text{H}_2\text{PO}_4)_3$, and $\text{Fe}_3(\text{PO}_4)_2$.

Phosphate- Fe(II)SO_4 interactions have also been observed in pine bark. Findings from multiple studies performed by Handreck (1991a, 1991b, 1992, 1996) have revealed that increasing the quantity of $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$ in pine bark-based substrates decreases the amount of diethylenetriaminepentaacetic acid (DTPA)-extractable P. However, Fe-P chemical speciation in pore-water of irrigated pine bark substrate has not been thoroughly investigated. Determining Fe-P species in effluent of irrigated pine bark would have environmental implications since many Fe-P species are water-insoluble (Sanyal and De Datta, 1991).

Phosphorus fractions

In natural waters, P fractionation is commonly utilized to assess the threat of P to water quality (Worsfold et al., 2016). In soil-water extracts, fractioning P provides insight into P lability for plant uptake. Orthophosphate (H_3PO_4 , H_2PO_4^- , HPO_4^{2-} or PO_4^{3-}) is the most bioavailable P species for uptake by plants, planktonic algae and bacteria (Boström et al., 1988). In water samples, orthophosphate is routinely determined via colorimetric methods (e.g., molybdate blue method) or ion chromatography (IC; Worsfold et al., 2016) after samples are filtered through a 0.22 or 0.45 μm membrane (Brober and Persson, 1988). However, Hens and Merckx (2002) and Maruo et al. (2016) reported release of OP from filterable colloids ($< 0.45 \mu\text{m}$) during colorimetric analysis ($\text{pH} < 1$) resulting in overestimation of OP concentrations. Hence, IC may be a more effective instrument for estimating actual OP concentrations compared to traditional colorimetric methods. The total dissolved P (TDP) fraction is operationally defined as that which passes through a 0.45 μm filter and includes orthophosphate and condensed, organic, and colloidal phosphates (McKelvie et al., 1995). If filtrate is first digested to convert all P into OP, TDP can be determined colorimetrically; however, analyzing TDP concentrations with inductively coupled plasma atomic emission spectroscopy (ICP-AES) may be a simpler approach since digestion is not required (Rowland and Haygarth, 1997; Van Moorlegheem et al., 2011). Orthophosphate-P and TDP values have been shown to diverge as dissolved organic carbon (DOC) content of the analyzed matrix increases (Zhang et al., 2004), purportedly due to the increased presence of dissolved organic P compounds. Hence, OP-P and TDP should not be interpreted interchangeably in solutions with relatively high DOC, which would be expected in water extracts or leachate from pine bark substrate. Particulate P (PP) fraction is determined by subtracting TDP from total P (TP) analyzed via ICP-AES after digestion of non-filtered sample.

Components in PP, operationally defined as the P retained by a 0.45 μm filter (McKelvie et al., 1995), include biological sources (e.g., material from plants, animals and bacteria), weathering products (e.g., primary and secondary minerals), inorganic P precipitates, coprecipitates and organic aggregates (Broberg and Persson, 1988).

Despite P fractions' varying degree of relevance to plant availability and surface water quality, few studies have measured P fractions in container-substrate extracts or leachate from containerized crop production. Yeager and Barrett (1984, 1985a) found that TP and TDP concentrations were the same in effluent of superphosphate-amended pine bark columns. In a study assessing DTPA extracts of pine bark from potted *Hakea francisiana* and *Hakea laurina* amended with crushed bone, rock phosphate, calcined rock phosphate or sewage sludge, Handreck (1996) observed minor differences between TP and OP-P concentrations in the range of 0 to 25 $\text{mg}\cdot\text{L}^{-1}$ P. Million et al. (2007) analyzed for TP and OP-P in runoff when producing containerized *Viburnum odoratissimum* grown in DL- and MF-amended substrate composed of 2 pine bark : 1 Sphagnum peat : 1 coarse sand (by volume). The authors found that 89% to 92% of TP in runoff was in the OP-P fraction. To our knowledge, PP, TDP, OP-P and non-orthophosphate dissolved P (NODP) concentrations and the impact of DL and MF on these P fractions has not been studied in effluent or water-extracts of pine bark substrate with incorporated CRF.

Literature Cited

- Altland, J.E., W. Zellner, and J.C. Locke. 2015. Substrate pH and butterfly bush response to dolomitic lime or steel slag amendment. *J. Environ. Hort.* 33:89–95.
- Argo, W.R. and J.A. Biernbaum. 1996a. The effect of lime, irrigation-water source, and water-soluble fertilizer on root-zone pH, electrical conductivity, and macronutrient management of container root media with impatiens. *J. Amer. Soc. Hort. Sci.* 121:442–452.
- Argo, W.R. and J.A. Biernbaum. 1996b. Availability and persistence of macronutrients from lime and preplant nutrient charge fertilizers in peat-based root media. *J. Amer. Soc. Hort. Sci.* 121:453–460.
- Bennett, E.M., S.R. Carpenter, and N.F. Caraco. 2001. Human impact on erodible phosphorus and eutrophication: A global perspective. *BioScience* 51:227-234
- Bilderback, T., C. Boyer, M. Chappell, G. Fain, D. Fare, C. Gilliam, B.E. Jackson, J. Lea-Cox, A.V. LeBude, A. Niemiera, J. Owen, J. Ruter, K. Tilt, S. Warren, S. White, T. Whitwell, R. Wright, and T. Yeager. 2013. *Best management practices: Guide for producing nursery crops*. 3rd ed. Southern Nursery Association, Acworth, GA.
- Bilderback, T.E., E.D. Riley, B.E. Jackson, H.T. Kraus, W.C. Fonteno, J.S. Owen, Jr., J.E. Altland, G.B. Fain. 2013. Strategies for developing sustainable substrates in nursery crop production. *Acta Hort.* 1013:43–56.
- Boesch, D. F., R. B. Brinsfield, and R.E. Magnien. 2001. Chesapeake Bay eutrophication: scientific understanding, ecosystem restoration, and challenges for agriculture. *J. Environ. Qual.* 30: 303-320.
- Boström B., G. Perrson, and B. Broberg. 1988. Bioavailability of different phosphorous forms in freshwater systems. *Hydrobiologia.*170:133–155.

- Broberg, O, and G. Persson. 1988. Particulate and dissolved phosphorus forms in freshwater: composition and analysis. *Hydrobiologia* 170:61-90.
- Broschat, T. 1995. Nitrate, phosphate, and potassium leaching from container-grown plants fertilized by several methods. *HortScience* 30:74–77.
- Carpernter, S.R. , N.F. Caraco, D.L, Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* 8:559–568.
- Cole, J.C., and J.M. Dole. 1997. Temperature and phosphorus source affect phosphorous retention by pine bark- based container medium. *HortScience.* 32:236–240.
- Correll, D.L. The role of phosphorous in eutrophication of receiving waters: A review. *J. Environ. Qual.* 27:261-266.
- Dennis, J.H., R.G. Lopez, B.K. Behe, C.R. Hall, C. Yue, B.L. Campbell. 2010. Sustainable production practices adopted by greenhouse and nursery plant growers. *HortScience* 45:1232-1237.
- Diara, C., L. Incrocci, G. Incrocci, G. Carmassi, A. Pardossi, P. Marzialetti, P. Cozzi, F. Fibbi, and R. Clemens. 2014. Reduction of nutrient run-off by the use of coated slow-release fertilizers on two container-grown nursery crops. *Acta Hort.* 1034:85–92.
- Fain, G.B., C.H. Gilliam, K.M. Tilt, J.W. Olive, and B. Wallace. 2000. Survey of best management practices in container production nurseries. *J. Environ. Hort.* 18:142–144.
- ytianos, K., E. Voudrias, and N. Raikos. 1998. Modeling of phosphorous removal from aqueous and wastewater samples using ferric iron. *Environmental Pollution.* 101:123–130.
- Gerke, J., and R. Hermann. 1992. Adsorption of orthophosphate to Humic-Fe-Complexes and to amorphous Fe-Oxide. *Z. Pflanzenernähr. Bodenk.* 155:233–236.

- Godoy, A., and J.C. Cole. 2000. Phosphorous source affects phosphorous leaching and growth of containerized spirea. *HortScience*. 35(7):1249–1252.
- Groves, K.M., S.L. Warren, and T.E. Bilderback. 1998. Irrigation volume, application, and controlled-release fertilizer. II. Effect on substrate solution nutrient concentration and water efficiency in containerized plant production. *J. Environ. Hort.* 16:182–188.
- Handreck, K.A. 1991a. Interactions between iron and phosphorus in the nutrition of *Banksia ericifolia* L.f. var. *ericifolia* (Proteaceae) in soil-less potting media. *Aust. J. Bot.* 39:373–84.
- Handreck, K.A. 1991b. Available phosphorus in potting media: Extractants and interpretation of extract data. *Commun. Soil Sci. Plant Anal.* 22:529–557.
- Handreck, K.A. 1992. Iron phosphorous interactions in in the nutrition of seedling macadamia in organic potting media. *Australian journal of experimental horticulture.* 32:773–779.
- Handreck, K.A. 1996. Comparison of phosphate-phosphorous and total phosphorus in DTPA extracts for assessing plant-available phosphorus in soilless potting media. *Commun. Soil Sci. Plant Anal.* 27:2125–2135.
- Havis, J.R., and J.H. Baker. 1985. Influence of liming rate and phosphorus leaching from a peat-sand medium. *J. Environ. Hort.* 3(2):74–76.
- Haynes, J.R. 1982. Leaching losses of nutrients and yield and nutrient uptake by container-grown begonia as affected by lime and fertilizer applications to a peat medium. *J. Sci. Food Agric.* 33:407–413.
- Hens, M., and R. Merckx. 2002. The role of colloidal particles in the speciation and analysis “dissolved” phosphorous. *Water Res.* 36:1483–1492.

- Hsu, P.H. 1976. Comparison of iron(III) and aluminum in precipitation of phosphate from solution. *Water Research*. 10: 903–907.
- Karaca, S., A. Gürses, M. Edjer, and M. Açıkyıldız. 2004. Kinetic modeling of liquid-phase adsorption and phosphate on dolomite. *J. Colloid Interface Sci*. 277:257–263.
- Karaca, S., A. Gürses, M. Edjer, and M. Açıkyıldız. 2006. Adsorptive removal of phosphate from aqueous solutions using raw and calcinated dolomite. *J. Hazard. Mater. B128*: 273–279.
- Khan, M.N. and F. Mohammad. 2014. Eutrophication: Challenges and Solutions. In Ed. Ansari, A.A., and S.S. Gill. *Eutrophication: Causes, Consequences and Control Volume 2*. Springer, Dordrecht Heidelberg London New York.
- Leonard, M., M. Orfanedes, and T. Yeager. 2007. Response of croton and coco plum to phosphorus. *Proc. South. Nurs. Assoc. Res. Conf*. 52:531–534.
- Lu, W., J.L. Sibley, C.H. Gilliam, J.S. Bannon, and Y. Zhang. 2006. Estimation of U.S. bark generation and implications for horticultural industries. *J. Environ. Hort*. 24(1);29–34.
- Michalak, A.M., E.J. Anderson, D. Beletsky, S. Boland, N.S. Bosch, T.B. Bridgeman, J.D. Chaffin, K.Cho, R. Confesor, I. Daloglu, J.V. DePinto, M.A. Evans, G.L. Fahnenstiel, L. He, J.C. Ho, L. Jenkins, T.H. Johengen, K.C. Kuo, E. LaPorte, X. Liu, M.R. McWilliams, M.R. Moore, D.J. Posselt, R.P. Richards, D. Scavia, A. L. Steiner, E. Verhamme, D.M. Wright, and M.A. Zagorski. 2015. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. *Proc. Natl. Acad. Sci*. 110:6448-6452
- Mack, R., J.S. Owen, A.X. Niemiera, and J. Latimer. 2017. Virginia nursery and greenhouse grower survey of best management practices. *HortTechnology* 27:386–392.

- Mangwandi, C., A. B. Albadarin, Y. Glocheux, G. M. Walker. Removal of ortho-phosphate from aqueous solution by adsorption onto dolomite. *J. Environ. Chem. Eng.* 2:1123–1130.
- Marconi, D.J. and P.V. Nelson. 1984. Leaching of applied phosphorous in container media. *Sci. Hort.* 22:275–285.
- Maruo, M. M. Ishimaru, Y. Azumi, Y. Kawasumi, O. Nagafuchi, and H. Obata. 2016. Comparison of soluble reactive phosphorous and orthophosphate concentrations in river waters. *Limnology.* 17:7–12.
- Matysiak, B. 2015. Growth response of container grown Japanese azalea and euonymus and concentration of nitrates and phosphates in the runoff water under different irrigation and fertilization. *J. Hort. Res.* 23:21–32.
- McGinnis, M.S., S.L. Warren, and T.E. Bilderback. 2009. Replacing conventional nursery crop nutrient inputs with vermicompost for container production of *Hibiscus moscheutos* L. ‘Luna Blush’. *HortScience* 44:1690-1703.
- McKelvie, I.D., D. M.W. Peat, and P.J. Worsfold. 1995. Techniques for the quantification and speciation of phosphorous in natural waters. *Anal. Proc. incl. Anal. Commun.* 32:437–445.
- Million, J., T. Yeager, and J. Albano. 2007. Consequences of excessive overhead irrigation on runoff during container production of sweet viburnum. *J. Environ. Hort.* 25(3): 117–125.
- Moore, P.A., Jr., and D.M. Miller. 1994. Decreasing phosphorus solubility in poultry litter with aluminum, calcium, and iron amendments. *J. Environ. Qual.* 23:325–330.
- Ogutu, R. A., and K.A. Williams. 2009. Phosphate sorption of calcined materials used as components of soilless root media characterized in laboratory studies. *HortScience.* 44(2):431-437.

- Owen, J.S., S.L. Warren, T.E. Bilderback, and J.P. Albano. 2008. Phosphorus rate, leaching fraction, and substrate influence on influent quantity, effluent nutrient content, and response of a containerized woody ornamental crop. *HortScience* 43:906–912.
- Owen, J.S., Jr., S.L. Warren, T.E. Bilderback, and J.P. Albano. 2007. Industrial mineral aggregate amendment affects physical and chemical properties of pine bark substrates. *HortScience* 42:1287-1294.
- Paradelo, R., M. Conde-Cid, M. Arias-Estévez, J.C. Nóvoa-Muñoz, E. Álvarez-Rodríguez, M.J. Fernández-Sanjurjo, A. Núñez-Delgado. 2017. Removal of anionic pollutants by pine bark influenced by the mechanism of retention. *Chemosphere* 167:139–145.
- Rich, D. 2005. The removal of total phosphorus from natural waters by precipitation. Ph.D. diss., Northwestern International Univ., Evanston, IL.
- Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2004. Nutrient uptake, partitioning and leaching losses from container-nursery production systems. *Acta Hort.* 630:321–328.
- Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2007. Nitrogen and phosphorus uptake efficiency and partitioning of container-grown azalea during spring growth. *J. Amer. Soc. Hort. Sci.* 132:563–572.
- Rowland, A.P. and P.M. Haygarth. 1997. Determination of total dissolved phosphorous in soil solutions. *J. Environ. Qual.* 26:410–415.
- Ruter, J.M. 2004. Rate of calcined clay incorporation influences phosphorus retention in a pine bark substrate. *Proc. South. Nurs. Assoc. Res. Conf.* 49:23-24.
- Sanyal, S.K., and S.K. De Datta. 1991. Chemistry of phosphorous transformations in soil. *Adv. Soil Sci.* 16:1–94.

- Schindler, D.W., R.E. Hecky, D.L. Findlay, M.P. Stainton, B.R. Parker, M.J. Paterson, K.G. Beaty, M. Lyng, and S.E.M. Kasian. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proc. Natl. Acad. Sci.* 105:11254-11258
- Tasistro, A. S., and D.E. Kissel. 2006. Composition of aqueous extracts of broiler litter treated with aluminum sulfate, ferrous sulfate, ferric chloride and gypsum. *J. Environ. Sci. Health B* 41(6); 989–1000.
- Tyler, H.H., S.L. Warren, and T.E. Bilderback. 1996. Reduced leaching fractions improve irrigation use efficiency and nutrient efficacy. *J. Environ. Hort.* 14:199–204.
- U.S. Department of Agriculture. 2014. 2012 Census of Agriculture: Census of horticultural specialties (2014). p. 24.
- U.S. Environmental Protection Agency. 2017. Nutrient pollution: Sources and solutions. <https://www.epa.gov/nutrientpollution/sources-and-solutions> (accessed 15 May 2018).
- Van Moorlegem, C., L. Six, F. Degryse, E. Smolders, and R. Merckx. 2011. Effect of organic P forms and P present in inorganic colloids on the determination of dissolved P in environmental samples by the diffusive gradient in thin films technique, ion chromatography, and colorimetry. *Anal. Chem.* 83:5317–5323. doi:10.1021/ac200748e.
- Warncke, D.D. 1986. Analyzing greenhouse growth media by the saturation extraction method. *HortScience* 21:223-225.
- Warren, S.L., T.E. Bilderback, and H.H. Kraus. 2001. Method of fertilizer application affects nutrient losses of controlled-release fertilizer. *Acta Hort.* 548:349–355.
- Warren, S.L., T.E. Bilderback, and H.H. Tyler. 1995. Efficacy of three nitrogen and phosphorus sources in container-grown azalea production. *J. Environ. Hort.* 13:147–151.

- Weir, C.C., and R. J. Soper. 1963. Interaction of phosphates with ferric organic complexes. *Can. J. Soil Sci.* 43:393–399.
- Worsfold, P., I. McKelvie, and P. Monbet. 2016. Determination of phosphorous in natural waters: a historical review. *Anal. Chim. Acta* 918:8–20.
- Wright, R.D. 1986. The pour-through nutrient extraction procedure. *HortScience* 21:227–229.
- Xu, N., H. Yin, Z. Chen, S. Liu, M. Chen, and J. Zhang. 2014. Mechanisms of phosphate retention by calcite: effects of magnesium and pH. *J. Soils Sediments* 14:495–503.
- Yeager, T. H., and J.E. Barrett. 1984. Phosphorus leaching from ³²P-superphosphate-amended soilless container media. *HortScience* 19:216–217.
- Yeager, T. H., and J.E. Barrett. 1985a. Phosphorous and sulfur leaching from an incubated superphosphate-amended soilless container medium. *HortScience* 20:671–672.
- Yeager, T. H., and J.E. Barrett. 1985b. Influence of incubation time on phosphorous leaching from a container medium. *J. Environ. Hort.* 3:186–187.
- Yeager, T.H., and R.D. Wright. 1982. Pine bark-phosphorus relationships. *Commun. Soil Sci. Plant Anal.* 13:57–66.
- Yeager, T.H. and R.D. Wright. 1982. Phosphorus requirement of *Ilex crenata* Thunb. cv. Helleri grown in a pine bark medium. *J. Amer. Soc. Hort. Sci.* 107:558–562.
- Yuan, X., W. Xia, J. An, W. Yang, and J. Yin. 2014. Removal of phosphate anions from aqueous solutions using dolomite as adsorbent. *Adv. Mater. Res.* 864–867:1454–1457.
- Yuan, X., X. Wentag, J. An, J. Yin, X. Zhou, and W. Yang. 2015. Kinetic and thermodynamic studies on the phosphate adsorption removal by dolomite mineral. *J. Chem.* 1–8.
- Zhang, M., R. Wright, D. Heaney, and D. Vanderwel. 2004. Comparison of different phosphorus extraction and determination methods used manured soils. *Can. J. Soil Sci.* 84: 469–475.

Chapter 2

Growth Response of Three Containerized Woody Plant Taxa to Varying Low Phosphorus Fertilizer Concentrations

Formatted to fit the style guide for publication in HortScience

Citation:

Shreckhise, J.H., J.S. Owen, Jr., and A.X. Niemiera. 2018. Growth response of three containerized woody plant taxa to varying low phosphorus fertilizer concentrations. HortScience (in press).

Abstract. Phosphorus (P) uptake efficiency (PUE; percent of applied P absorbed by roots) for containerized crops is \approx 27% to 62%. Reducing P fertilization may increase PUE without decreasing growth and may reduce P leaching from containers, thus mitigating the environmental impact of containerized production while potentially reducing fertilizer input costs for growers. The objective of this study was to determine the minimum P application concentration and the resulting substrate pore-water (i.e., solution residing within and between substrate particles) P concentration that maintains maximal growth of three containerized woody plant taxa grown in pine bark substrate. *Hydrangea paniculata* Sieb. ‘Limelight’ (hydrangea), *Ilex crenata* Thunb. ‘Helleri’ (holly) and *Rhododendron* L. ‘Karen’ (azalea) were potted in pine bark substrate amended with dolomite and micronutrients and grown for 81 days in an open-wall greenhouse. Plants received either one of five constant liquid feed treatments with varying P concentrations [80 mg·L⁻¹ nitrogen (N), 50 mg·L⁻¹ potassium (K) and 0.5, 1.0, 2.0, 4.0 or 6.0 mg·L⁻¹ P] or a single application of controlled-release fertilizer (CRF; control) at experiment initiation. Calculated lowest P-fertilizer concentration that sustained maximal shoot dry weight (SDW) in hydrangea and azalea was 4.7 and 2.9 mg·L⁻¹, respectively, and holly SDW was the same across all liquid fertilizer treatments. In all three taxa, CRF-fertilized plants achieved < 50% of maximal SDW observed in liquid-fertilized plants. Hydrangea root dry weight (RDW) nearly doubled as fertilizer-P increased from 0.5 to 2.0 mg·L⁻¹ P, but higher P concentrations did not further increase RDW. Holly RDW was unaffected by liquid P treatment. Pore-water P concentrations of treatments that sustained maximal SDW of hydrangea and azalea were as low as 0.6 and 2.2 mg·L⁻¹ P, respectively. Our findings suggest that when using constant liquid feed, applied P levels more accurately predict plant growth responses than pore-water P levels.

Rising fertilizer prices and environmental implications of fertilizer runoff substantiates the need to select fertilization rates that optimize crop growth and nutrient use efficiency yet minimize the effect on the surrounding ecosystem. From 2007 to 2008, the U.S. farm price of phosphate (PO_4^{3-}) fertilizer nearly doubled, reaching an all-time high of \approx \$909 per tonne (\$825 per short ton; USDA, 2013). By 2013, U.S. PO_4^{3-} fertilizer prices were 56% higher than those in 2007 (USDA, 2013), and this trend of increasing phosphorus (P) fertilizer prices is expected to continue as quality rock phosphate reserves are depleted (Smil, 2000) and global crop demand continues to rise with the world's growing population. Agriculture, including container nurseries, is a "primary" contributor of P pollution in U.S. surface waters (USEPA, 2017). Phosphorus is often the greatest contributing factor for eutrophic conditions in freshwater ecosystems (USEPA, 2002) and is therefore a focus of federal and state monitoring and regulation within impaired watersheds (e.g., the Chesapeake Bay Watershed and the Everglades Watershed). Thus, to preserve water quality or facilitate remediation of surface waters, agricultural nutrient management programs are being implemented throughout the U.S.

Fertilizing containerized nursery crops via controlled-release fertilizer (CRF) in complete or partial substitution for soluble granular or aqueous forms is a best management practice (BMP; Bilderback et al., 2013) and has been widely adopted by containerized-crop growers (Dennis et al., 2010). When utilizing CRF to fertilize containerized nursery crops grown in a pine bark based substrate, P uptake efficiency (PUE; percent of applied P absorbed by roots) has been reported to be \approx 27% to 62% (Owen et al., 2008; Tyler et al., 1996; Warren et al., 1995; Warren et al. 2001). The observed low PUE is in part because containerized crop producers use soilless substrates (e.g., pine bark) that have low anion exchange capacity, high total porosity, and relatively low specific surface area for P adsorption compared to soils used in conventional

agricultural production. Continuous fertilization with controlled-release, soluble granular or liquid fertilizer coupled with daily irrigation, commonplace in container nurseries, give rise to P losses via leaching and subsequent runoff from the growing area.

To reduce nutrient leaching, BMPs recommend applying the minimum amount of fertilizer that achieves the desired growth and quality (Bilderback et al., 2013). Phosphorus uptake efficiency of containerized crops improves with a marginal decrease in P fertilization amount (Ku and Hershey 1997; Lea-Cox and Ristvey 2003; Owen et al., 2008; Ristvey et al., 2004; Ristvey et al., 2007). Ristvey et al. (2007), when growing liquid-fed *Rhododendron L.* ‘Karen’ in a substrate comprised of 1 composted pine bark : 1 *Sphagnum* peat : 1 rice hulls (by volume), increased PUE from $\approx 15\%$ to 49% without decreasing shoot dry weight (SDW) by reducing P fertilizer from 25 to 5 mg per week. Owen et al. (2008) increased PUE from 21% to 34% in *Cotoneaster dammeri* Schnied. ‘Skogholm’ grown in pine bark amended with 11% builder’s sand (by volume) with no reduction in total dry weight when P in CRF was reduced by half. Reduced PUE in response to superfluous P fertilization of container-grown plants can be explained by the relatively large proportion of applied P that is inaccessible to roots for absorption. Much of the fertilizer-P is inaccessible due to the slow rate of P diffusion from bulk solution to roots (i.e., 10^{-8} to 10^{-11} $\text{cm}^2 \cdot \text{s}^{-1}$; Heinrich and Patrick, 1985; Jungk and Claassen, 1997) coupled with the spatial separation between the roots and much of the P supply (i.e., that which is not within 0.4 cm of the roots; Jungk and Claassen, 1997; Temple-Smith and Menary, 1977). Thus, as P fertilization increases, the ratio of available P to non-available P decreases, concurrently reducing PUE. These studies imply that fertilizing with minimally sufficient P levels for maximal plant growth may optimize PUE when all other factors (e.g., leaching fraction, amendments, substrate physical properties, etc.) are held constant.

Fertilizer recommendations for container-grown crops are often based on nutrient levels found in solution extracted via the pour-through method (Wright, 1986). Currently, BMPs suggest 5 to 15 mg·L⁻¹ P be maintained in pour-through-extracted substrate pore-water (Bilderback et al., 2013). This recommendation is, in part, based on studies by Yeager and Wright (1982) and Wright and Niemiera (1985) who grew *Ilex crenata* Thumb. ‘Helleri’ in 100% pine bark and 100% quartz sand, respectively. In both studies, shoot growth increased as applied P was increased from 0 to 5 mg·L⁻¹, but ≥ 5 mg·L⁻¹ P did not increase shoot growth. Despite BMP recommendations, there is evidence that < 5 mg·L⁻¹ P in substrate pore-water is sufficient for growing salable woody plants in soilless substrates. In a substrate consisting of 2 perlite : 1 peat (by volume), Havis and Baker (1985) observed maximal growth of *Rhododendron* L. ‘Victor’ when liquid-fertilizing with 2.5 mg·L⁻¹ P. Million et al. (2007) observed no change in plant size index [i.e., (plant height + plant width) ÷ 2] of CRF-fertilized *Viburnum odoratissimum* Ker Gawl. grown in 2 aged pine bark : 1 sphagnum peat moss : 1 coarse sand (by volume) when doubling the irrigation rate reduced pour-through-extracted P concentration ranges from 5 to 11 mg·L⁻¹ down to 1 to 4 mg·L⁻¹. When growing CRF-fertilized *Cotoneaster dammeri* Schnied. ‘Skogholm’ in 8 pine bark : 1 sand (by volume), Groves et al. (1998) observed no apparent plant growth reduction even though pour-through extracted P levels were consistently lower than 5 mg·L⁻¹.

Based on the preponderance of previous research, maximal growth of containerized woody plants should be achievable with applied P concentrations between 0 and 5 mg·L⁻¹. Therefore, our objective was to determine the minimum fertilizer-P concentration and the resulting substrate pore-water P concentration that maintains maximal growth of three containerized woody plant taxa grown in pine bark substrate.

Materials and Methods

This study was performed at the Hampton Roads Agriculture Research and Extension Center in Virginia Beach, Virginia (latitude 36°53'31"N; longitude 76°10'44"W; USDA Plant Hardiness Zone 8a). The three plant taxa used were *Rhododendron* L. 'Karen' (azalea), *Ilex crenata* Thunb. 'Helleri' (holly) and *Hydrangea paniculata* Sieb. 'Limelight' (hydrangea). On 10 April 2014, liners were acquired in 36-cell flats with either two rooted stem cuttings (hydrangea; Bennett's Creek Nursery, Suffolk, VA) or three rooted stem cuttings (holly and azalea; Saunders Brothers Nursery, Piney River, VA) per cell. Rooted stem cuttings within each cell were separated and roots rinsed with a high-pressure water stream to remove existing substrate and fertilizer. Single-plant bare-root liners were then potted individually into 3.8 L black plastic containers (#1 gal, Nursery Supplies, Chambersburg, PA). Hydrangea and holly were potted in an aged pine bark substrate (pH 4.3 ± 0.06 SE; 1.6 cm screen, Carolina Bark Products, Seaboard, NC) amended with $0.89 \text{ kg}\cdot\text{m}^{-3}$ granular micronutrient fertilizer (Micromax, Everris, Dublin, OH), $2.37 \text{ kg}\cdot\text{m}^{-3}$ ground dolomite [97% calcium carbonate equivalent (CCE), Rockydale Quarries Corporation, Roanoke, VA] and $2.37 \text{ kg}\cdot\text{m}^{-3}$ pulverized dolomite (94% CCE, Old Castle Lawn and Garden, Thomasville, PA). Azalea was potted in the same pine bark substrate but amended with $1.19 \text{ kg}\cdot\text{m}^{-3}$ ground dolomite and no pulverized dolomite to maintain crop-specific low pH. Dolomite was selected as a lime source to ensure calcium (Ca) and magnesium (Mg) were not growth-limiting. Container capacity and air space of the substrate were 33% and 36% (by volume), respectively, and bulk density was $0.24 \text{ g}\cdot\text{cm}^{-3}$ (NCSU porometer method; Fonteno et al., 1995). All amendments were incorporated using a 11.5 m^3 ribbon mixer for a duration of 5 min. Prior to treatment initiation, plants were fertilized with a single 350-mL

aliquot of P-free nutrient solution [80 mg·L⁻¹ nitrogen (N) from ammonium nitrate (NH₄NO₃) and 50 mg·L⁻¹ potassium (K) from potassium sulfate (K₂SO₄)] every 7 d for 35 d.

Experiment initiation. On 16 May 2014, all plants were moved into an 8 m × 29 m greenhouse running northwest to southeast and roofed with a polyolefin reflective shade cover [FLS 50 W/B; Svensson, Kinna, Sweden (49% direct photosynthetically active radiation transmission)]. Greenhouse walls remained rolled up approximately 1 m above the ground to facilitate passive cooling throughout the study. The experiment was arranged in a randomized complete block design (RCBD) in a 3 (plant taxa) × 6 (fertilizer treatments) factorial treatment arrangement with three replications and three plants per replication. This experiment was blocked to account for potential differences in shading from the greenhouse structure. Plants were placed in 18 rows running perpendicular to the greenhouse length, each row containing three plants per taxon, totaling 54 plants per taxon. Every six rows of plants from northwest to southeast were one complete block.

Injectors (D25F1; Dosatron Intl., Clearwater, FL), which had a 1:100 fixed injection rate, were used to formulate target fertilizer concentrations by diluting each of five concentrated nutrient solutions with potable water. Post-injector nutrient solutions were delivered to plants via pressure-compensating spray stakes [01PSDS-PL1-B; Netafim, Fresno, CA (202 mL·min⁻¹)] every three days or as needed in two consecutive two-minute intervals with ≈ 34 min between intervals. To ensure full pore-water exchange with fertilizer solution and prevent salt accumulation, leaching fractions (i.e., mL leached divided by mL applied) for hydrangea, azalea and holly were maintained at 0.84 ± 0.01 SE, 0.88 ± 0.01 SE and 0.88 ± 0.01 SE, respectively. Target fertilizer-nutrient concentrations were 80 mg·L⁻¹ N (NH₄NO₃), 50 mg·L⁻¹ K [K₂SO₄ and

monopotassium phosphate (KH_2PO_4)] and one of five P concentrations (i.e., treatments): 0.5, 1.0, 2.0, 4.0 or 6.0 $\text{mg}\cdot\text{L}^{-1}$ P (KH_2PO_4).

Irrigation source-water was sent to Brookside Laboratories (New Bremen, OH) for complete water analysis. Source-water contained $< 0.20 \text{ mg}\cdot\text{L}^{-1}$ P, $4.49 \text{ mg}\cdot\text{L}^{-1}$ K, $3.69 \text{ mg}\cdot\text{L}^{-1}$ N, $< 0.20 \text{ mg}\cdot\text{L}^{-1}$ aluminum (Al), $< 0.10 \text{ mg}\cdot\text{L}^{-1}$ iron (Fe), $15.92 \text{ mg}\cdot\text{L}^{-1}$ Ca and $24.19 \text{ mg}\cdot\text{L}^{-1}$ alkalinity. In addition, spray stake-emitted nutrient solution was periodically analyzed for N, P and K concentration to confirm target nutrient concentrations were being applied. Observed applied N and K concentrations were $64.5 \pm 3.44 \text{ SE}$ and $42.3 \pm 2.23 \text{ SE} \text{ mg}\cdot\text{L}^{-1}$, respectively, and observed applied P concentrations were $0.4 \pm 0.11 \text{ SE}$, $1.3 \pm 0.11 \text{ SE}$, $2.0 \pm 0.24 \text{ SE}$, $4.2 \pm 0.63 \text{ SE}$ and $6.3 \pm 0.41 \text{ SE} \text{ mg}\cdot\text{L}^{-1}$ P, in order of lowest to highest P treatment.

Injector mixing chambers and suction tubes were wrapped in aluminum foil to reduce biofilm accumulation. Every 2 to 3 weeks, suction tubes and strainers were rinsed with deionized water and concentrated fertilizer solutions were made. Injection rate was checked 1, 42 and 84 day(s) after experiment initiation (DAI) to ensure a 1:100 dilution factor remained constant.

On 17 May 2014 (day 0), plants within the control treatment were top-dressed with 17.0 g of 18N–2.6P–10.0K polymer-coated, 8 to 9 month CRF (Harrell's LLC, Lakeland, FL), a standard fertilization practice in the southeastern U.S. nursery industry. In the CRF treatment, 93% of N was derived from polymer-coated NH_4NO_3 and 7% from polymer-coated monoammonium phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$). Immediately after top-dressing, CRF was incorporated into the top 2 to 3 cm of the substrate surface to ensure consistent fertilizer release via full contact of granules with substrate and to prevent loss from other factors (e.g., spillage). Irrigation regime was similar to those liquid-fertilized, except CRF-fertilized plants received only one 2-

min irrigation event to reduce leaching and mimic irrigation practices used in conventional nursery production. Leaching fractions for the CRF treatment were the same across taxa, averaging 0.55 ± 0.02 SE.

Hydrangea flowers were removed to ensure continued vegetative growth and were inspected weekly for flower bud swelling. Stems with swollen flower buds were pruned just above the first node below the basal-most flower bud. Removed plant tissue was dried at 58°C and added to the final shoot dry weight (SDW). In this experiment, SDW indicates the above ground portion of the plant.

Data collection and analysis. Pore-water extracts were collected via the pour-through method (Wright, 1986) from one randomly selected plant per replication 0, 20, 41, 62 and 81 DAI. Pour-throughs were performed ≈ 1 h following a normal fertigation event by hand-pouring 120 mL deionized water over the surface of the substrate and collecting ≥ 50 mL of subsequent leachate. Using a benchtop meter (Orion 4-Star Plus pH/Conductivity Meter, Thermo Fisher Scientific Inc., Beverly, MA), substrate pH and electrical conductivity (EC) were measured on all five sampling dates within 12 h of pour-through extraction.

At 41 and 81 DAI, an 8-mL aliquot of each pore-water extract and each spray stake-emitted fertilizer solution was filtered ($0.2 \mu\text{m}$, 30 mm syringe filter; Thermo Fisher Scientific) and stored at -18°C for later ion analysis. Samples were thawed and analyzed at $\approx 25^{\circ}\text{C}$ for ammonium (NH_4^+), nitrate (NO_3^-), nitrite (NO_2^-), PO_4^{3-} and K^+ , concentration using an ion chromatography (IC) system (ICS-2100; Thermo Fisher Scientific). Anion- and cation-exchange columns as well as the autosampler utilized by the IC system were similar to those described in Hoskins et al. (2014b), except both columns were 4×250 mm (i.d. \times length) and a different anion-exchange column (AS19; Thermo Fisher Scientific) was used.

Growth index [GI; (widest width + perpendicular width + height)/3] was determined for holly and azalea at 0, 19, 40, 61 and 80 DAI. Hydrangea GI was not recorded since flower pruning often altered width and height. While measuring GI, symptoms of potential nutrient deficiency and pest damage were recorded. Pests (e.g., spider mites, beetles) were controlled via hand removal or pesticide sprays or both. Bifenazate [Floramite; Mainland, PA (0.65 mL·L⁻¹)] was applied 45 DAI to all hydrangea and holly using a handheld, carbon dioxide-pressurized sprayer (276 kpa) with a boom nozzle.

Eighty-two DAI, foliar tissue samples were collected from each plant for nutrient concentration analysis. Four 5.1-cm ($\approx 11 \pm 0.8$ SE leaves) and nine 6.1-cm ($\approx 11 \pm 0.3$ SE leaves) apical stem cuttings were taken from young stems of each azalea and holly, respectively. For hydrangea, 30 leaf blade samples were taken from most recently matured leaves across three plants per replication. Tissue sample collection per species was done according to Bryson et al. (2014). All tissue samples were rinsed with deionized water, oven-dried (58 °C), weighed and analyzed for N, P and K concentrations (Brookside Laboratories, New Bremen, OH). Bryson et al. (2014), who provides foliar nutrient sufficiency ranges for a multitude of taxa based on survey data reported in the literature and by analytical laboratories, was used to determine if foliar nutrient levels were sufficient or growth limiting. Foliar sample dry weight and shoot weight were combined to provide final SDW.

After foliar sampling was complete, plants were severed at substrate level. Shoots were oven-dried (58 °C) for a minimum of 72 h and weighed. Hydrangea and holly roots were first washed with a high-pressure stream of tap water over a screen to remove all substrate particles while minimally losing root mass. Roots were then oven-dried (58 °C) for a minimum of 72 h

and weighed for root dry weight (RDW). Roots of azalea were not harvested due to difficulty in separating substrate from fine roots without losing root mass.

Statistical analysis. Data were subjected to analysis of variance (ANOVA) and *post-hoc* comparison of means was accomplished using the Tukey-Kramer Honest Significant Difference test or a t-test. Dunnett's test was used to compare the response of liquid-fertilized crops to those fertilized with CRF (control). The block effect was insignificant in all ANOVA and was removed from analysis to simplify the model. Correlations were determined using the Pearson correlation coefficient (r).

To determine the appropriate non-linear model to regress SDWs and foliar P concentrations over P treatment, Akaike Information Criterion (AIC) values were compared across exponential and logistic growth curves. Non-linear models were used to calculate optimal P fertilizer concentrations for each taxon (i.e., the lowest pore-water P concentration that sustains maximal SDW). This was accomplished via the method proposed by Mischan et al. (2011) for calculating the asymptotic deceleration point in which data were fitted to a three-parameter logistic growth curve:

$$y = \frac{c}{\{1 + \text{Exp}[-a(x - b)]\}}$$

where y = SDW; x = fertilizer-P concentration; a = growth rate; b = inflection point; and c = asymptote. Computer-generated estimates for parameters a, b and c were obtained via JMP Pro, then the fourth derivative equation was solved for x'''' by equating y'''' to zero to obtain the asymptotic deceleration point. Optimal foliar P concentrations were calculated by solving the logistic growth equation for foliar P over applied P concentration, with “x” equal to the calculated optimal applied P concentration for SDW. JMP Pro (Version 11.0; SAS Institute) was used to process all data.

Results and Discussion

Growth index. Highest GI values of holly at harvest were achieved with 1.0 to 6.0 mg·L⁻¹ P ($P = 0.0413$; $F = 3.92$; data not shown) whereas azalea attained highest GI values when fertilized with 4.0 to 6.0 mg·L⁻¹ P ($P = 0.0005$; $F = 15.11$; data not shown). Growth index of holly and azalea fertilized with 2.0 to 6.0 mg·L⁻¹ P increased linearly over time after 20 d (Fig. 1). Growth index of holly fertilized with 0.5 to 1.0 mg·L⁻¹ P and azalea fertilized with 1.0 mg·L⁻¹ P increased until 61 DAI, then plateaued, whereas GI of azalea fertilized with 0.5 mg·L⁻¹ P plateaued 40 DAI (Fig. 1). The finding that GI values were the same across all liquid fertilizer treatments for at least the first half of the experiment suggests that the P supply of the low-P treatments was adequate for the relatively low GI values of both taxa. As GI values increased for the higher P treatments (but not the lower treatments), a higher P supply was required. A possible implication for this finding is that growers utilizing liquid fertilization can use relatively low P concentrations when plants are small and then increase concentrations commensurate with plant size. In support of this contention, Ingestad and Lund (1986) proposed that to achieve steady state plant nutrition, nutrient solution concentrations be increased over time to correspond with the increasing plant growth rate and concomitant increase in nutrient requirement.

Growth index was the same until 61 DAI for holly and azalea fertilized with CRF, then increased 246% and 206%, respectively (Fig. 1). The belated growth response in CRF-fertilized plants is likely a result of delayed N release from CRF as indicated by lower substrate pore-water N concentrations for the CRF treatment at 41 relative to 81 DAI in all three taxa (Table 1). In addition, from 61 to 81 DAI, EC increased 63% and 41% in holly and azalea (Fig. 2), respectively, supporting the notion that CRF-N was withheld for the first 61 d. A lag in N release

or availability from CRF has also been reported by Merhaut et al. (2006) and Newman et al. (2006).

Carbon allocation. Final SDW of hydrangea and azalea was highest when liquid fertilized with 4.0 to 6.0 mg·L⁻¹ P, whereas holly SDW was unaffected by fertilizer P concentration (Table 2). Yeager and Wright (1982) and Wright and Niemiera (1985) observed maximal shoot growth of ‘Helleri’ holly when fertilized with 5 to 15 mg·L⁻¹ P; however, P fertilizer concentrations between 0 and 5 mg·L⁻¹ P were not investigated. Havis and Baker (1985) found maximal shoot growth in *Rhododendron* ‘Victor’ and *Cotoneaster adpressus* var. *praecox* Bois & Berthault when fertilized with 2.5 and 10 mg·L⁻¹ P, respectively. Shoot dry weights of CRF-fertilized hydrangea, holly and azalea were lower than in those liquid-fertilized with 4.0 to 6.0 mg·L⁻¹ (Table 2), indicating liquid-fertilizing with non-limiting nutrient levels may generate more shoot growth than CRF in the first 80 d of production. Higher SDW of liquid-fertilized plants compared to those given CRF can be attributed to a relatively constant, non-limiting nutrient supply in a liquid fertilization regime (Huett, 1997). In contrast to liquid fertilization, pore-water nutrient levels for CRF-fertilized plants depend on the rate and degree of nutrient diffusion out of CRF granules; CRF nutrient release may also be delayed due to low temperatures (Huett and Gogel, 2000; Husby et al., 2003).

Hydrangea and azalea SDW increased with increasing applied liquid P concentration in a non-linear, logistic (sigmoidal) manner (Fig. 3). A sigmoid growth response to increasing P levels in field soils has also been observed in *Zea maize* L. and *Phaseolus vulgaris* L. (Postma and Lynch, 2011). Applied P concentrations in the 1.0 to 4.0 mg·L⁻¹ P range resulted in greatest increase in SDW (i.e., grams SDW per mg·L⁻¹ P applied) in hydrangea and azalea when compared to low (i.e., 0.5 to 1.0 mg·L⁻¹ P) or high applied P concentrations (i.e., 4.0 to 6.0 mg·L⁻¹

¹ P; Fig. 3). Non-linear regression was used to describe the pattern of SDW increase over applied P treatments (Fig. 3) and estimate the optimal pore-water P concentration for each taxon (Table 3). Calculated optimal applied P concentrations (i.e., asymptotic deceleration point) for hydrangea and azalea SDW were 4.7 and 2.9 mg·L⁻¹ P, respectively. However, since there was an increasing trend in SDW from 4.0 to 6.0 mg·L⁻¹ P for hydrangea, SDW may have continued to increase if fertilized with P concentrations > 6.0 mg·L⁻¹. Regression was not performed for holly since SDW was unaffected by P concentration (Table 2).

Increasing P fertilizer level from 0.5 to 2.0 mg·L⁻¹ P increased RDW 92% in hydrangea, but > 2.0 mg·L⁻¹ P did not further increase RDW (Table 4). Similar to SDW, RDW of CRF-fertilized hydrangea was lower than in those that received the 4.0 to 6.0 mg·L⁻¹ P treatments. In contrast, holly RDW was the same across all fertilizer treatments. Havis and Baker (1985) observed increased root growth of *Cotoneaster adpressus* var. *praecox* when P fertilizer concentration was increased from 0 to 10 mg·L⁻¹ P. In one of the three experiments performed by Yeager and Wright (1982), RDW of *Ilex crenata* ‘Helleri’ was unaffected when applied P concentration was increased from 0 to 20 mg·L⁻¹ in 5 mg·L⁻¹ increments.

In hydrangea and holly, root : shoot values decreased by 43% and 46%, respectively, as applied P concentration increased from 0.5 to 4.0 mg·L⁻¹ P (Table 4), reflecting relatively high increases in SDW compared to RDW. Similarly, Kim and Li (2016) observed decreasing root : shoot values in *Lantana camara* L. ‘New Gold’ when P fertilization was increased from 1 to 30 mg·L⁻¹. Yeager and Wright (1981) found that root : shoot values of *Ilex crenata* ‘Helleri’ were the same when fertilized with 17, 42, or 85 mg·L⁻¹ P; however, absence of response was attributed to all P treatments being sufficient for maximal growth. These studies agree with our

findings that fertilizing crops with P levels higher than those necessary for maximal shoot growth does not further increase root growth.

Pore-water P, N and K concentrations. The 4.0 and 6.0 mg·L⁻¹ P fertilizer treatments, which achieved maximal SDW in hydrangea and azalea, had 0.6 to 2.0 mg·L⁻¹ P in substrate pore-water for hydrangea and 2.2 to 5.7 mg·L⁻¹ P in substrate pore-water for azalea (Table 5). These data suggest that the minimum recommended pour-through P concentration of 5 mg·L⁻¹ (Bilderback et al., 2013) is higher than that necessary to produce hydrangea and azalea without limiting growth. Measured pore-water P concentrations were consistently lower than applied P levels. Lower P concentrations in pour-through leachate compared to the fertilizer solution has also been observed in containerized *Tagetes erecta* L. ‘Inca Gold’ (Tolman et al., 1990) and *Euphorbia pulcherrima* ‘Freedom Red’ Willd. Ex Klotzch. (Cavins et al., 2004). Phosphorus removal from substrate pore-water is likely due to uptake by roots prior to pour-through sampling. Although statistical analysis was not used to compare taxa, pore-water P concentrations within each treatment appeared to be lower in hydrangea than in holly or azalea which may be attributed to genotypic variation in nutrient uptake characteristics across taxa (Marschner, 2012). Differences in growth (hydrangea > azalea > holly; according to SDW data) across taxa may also have contributed to apparent variation in pore-water P concentrations since growth is proportional to rate of nutrient uptake (Ingestad and Lund, 1986). Although P chemical fate was not determined in this study, P adsorption to dolomite or precipitation with dolomite and micronutrient amendment dissolution products was not likely a major contributor to relatively low pore-water P concentrations since potential sorption sites were routinely flushed with fertilizer solution prior to the first pour-through extraction 41 DAI. Substrate pore-water P concentrations increased between 41 and 81 DAI for holly fertilized with 1.0 mg·L⁻¹ P or 4.0 mg·L⁻¹ P as well as azalea

fertilized with 2.0 mg·L⁻¹ P (Table 5). For all other treatments within each taxon, pore-water P levels were the same over time. At 81 DAI, increasing the fertilizer treatment from 0.5 to 4.0 mg·L⁻¹ P in hydrangea or from 0.5 to 2.0 mg·L⁻¹ P in holly and azalea did not increase pore-water P levels (Table 5). Correlation values between pore-water P and SDW ($r = 0.71, 0.53$ and 0.73 for hydrangea, holly and azalea, respectively; data not shown) were lower than those between applied P concentrations and SDW ($r = 0.97, 0.57$ and 0.85 for hydrangea, holly and azalea, respectively; data not shown). These correlations indicate applied P concentrations more accurately predict growth responses than pore-water P concentrations.

Nitrogen (i.e., sum of NH₄⁺-N, NO₃⁻-N and NO₂⁻-N) and K⁺ pore-water concentrations were unaffected by liquid P fertilizer treatment (data not shown). Thus, pore-water N and K⁺ concentrations were pooled across liquid fertilizer treatments (Table 1) and collectively referred to as liquid fertilizer. At 41 DAI, pore-water N concentrations were 45%, 51% and 16% lower than the measured applied N concentration (64.5 ± 3.44 SE mg·L⁻¹ N) in liquid-fertilized hydrangea, holly and azalea, respectively. Relatively low N concentrations in pour-through leachate compared to applied levels have also been reported for containerized *Citrus* species (64% to 80% of applied; Maust and Williamson, 1994) and *Tagetes erecta* ‘Inca Gold’ (53% to 80% of applied; Tolman et al., 1990). Similar to pore-water P, relatively low pore-water N concentrations were attributed to plant uptake prior to pour-through sampling. Seemingly higher pore-water N concentrations (i.e., less N removal) for azalea relative to hydrangea and holly may be due to lower nutrient demand which has been reported for *Rhododendron* sp. (Bilderback et al., 2013). In holly and azalea, pore-water N (primarily as NO₃⁻; data not shown) concentrations of liquid-fertilized plants were higher at 81 DAI than at 41 DAI (Table 1). This finding is most likely due to the N supply exceeding plant demand, resulting in a slow buildup of NO₃⁻. Since N

was the nutrient in highest concentration in the fertilizer solution and since NO_3^- is a leading contributor to the bulk EC relative to other fertilizer ions (e.g., SO_4^{2-} , K^+ , PO_4^{3-} , Mg^{2+} and Ca^{2+} ; Hoskins et al., 2014a), NO_3^- buildup is likely why EC increased over time (Fig. 2).

Pore-water N and K^+ concentrations of CRF-fertilized plants were consistently lower than that of liquid-fertilized plants for all three taxa except for N in holly at 81 DAI in which the values were the same (Table 1). Pore-water K^+ concentrations of CRF-fertilized plants were within or above the BMP recommended range (10 to 20 $\text{mg}\cdot\text{L}^{-1}$ K^+ ; Bilderback et al., 2013) and therefore deemed sufficient for maximal vegetative growth for all three taxa and at both sampling dates. However, observed pore-water N concentrations for CRF-fertilized hydrangea (41 and 81 DAI) and holly (41 DAI) were less than the BMP recommendation (15 to 25 $\text{mg}\cdot\text{L}^{-1}$ NO_3^- -N; Bilderback et al., 2013). Therefore, consistently lower concentrations of pore-water N for CRF-fertilized plants compared to those liquid-fertilized may partially explain why plants were smaller when given the CRF treatment. Pore-water N concentrations at 81 DAI for CRF-fertilized hydrangea, holly and azalea were 3-, 5-, and 5-fold higher, respectively, than at 41 DAI (Table 1), indicating a major release of N following the lag in N release from CRF granules as previously stated. Similar to the liquid-fertilizer treatments, the observed increase in N, primarily in the form of NO_3^- (data not shown), suggests NO_3^- is responsible for the increase in EC over time in the CRF treatment (Fig. 2).

Foliar P, N and K concentrations. In all three taxa, foliar P concentrations increased with increasing P supply in a logistic fashion (Fig. 3). The calculated foliar P concentrations (\pm 95% confidence interval) for optimal SDW in hydrangea and azalea are $0.23 \pm 0.03\%$ and $0.23 \pm 0.01\%$, respectively (Table 3).

Foliar P and SDW had a strong positive correlation in hydrangea ($r = 0.88$) and azalea ($r = 0.89$), whereas in holly, this correlation was relatively weak ($r = 0.52$) since SDW stayed the same despite increasing foliar P. The increase in foliar P concentration to levels higher than those required for maximal SDW in holly indicated luxury consumption. Superfluous foliar P accumulation has been reported for multiple containerized woody plant taxa, including *Rhododendron* 'Karen' (Ristvey et al., 2007), *Rhododendron* 'Victor' (Havis and Baker, 1985), *Rhododendron indicum* L. 'Formosa' (Dickey et al., 1967) and *Ilex crenata* 'Helleri' (Yeager and Wright, 1982), and several herbaceous taxa, such as *Rudbeckia fulgida* var. *sullivantii* Ait. 'Goldsturm' (Kraus et al., 2011) and *Lantana camara* L. 'New Gold' (Kim and Li, 2016).

Foliar P sufficiency ranges (Bryson et al., 2014) for *Hydrangea paniculata* Siebold., *Ilex crenata* 'Helleri' and *Rhododendron* L. 'Herbert' are 0.23% to 0.59%, 0.08% to 0.11%, and 0.14% to 0.22%, respectively. Thus, foliar P concentrations were within the sufficiency range at harvest when fertilized with 6.0 mg·L⁻¹ P in hydrangea or 1.0 to 6.0 mg·L⁻¹ P in holly and azalea. These data also imply that optimal foliar P concentrations may vary between cultivars of the same interspecific hybrid since the calculated optimal foliar P level in 'Karen' azalea is higher than the upper threshold of the sufficiency range for another Gable hybrid azalea, *Rhododendron* 'Herbert', listed in Bryson et al. (2014). Cultivar-specific foliar nutrient sufficiency levels have been documented for *Vaccinium corymbosum* L. (Wilber and Williamson, 2008). In hydrangea, holly and azalea fertilized with CRF, foliar P concentrations were the same as in those that received the constant liquid feed treatments that resulted in maximal growth (Table 6) despite having lower than optimal SDW. Bi et al. (2007) demonstrated that following N fertilization of *Rhododendron* L. 'H-1 P.J.M' or *Rhododendron* L. 'Cannon's Double', biomass increased 2 to 4 weeks after tissue-N accumulation. The delayed nutrient release from the CRF when growing

hydrangea and holly may have allowed for adequate uptake and translocation of nutrients to leaves, but plants were harvested before shoot growth could respond to increased nutrient availability.

Foliar N concentration had a strong, positive correlation with foliar P in holly and azalea ($r = 0.93$ and $r = 0.81$, respectively), but was poorly and negatively correlated with foliar P concentrations in hydrangea ($r = -0.39$). As applied P concentration increased from 0.5 to 6.0 $\text{mg}\cdot\text{L}^{-1}$, foliar N concentrations of holly and azalea increased 45% and 9%, respectively. Though not measured, average leaf size appeared to decrease with decreasing P treatment in all taxa, and reduced leaf expansion is a common P deficiency symptom (Fredeen et al., 1989). In the case of holly and azalea, in which tissue samples were taken as terminal stem cuttings, increasing P fertilization may have resulted in a higher leaf to stem ratio in plants treated with higher P concentrations. Since nearly 66% of plant tissue N in *Rhododendron* 'Karen' has been shown to be partitioned in the leaves (Ristvey et al., 2007), the effect of P deficiency on leaf expansion may explain why foliar N in holly and azalea had a strong positive correlation with foliar P. Kraus et al. (2011) also observed an increase in foliar N concentration in *Rudbeckia fulgida* Aiton var. *sullivantii* 'Goldsturm' and *Hibiscus moscheutos* L. in response to increasing P fertilizer concentration. Foliar N concentrations in all three taxa and all six treatments were within the foliar N sufficiency range (Bryson et al., 2014). Similar to foliar P, foliar N concentrations in CRF-fertilized hydrangea, holly and azalea were the same as in those that received constant liquid feed treatments that resulted in optimal growth (Table 6).

Foliar K concentration had a strong positive correlation with foliar P concentration in hydrangea ($r = 0.92$) and holly ($r = 0.95$), but a strong negative correlation with foliar P concentration in azalea ($r = -0.82$). Foliar K concentration increased 143% and 50% in

hydrangea and holly, respectively, and decreased 22% in azalea as applied P concentration increased from 0.5 to 6.0 mg·L⁻¹ (Table 6). Similar to the trend observed in azalea, tissue P and K levels in *Rudbeckia fulgida* var. *sullivantii* ‘Goldsturm’ linearly increased and decreased, respectively, in response to increasing P fertilizer concentrations (Kraus et al., 2011). In hydrangea, foliar K levels were below the sufficiency range (Bryson et al., 2014) in all treatments except 4.0 mg·L⁻¹ P, but were within or above the sufficiency range in holly and azalea. Thus, although foliar K levels varied with P treatment, pore-water K concentration was not likely growth limiting in holly and azalea. Similar to foliar P and N, foliar K concentrations in all three taxa fertilized with CRF were the same as in plants that received constant liquid feed treatments that resulted in optimal growth. *Pore-water pH*. Substrate pH was the same across liquid fertilizer treatments within each taxon and sampling date (data not shown); therefore, data were pooled across liquid fertilizer treatments within each taxon and sampling date (Fig. 4). For CRF- and liquid-fertilized hydrangea and holly, substrate pore-water pH increased approximately 0.3 units, from 6.9 ± 0.02 SE to 7.2 ± 0.02 SE, in the first 20 d. After 20 d, pH decreased until 81 DAI to 6.4 ± 0.05 SE (hydrangea) or 6.6 ± 0.07 SE (holly) when liquid fertilized and to 6.8 ± 0.04 SE (hydrangea) or 7.0 ± 0.02 SE (holly) when given the CRF treatment. In the CRF treatment, 93% of N was derived from NH₄NO₃ (610 kg·t⁻¹ CaCO₃ fertilizer potential acidity; Mortvedt and Sine, 1994) and the remainder from monoammonium phosphate (560 kg·t⁻¹ CaCO₃ fertilizer potential acidity; Mortvedt and Sine, 1994), and the liquid-fertilizer N source was entirely from NH₄NO₃; thus, the potential acidity of the N sources was similar in both treatments. The 0.4 unit lower pH in the liquid-fertilizer treatment relative to the CRF treatment at 81 DAI may be explained by the greater quantity of NH₄NO₃ applied to liquid-fertilized plants than CRF-fertilized plants over the course of the study. Substrate pore-

water pH of liquid- and CRF-fertilized azalea ($3.6 \text{ kg} \cdot \text{m}^{-3}$ less dolomite than for hydrangea and holly) generally decreased throughout the course of the study, ranging from $5.0 \pm 0.03 \text{ SE}$ to $4.7 \pm 0.04 \text{ SE}$ and $5.2 \pm 0.04 \text{ SE}$ to $4.7 \pm 0.10 \text{ SE}$, respectively (Fig. 4).

Conclusions

Containerized hydrangea, holly and azalea can be grown with less than $5 \text{ mg} \cdot \text{L}^{-1} \text{ P}$ in substrate pore-water, the current minimum pore-water P concentration recommended by BMPs, when fertigated with a complete liquid fertilizer containing $\approx 65 \text{ mg} \cdot \text{L}^{-1} \text{ N}$ and $42 \text{ mg} \cdot \text{L}^{-1} \text{ K}$. However, minimum applied P requirements are taxa- and growth stage-specific. According to our calculations, hydrangea and azalea grown in 3.8-L containers may be fertilized with 4.7 and $2.9 \text{ mg} \cdot \text{L}^{-1} \text{ P}$, respectively, without affecting SDW, whereas in holly, $0.5 \text{ mg} \cdot \text{L}^{-1} \text{ P}$ was sufficient for growth. Identifying optimal P concentrations by calculating the asymptotic deceleration point for logistic increase in SDW over P concentration is an informative method that should be evaluated and utilized to identify nutrient sufficiency in other ornamental plant taxa. When fertigating, applied P concentration is a better predictor of growth response than pour-through P levels. Although pour-through extraction is a simple method growers can use as a proxy for substrate pore-water fertility, nutrient concentrations in extracted solution may not accurately predict actual nutrient levels available for root uptake after fertigation. Our findings suggest pour-through P levels as low as 1.0 and $2.3 \text{ mg} \cdot \text{L}^{-1}$ for hydrangea and azalea, respectively, are adequate for maximal shoot growth; these values are lower than the BMP recommendation of 5 to $15 \text{ mg} \cdot \text{L}^{-1} \text{ P}$. Variables not considered in this study, including N : P : K ratio, container size and frequency of fertilization and irrigation, can influence critical nutrient levels in plant tissue and may therefore also affect the optimal fertilizer level.

Literature cited

- Bi, G., C.F. Scagel, L.H. Fuchigami, and R.P. Regan. 2007. Differences in growth, and nitrogen uptake and storage between two container-grown cultivars of *Rhododendron*. *J. Environ. Hort.* 25:13–20.
- Bilderback, T., C. Boyer, M. Chappell, G. Fain, D. Fare, C. Gilliam, B.E. Jackson, J. Lea-Cox, A.V. LeBude, A. Niemiera, J. Owen, J. Ruter, K. Tilt, S. Warren, S. White, T. Whitwell, R. Wright, and T. Yeager. 2013. *Best management practices: Guide for producing nursery crops*. 3rd ed. Southern Nursery Association, Acworth, GA.
- Bryson, G.M., H.A. Mills, N.D. Sasseville, J.B. Jones, Jr., and A.B. Barker. 2014. *Plant analysis handbook III: A guide to sampling, preparation, analysis, interpretation and use of results of agronomic and horticultural crop plant tissue*. Macro-Micro Publishing, Inc., Athens, GA.
- Cavins, T.J., B.E. Whipker, and W.C. Fonteno. 2004. Establishment of calibration curves for comparing pour-through and saturated media extract nutrient values. *HortScience* 39:1635–1639.
- Dennis, J.H., R.G. Lopez, B.K. Behe, C.R. Hall, C. Yue, and B.L. Campbell. 2010. Sustainable production practices adopted by greenhouse and nursery plant growers. *HortScience* 45:1232–1237.
- Dickey, R.D., R.T. Poole and J.N. Joiner. 1967. Effect of nitrogen, phosphorus and potassium levels on growth and chemical composition of *Rhododendron indicum* 'Formosa' and *Viburnum suspensum*. *Proc. Amer. Soc. Hort. Sci.* 91:762–770.

- Fonteno, W.C., C.T. Hardin, and J.P. Brewster. 1995. Procedures for determining physical properties of horticultural substrates using the NCSU Porometer. Horticultural Substrates Laboratory. NC State University, Raleigh, NC.
- Fredeen, A.L., I.M. Rao, and N. Terry. 1989. Influence of phosphorus nutrition on growth and carbon partitioning of *Glycine max*. *Plant Physiol.* 89:225–230.
- Groves, K.M., S.L. Warren, and T.E. Bilderback. 1998. Irrigation volume, application, and controlled-release fertilizer. II. Effect on substrate solution nutrient concentration and water efficiency in containerized plant production. *J. Environ. Hort.* 16:182–188.
- Havis, J.R. and J.H. Baker. 1985. Phosphorus requirement of *Rhododendron* 'Victor' and *Cotoneaster adpressa praecox* grown in a perlite-peat medium. *J. Environ. Hort.* 3:63–64.
- Heinrich, P.A., and J.W. Patrick. 1985. Phosphorus acquisition in the soil-root system of *Eucalyptus pilularis* Sm. Seedlings. I Characteristics of the soil system. *Aust. J. Soil Res.* 23:223–236.
- Hoskins, T.C., J.S. Owen, Jr., and A.X. Niemiera. 2014a. Controlled-release fertilizer placement affects the leaching pattern of nutrients from nursery containers during irrigation. *HortScience* 49:1341–1345.
- Hoskins, T.C., J.S. Owen, Jr., J.S. Fields, J.E. Altland, Z.M. Easton, and A.X. Niemiera. 2014b. Solute transport through a pine bark-based substrate under saturated and unsaturated conditions. *J. Amer. Soc. Hort. Sci.* 139:634–641.
- Huett, D.O. 1997. Fertiliser use efficiency by containerized nursery plants 1. plant growth and nutrient uptake. *Aust. J. Agric. Res.* 48:251–258.

- Huett, D.O., and B.J. Gogel. 2000. Longevities and nitrogen, phosphorus, and potassium release patterns of polymer-coated controlled-release fertilizers at 30°C and 40°C. *Commun. Soil Sci. Plant Anal.* 31:959–973.
- Husby, C.E., A.X. Niemiera, J.R. Harris, and R.D. Wright. 2003. Influence of diurnal temperature on nutrient release patterns of three polymer-coated fertilizers. *HortScience* 38:387–389.
- Ingestad, T. and A. Lund. 1986. Theory and techniques for steady state mineral nutrition and growth of plants. *Scand. J. For. Res.* 1:439–453.
- Jungk, A., and N. Claassen. 1997. Ion diffusion in the soil-root system. *Adv. Agron.* 61:53–110.
- Kim, H and X. Li. 2016. Effects of phosphorus on shoot and root growth, partitioning, and phosphorus utilization efficiency in lantana. *HortScience* 51:1001–1009.
- Kraus, H.T., S.L. Warren, G.J. Bjorkquist, A.W. Lowder, C.M. Tchir, and K.N. Walton. 2011. Nitrogen : phosphorus : potassium ratios affect production of two herbaceous perennials. *HortScience* 46:776–783.
- Ku, C.S. and D.R. Hershey. 1997. Growth response, nutrient leaching, and mass balance for potted poinsettia. II. Phosphorus. *J. Amer. Soc. Hort. Sci.* 122:459–464.
- Lea-Cox, J. D., A.G. Ristvey. 2003. Why are nutrient uptake efficiencies so low in ornamental plant production. *Proc. South. Nurs. Assoc. Res. Conf.* 48:116–122.
- Marschner, H. 2012. *Mineral nutrition of higher plants*. 3rd ed. Academic Press, San Diego, CA.
- Maust, B.E. and J.G. Williamson. 1994. Nitrogen nutrition of containerized citrus nursery plants. *J. Amer. Soc. Hort. Sci.* 119:195–201.
- Merhaut, D.J., E.K. Blythe, J.P. Newman, and J.P. Albano. 2006. Nutrient release from controlled-release fertilizers in acid substrate in a greenhouse environment: I. leachate

- electrical conductivity, pH, and nitrogen, phosphorus, and potassium concentrations. *HortScience* 41:780–787.
- Million, J., T. Yeager, and J. Albano. 2007. Consequences of excessive overhead irrigation on runoff during container production of sweet viburnum. *J. Environ. Hort.* 25:117–125.
- Mischan, M.M., S.Z.D Pinho, and L.R.D Carvalho. 2011. Determination of a point sufficiently close to the asymptote in nonlinear growth functions. *Sci. Agric.* 68(1):109–114.
- Mortvedt, J.J., and C. Sine, eds. 1994. Fertilizer dictionary. In: *Farm chemicals handbook*. Willoughby, OH: Meister Publishing.
- Newman, J.P., J.P. Albano, D.J. Merhaut, and E.K. Blythe. 2006. Nutrient release from controlled-release fertilizers in a neutral-pH substrate in an outdoor environment: I. leachate electrical conductivity, pH, and nitrogen, phosphorus, and potassium concentrations. *HortScience* 41:1674–1682.
- Owen, J.S., S.L. Warren, T.E. Bilderback, and J.P. Albano. 2008. Phosphorus rate, leaching fraction, and substrate influence on influent quantity, effluent nutrient content, and response of a containerized woody ornamental crop. *HortScience* 43:906–912.
- Postma, J.A. and J.P. Lynch. 2011. Theoretical evidence for the functional benefit of root cortical aerenchyma in soils with low phosphorus availability. *Ann. Bot.* 107:829–841.
- Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2004. Nutrient uptake, partitioning and leaching losses from container-nursery production systems. *Acta Hort.* 630:321–328.
- Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2007. Nitrogen and phosphorus uptake efficiency and partitioning of container-grown azalea during spring growth. *J. Amer. Soc. Hort. Sci.* 132:563–572.

- Smil, V. 2000. Phosphorus in the environment: natural flows and human interferences. Annual Review of Energy and the Environment 25:53–88.
- Temple-Smith, M.G., and R.C. Menary. 1977. Movement of ³²P to roots of cabbage and lettuce grown in two soil types. Comm. Soil Sci. Plant Anal. 8:67–79.
- Tolman, D.A., A.X. Niemiera, and R.D. Wright. 1990. Influence of plant age on nutrient absorption for marigold seedlings. HortScience 25:1612–1613.
- Tyler, H.H., S.L. Warren, and T.E. Bilderback. 1996. Reduced leaching fractions improve irrigation use efficiency and nutrient efficacy. J. Environ. Hort. 14:199–204.
- USDA ERS. 2013. Fertilizer use and price. Table 8. Fertilizer price indexes. 7 April 2017. <<https://www.ers.usda.gov/data-products/fertilizer-use-and-price/>>.
- USEPA. 2002. Mid-Atlantic Integrated Assessment 1997–98 Summary Report, EPA/620/R-02/003. U.S. Environmental Protection Agency, Atlantic Ecology Division, Narragansett, RI.
- USEPA. 2017. Nutrient pollution: Sources and solutions. 15 May 2018. <<https://www.epa.gov/nutrientpollution/sources-and-solutions>>.
- Warren, S.L., T.E. Bilderback, and H.H. Kraus. 2001. Method of fertilizer application affects nutrient losses of controlled-release fertilizer. Acta Hort. 548:349–355.
- Warren, S.L., T.E. Bilderback, and H.H. Tyler. 1995. Efficacy of three nitrogen and phosphorus sources in container-grown azalea production. J. Environ. Hort. 13:147–151.
- Wilber, W.L. and J.G. Williamson. 2008. Effects of fertilizer rate on growth and fruiting of containerized southern highbush blueberry. HortScience 43:143–145.
- Wright, R.D. and A.X. Niemiera. 1985. Influence of N, P and K fertilizer interactions on growth of *Ilex crenata* Thunb. 'Helleri'. J. Environ. Hort. 3:8–10.

- Wright, R.D. 1986. The pour-through nutrient extraction procedure. HortScience 21:227–229.
- Yeager, T.H. and R.D. Wright. 1981. Influence of nitrogen and phosphorus on shoot: root ratio of *Ilex crenata* Thunb. 'Helleri'. HortScience 16:564–565.
- Yeager, T.H. and R.D. Wright. 1982. Phosphorus requirement of *Ilex crenata* Thunb. cv. Helleri grown in a pine bark medium. J. Amer. Soc. Hort. Sci. 107:558–562.

Table 1. Substrate pore-water nitrogen (N) and potassium (K) concentrations ($\text{mg}\cdot\text{L}^{-1}$) in pour-through leachate at 41 and 81 days after initiation (DAI) for hydrangea, holly and azalea potted in pine bark amended with $0.89 \text{ kg}\cdot\text{m}^3$ micronutrients and either $4.75 \text{ kg}\cdot\text{m}^3$ (hydrangea and holly) or $1.19 \text{ kg}\cdot\text{m}^3$ (azalea) of dolomitic limestone and fertilized with controlled-release fertilizer (CRF; control; $n = 3$) or liquid fertilizer (LF; $n = 14$).

Treatment	Nutrient concentrations in pour-through leachate ($\text{mg}\cdot\text{L}^{-1}$)					
	N			K		
	41 DAI	81 DAI	P-value	41 DAI	81 DAI	P-value
	hydrangea			hydrangea		
CRF	5.1	15.0	0.0215	18.0	17.2	0.7397
LF ^z	35.6	44.5	0.0770	50.8	43.0	0.0770
P value	0.0010	0.0009		<0.0001	0.0040	
	holly			holly		
CRF	6.3	32.4	0.0146	22.2	30.0	0.0125
LF	31.3	44.9	0.0197	50.2	47.6	0.6010
P value	0.0015	0.2562		0.0031	0.0351	
	azalea			azalea		
CRF	7.7	44.7	0.0145	29.3	30.2	0.8889
LF	54.2	66.7	0.0133	56.4	54.4	0.7095
P value	0.0003	0.0082		0.0062	0.0277	

^z Pour-through leachate concentrations of N and K were not different between liquid fertilizer treatments, 0.5, 1.0, 2.0, 4.0 and 6.0

$\text{mg}\cdot\text{L}^{-1}$ at the $\alpha = 0.05$ level. Thus, values indicate mean of liquid fertilizer treatments.

Table 2. Effect of applied phosphorus (P) concentration versus controlled-release fertilizer (CRF; control) on shoot dry weight of hydrangea, holly and azalea grown for 82 d in pine bark amended with 0.89 kg·m³ micronutrients and either 4.75 kg·m³ (hydrangea and holly) or 1.19 kg·m³ (azalea) of dolomitic limestone.

Treatment (mg·L ⁻¹ P) ^y	Shoot dry weight (g) ^z		
	hydrangea	holly	azalea
CRF	11.53	5.71	4.72
0.5	4.95 a ^x **	8.08	4.43 a
1.0	6.71 a *	10.11 **	5.60 a
2.0	12.16 b	8.69 *	9.77 b **
4.0	24.09 c **	11.06 **	12.99 c **
6.0	27.52 c **	10.56 **	12.30 bc **
<i>P</i> value	<0.0001	0.0654	<0.0001

^z Shoot dry weight includes all portions of plant above substrate line.

^y n = 3 (three-plant mean for each of three blocks) for control and treatments 0.5, 2.0, 4.0 and 6.0; n = 2 for treatment 1.0 (3 plant mean for each of two blocks).

^x Letters within columns separate means by Tukey's Honest Significant Difference at $P \leq 0.05$ (control not included).

* or ** indicates significant difference from than control at $P \leq 0.05$ and 0.01, respectively (Dunnett's test).

Table 3. Non-linear logistic prediction models to describe shoot dry weight (SDW) and foliar phosphorus (P) response to P fertilizer concentrations for hydrangea (Fig. 3 a and d, respectively) and azalea (Fig. 3 c and f, respectively). Optimal P fertilizer concentration

was determined by calculating the asymptotic deceleration point for each SDW curve; foliar P and SDW at optimal P fertilizer concentration was determined by solving respective prediction equations when $x = \text{optimal fertilizer P}$.

Taxa	SDW Prediction model ^z	Foliar P prediction model	Optimal fertilizer P ($\text{mg}\cdot\text{L}^{-1}$)	Foliar P ^y (%)	SDW ^x (g)
hydrangea	$28.6/(1+e^{-0.929*(x-2.27)})$	$0.237/(1+e^{-0.997*(x-1.48)})$	4.74	0.23	25.9
azalea	$12.8/(1+e^{-1.28*(x-1.08)})$	$0.250/(1+e^{-1.05*(x-0.759)})$	2.88	0.23	11.6

^z $x = \text{fertilizer P concentration (mg}\cdot\text{L}^{-1})$.

^y Predicted foliar P concentration when fertilizer P is optimal for SDW.

^x Predicted SDW when fertilizer P is optimal for SDW.

Table 4. Effect of applied phosphorus (P) concentration versus controlled-release fertilizer (CRF; control) on root : shoot ratio and root dry weight of hydrangea, holly and azalea grown for 82 d in pine bark amended with 0.89 kg·m³ micronutrients and either 4.75 kg·m³ (hydrangea and holly) or 1.19 kg·m³ (azalea) of dolomitic limestone.

Treatment (mg·L ⁻¹ P)	Root dry weight (g) ^z	Root : shoot ^y
	hydrangea	
CRF	3.11	0.28
0.5	2.12 a ^x	0.41 d **
1.0	2.25 a	0.34 cd
2.0	4.08 ab	0.30 bc
4.0	5.31 b *	0.22 ab
6.0	5.48 b *	0.21 a
<i>P</i> value	0.0026	0.0001
	holly	
CRF	2.90	0.52
0.5	3.35	0.44 c
1.0	4.11	0.40 c
2.0	3.03	0.36 bc *
4.0	2.78	0.25 ab **
6.0	2.56	0.24 a **
<i>P</i> value	0.1083	0.0007

^z Root dry weight include all portions of plant below substrate level. For all treatments except 1.0, n = 3 (two-plant mean for each of three blocks). For treatment 1.0, n = 2 (two-plant mean for each of two blocks).

^y Root : shoot = root dry weight (g) ÷ shoot dry weight (g).

^x Letters within columns separate means by Tukey's Honest Significant Difference at $P \leq 0.05$ (control not included).

* or ** indicates significant difference from than control at $P \leq 0.05$ and 0.01 , respectively (Dunnett's test).

Table 5. Observed pore-water phosphorus (P) concentrations in pour-through leachate of hydrangea, holly and azalea fertilized with controlled-release fertilizer (CRF; control) or one of five liquid P treatments at 41 and 81 days after initiation (DAI). Substrate was amended with $0.89 \text{ kg}\cdot\text{m}^3$ micronutrients and either $4.75 \text{ kg}\cdot\text{m}^3$ (hydrangea and holly) or $1.19 \text{ kg}\cdot\text{m}^3$ (azalea) of dolomitic limestone.

Treatment ^z ($\text{mg}\cdot\text{L}^{-1}$ P applied)	Pore-water P ($\text{mg}\cdot\text{L}^{-1}$)		<i>P</i> value
	41 DAI	81 DAI	
	hydrangea		
CRF	0.16	0.24	0.4528
0.5	0.08 ^y a ^x	0.08 a	NA ^w
1.0	0.08 a	0.08 a	0.4226
2.0	0.59 ab	0.18 a	0.1501
4.0	1.43 bc **	0.55 ab	0.0963
6.0	2.02 c **	1.52 b *	0.4203
<i>P</i> value	0.0016	0.0088	
	holly		
CRF	0.08	2.49	0.0140
0.5	0.08 a	0.08 a **	NA
1.0	0.08 a	0.18 a **	0.0002
2.0	0.27 a	0.85 a *	0.0898
4.0	1.11 a	2.64 b	0.0014
6.0	4.22 b *	5.03 c **	0.4494
<i>P</i> value	<0.0001	<0.0001	
	azalea		
CRF	1.68	2.26	0.7419
0.5	0.08 a	0.08 a **	NA
1.0	0.08 a	0.14 a **	0.4226
2.0	0.15 a	0.71 a **	0.0227
4.0	2.39 a	2.18 b	0.7233
6.0	5.71 b *	4.58 c **	0.3486
<i>P</i> value	0.0002	<0.0001	

^z For all treatments except 1.0, pore-water samples were collected from one randomly selected replication from each block on each sampling date (n = 3). For treatment 1.0, pore-water samples were collected from one randomly selected replication from two blocks each sampling date (n = 2).

^y Detection limit was 0.08 mg·L⁻¹. For means comparisons, non-detectable values were assumed to be 0.08 to reduce Type I error.

^x Letters within columns separate means by Tukey's Honest Significant Difference at P ≤ 0.05 (control not included).

^w All pore-water P concentrations were below detection limits at 41 and 81 DAI.

* or ** indicates significant difference from than control at P ≤ 0.05 and 0.01, respectively (Dunnnett's test).

Table 6. Effect of applied phosphorus (P) concentration versus controlled-release fertilizer (CRF; control) on foliar nitrogen (N), P and potassium (K) concentrations of hydrangea, holly and azalea grown for 82 d in pine bark amended with 0.89 kg·m³ micronutrients and either 4.75 kg·m³ (hydrangea and holly) or 1.19 kg·m³ (azalea) of dolomitic limestone.

Treatment ^z (mg·L ⁻¹ P applied)	Foliar nutrient concentration (%)		
	N	P	K
		hydrangea	
CRF	3.53	0.212	2.06
0.5	3.52	0.057 a ^x **	0.88 a **
1.0	3.84	0.096 ab **	1.17 a **
2.0	3.46	0.155 bc	1.84 b
4.0	3.30	0.205 c	2.17 b
6.0	3.42	0.243 c	2.13 b
<i>P</i> value	0.2361	0.0004	<0.0001
		holly	

CRF	2.35	0.202	1.29
0.5	1.44 a **	0.069 a **	0.98 a *
1.0	1.63 ab **	0.095 a **	1.06 a
2.0	1.88 bc **	0.156 b *	1.19 a
4.0	1.99 c **	0.207 c	1.46 b
6.0	2.09 c *	0.219 c	1.47 b
<i>P</i> value	<0.0001	<0.0001	<0.0001
azalea			
CRF	2.16	0.237	1.01
0.5	2.04 a *	0.103 a **	1.16 b *
1.0	2.03 a *	0.151 ab **	1.04 ab
2.0	2.12 ab	0.196 bc	0.91 a
4.0	2.15 ab	0.236 cd	0.89 a
6.0	2.23 b	0.255 d	0.90 a
<i>P</i> value	0.0029	<0.0001	0.0008

^z n = 3 (three-plant mean for each of three blocks) for control and treatments 0.5, 2.0, 4.0 and

6.0; n = 2 for treatment 1.0 (three plant mean for each of two blocks).

^y Samples for foliar nutrient concentrations were obtained from 5.1- and 6.1-cm stem tip cuttings for azalea and holly, respectively; recently matured leaf blades were used for hydrangea.

^x Letters within columns separate means by Tukey's Honest Significant Difference at $P \leq 0.05$ (control not included).

* or ** indicates significant difference from than control at $P \leq 0.05$ and 0.01, respectively

(Dunnnett's test)

Figure captions

Fig. 1. Growth index [(widest width + perpendicular width + height) ÷ 3] over time (days after initiation) for holly (A) and azalea (B) fertilized with controlled-release fertilizer (CRF; control) or one of five liquid phosphorus (P) fertilizer treatments: 0.5, 1.0, 2.0, 4.0 or 6.0 mg·L⁻¹ P.

Vertical bars indicate SE.

Fig. 2. Substrate pore-water electrical conductivity [EC ($\mu\text{S}\cdot\text{cm}^{-1}$)] of hydrangea (A), holly (B) and azalea (C) amended with $0.89\text{ kg}\cdot\text{m}^{-3}$ micronutrients and either $4.75\text{ kg}\cdot\text{m}^{-3}$ (hydrangea and holly) or $1.19\text{ kg}\cdot\text{m}^{-3}$ (azalea) of dolomitic limestone fertilized with either controlled-release fertilizer (CRF; —) or liquid fertilizer (LF; - - -) pooled across liquid fertilizer treatments, 0.5, 1.0, 2.0, 4.0 and $6.0\text{ mg}\cdot\text{L}^{-1}$ P over duration of study. Vertical bars indicate SE.

Fig. 3. Non-linear logistic models describing the effect of applied phosphorus (P) concentration on shoot dry weight (SDW) of hydrangea (A), holly (B) and azalea (C), and foliar P concentration of hydrangea (D), holly (E) and azalea (F) at harvest. Model equations are listed in Table 3.

Fig. 4. Substrate pore-water pH of hydrangea (A), holly (B) and azalea (C) amended with $0.89\text{ kg}\cdot\text{m}^{-3}$ micronutrients and either $4.75\text{ kg}\cdot\text{m}^{-3}$ (hydrangea and holly) or $1.19\text{ kg}\cdot\text{m}^{-3}$ (azalea) of dolomitic limestone fertilized with either controlled-release fertilizer (CRF; —) or liquid fertilizer (LF; - - -) pooled across five phosphorus (P) concentration treatments ($0.5, 1.0, 2.0, 4.0$ and $6.0\text{ mg}\cdot\text{L}^{-1}$ P) over duration of study (80 d). Vertical bars indicate SE.

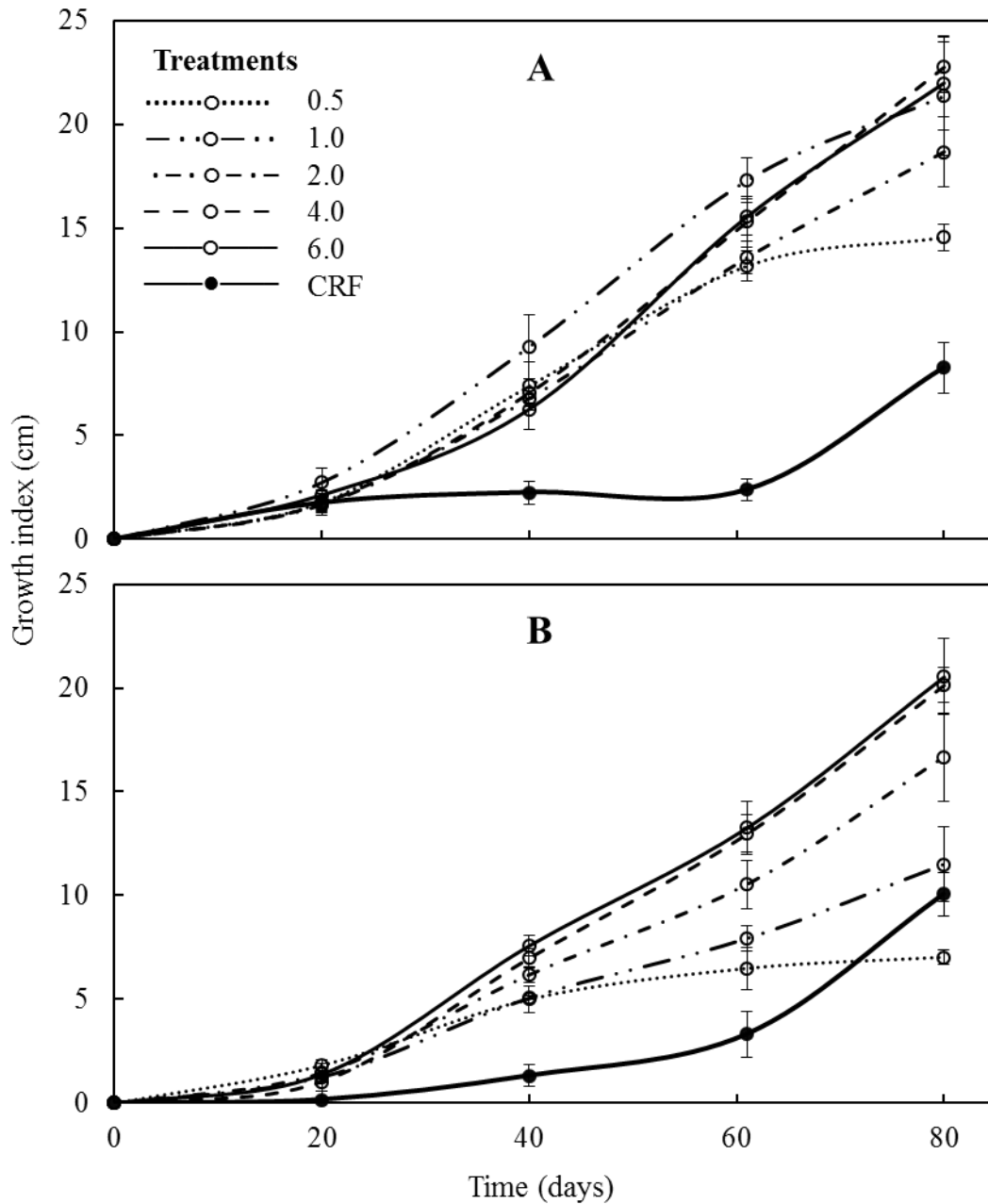


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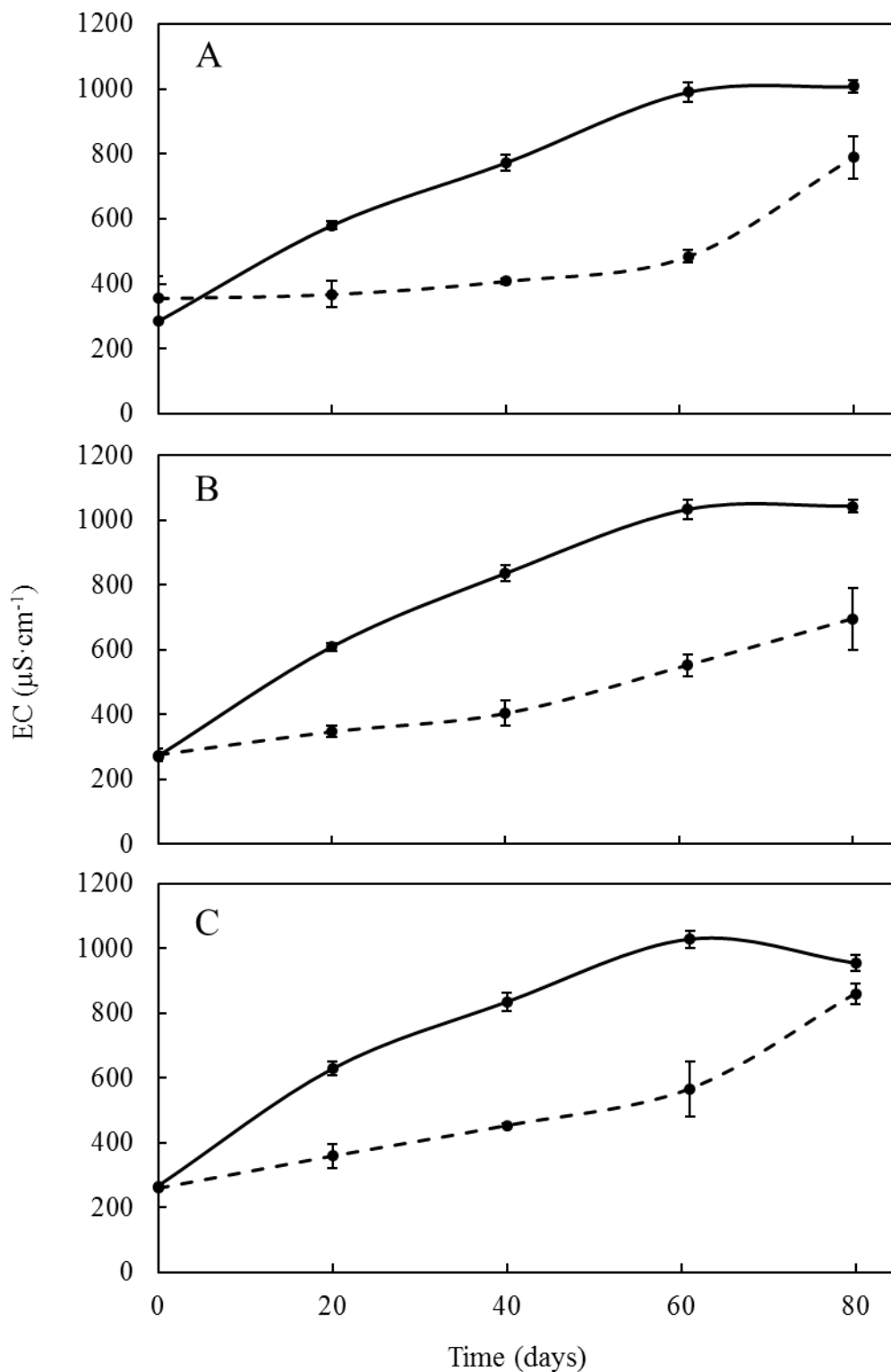


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holly) or 1.19 kg·m³ (azalea) of dolomitic limestone fertilized with either controlled-release fertilizer (CRF; —) or liquid fertilizer (LF; - - -) pooled across liquid fertilizer treatments, 0.5, 1.0, 2.0, 4.0 and 6.0 mg·L⁻¹ P over duration of study. Vertical bars indicate SE.

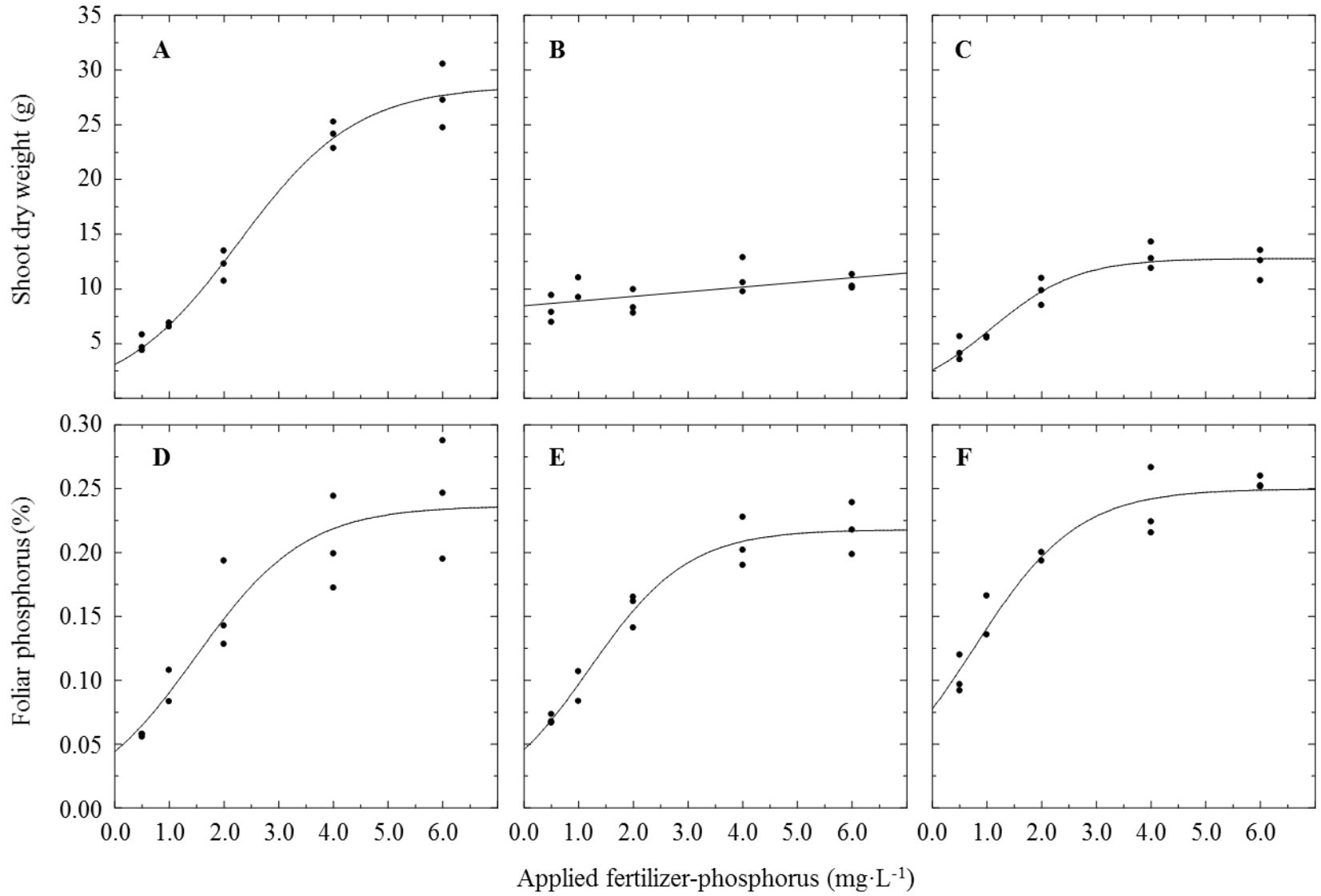


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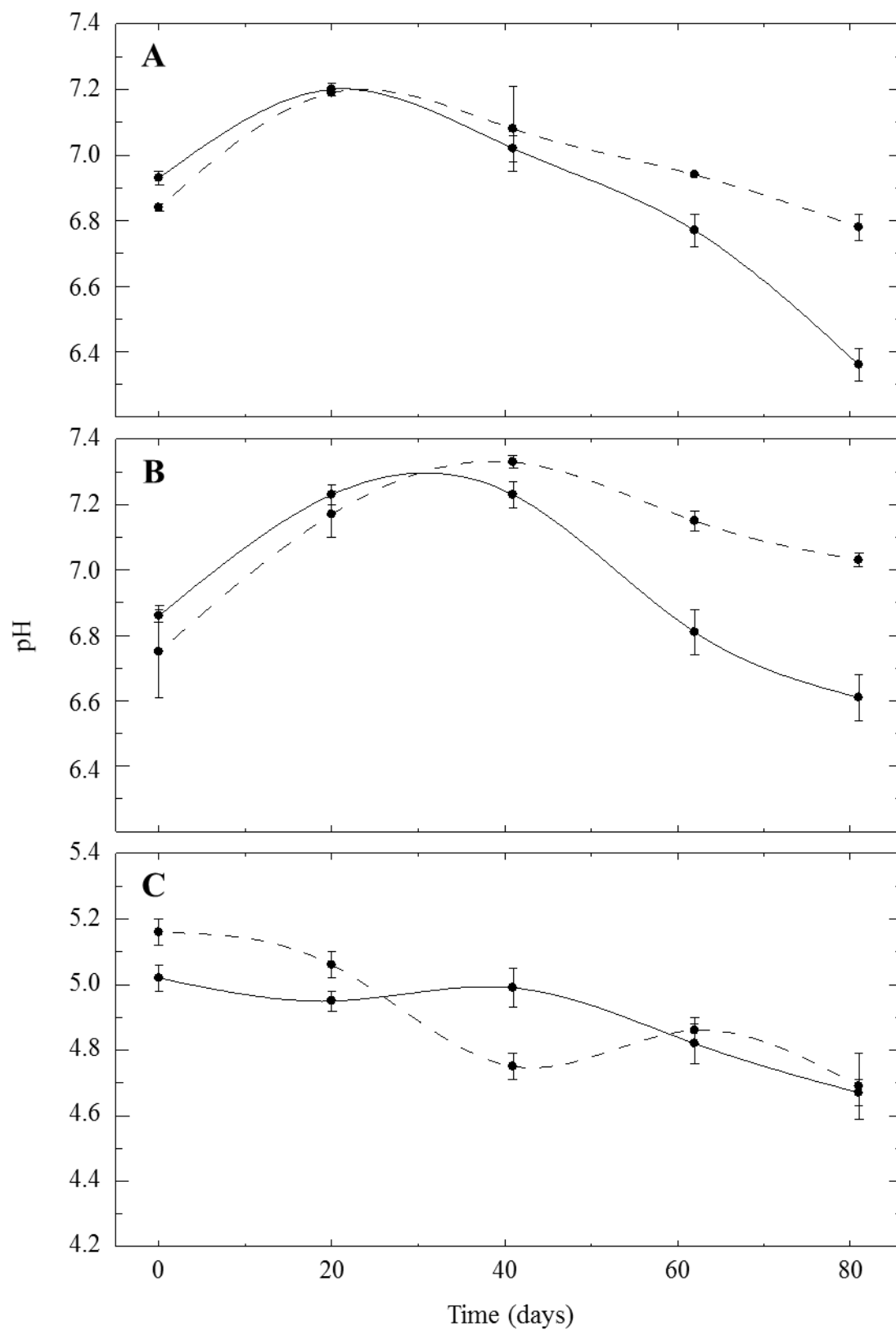


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Chapter 3

Growth Response of *Hydrangea macrophylla* and *Ilex crenata* Cultivars to Low-phosphorus Controlled-release Fertilizers

Abstract

In containerized nursery-crop production, conventional phosphorus (P) fertilization amounts are reported to be in excess of plant needs which has resulted in poor P use efficiency (PUE) and subsequent P leaching from containers. Phosphorus leaching can be reduced and PUE improved without affecting plant growth by reducing P fertilization. The objective of this study was to identify the lowest controlled-release fertilizer (CRF)-P content and subsequent pour-through-extracted substrate pore-water P (PWP) concentration that produces maximal shoot growth of two common container-grown nursery crop species, *Hydrangea macrophylla* ‘P11HM-11’ (hydrangea) and *Ilex crenata* ‘Helleri’ (holly), in a pine bark substrate. Hydrangea and holly liners were potted into 3.8-L containers containing a pine bark substrate and grown simultaneously in two different Virginia ecoregions, Middle Atlantic Coastal Plain (MACP) and Ridge and Valley (RV). Plants were fertilized with one of five CRF formulations, each containing equal nitrogen (N) and potassium (K) and 0.4%, 0.9%, 1.3%, 1.7% or 2.6% (control) P to supply containers with 0.1, 0.2, 0.3, 0.4 or 0.6 g P, respectively. In both ecoregions, hydrangea shoot dry weight (SDW) and growth index [i.e., (widest width + perpendicular width + height) ÷ 3, GI] values were maximal in plants fertilized with 0.3 to 0.4 g P or the control. The lowest CRF-P rate needed for maximal SDW and GI of holly was 0.2 g P at the RV site and 0.4 g P at the MACP site. Mean PWP concentrations that corresponded with highest SDW were as low as 0.8 and 1.2 mg·L⁻¹ for hydrangea and holly, respectively. Results from this research suggest hydrangea requires approximately half the P rate supplied by recommended rates of conventional CRFs. Since the growth response of holly to CRF-P rate at the MACP site was inconsistent with results observed at the RV site and findings in scientific literature, further research is needed to determine the minimum required CRF-P rate for this taxon.

Keywords: Hydrangea macrophylla, Ilex crenata, phosphorus, controlled-release fertilizer, pine bark, substrate pore-water

1. Introduction

Phosphorus (P) management in crop production is becoming increasingly critical from an environmental perspective. From 2002 to 2010, agriculture was estimated to contribute 38% of anthropogenically sourced P loads in fresh water across the world (Mekonnen and Hoekstra, 2017). In the US, agricultural inputs of nitrogen (N) and P are a leading source of nutrient pollution and resulting eutrophication in surface waters (USEPA, 2017). Establishment of the Impaired Waters and Total Maximum Daily Load (TMDL) Program, section 303d of the Clean Water Act, resulted in increased efforts by US state legislatures to reduce N and P loads to waterbodies. In the Chesapeake Bay Watershed (CBW), which spans six states and the District of Columbia, agriculture is the leading source of N and P loads (USEPA, 2010a), and approximately 29% of agricultural P-inputs are from chemical fertilizers (USEPA, 2010b). To support achieving TDMLs for the CBW, Maryland passed a law in 1998 requiring agricultural operations grossing \geq \$2,500 per year, including container-grown plant nurseries, to submit a nutrient management plan and subsequent annual reports on N and P applications (Lea-Cox and Ross, 2001; Lea-Cox et al., 2001). Accordingly, if forthcoming N and P TMDL milestones are not met for the CBW and other impaired waterways, more stringent nutrient load regulations can be expected for agricultural producers therein (Majsztrik and Lea-Cox, 2013).

Agricultural operations can voluntarily contribute to achieving TMDLs by implementing Best Management Practices (BMPs). In containerized crop production, fertilizing with controlled-release fertilizer (CRF) instead of aqueous or soluble granular forms is among the most widely employed BMPs for fertilizer management by growers in Virginia (Mack et al.,

2017) and Alabama (Fain et al., 2000). Relative to other fertilization methods used in nursery crop production, CRFs have been shown to promote higher phosphorus use efficiency (PUE; percent of fertilizer P taken up by plants; Naik et al., 2017) and decrease P leaching from containers (Broschat, 1995; Diara, 2014) and subsequent P runoff from a given production area (Sharma and Bolques, 2007).

Despite improved PUE when using CRF relative to other fertilization methods, PUE of CRF-fertilized container-grown nursery crops has been reported to remain between $\approx 27\%$ and 62% (Owen et al., 2008; Tyler et al., 1996; Warren et al., 1995; Warren et al. 2001). Multiple containerized crop production studies have shown that P readily leaches from pine bark substrates during irrigation (Yeager and Barrett, 1984; Yeager and Barrett, 1985; Yeager and Wright, 1982), and current P fertilization amounts exceed plant needs (Ristvey et al., 2007). Thus, relatively low PUE is a consequence of superfluous P fertilization amounts and inaccessibility of P to roots due to P leaching from containers.

Phosphorus use efficiency of containerized crops can be improved without affecting plant growth by reducing P fertilization. In two studies utilizing liquid-fertilization, Ristvey et al. (2004, 2007) observed increased PUE of containerized *Rhododendron* L. 'Karen' grown in a pine bark-peat substrate from $\approx 15\%$ to $\approx 45\%$ by decreasing P from 25 to 5 mg per week when applied N was non-limiting for growth (i.e., 100 to 250 mg·L⁻¹ N). In both studies, shoot growth was unaffected by P fertilizer level. When using a CRF, Owen et al. (2008) found that reducing P from 1.0 to 0.5 g per container improved PUE of *Cotoneaster dammeri* Schneid. 'Skogholm' by 11% without affecting shoot growth when grown in a pine bark substrate amended with 11% builder's sand (by volume).

Best Management Practices for CRF-fertilized container nursery crops (Bilderback et al., 2013) cite that pore-water P (PWP) concentrations, as measured using the pour-through extraction method, of 5 to 10 mg·L⁻¹ P be maintained for non-limiting crop growth. However, studies have shown that some woody taxa can sustain maximal growth when pour-through leachate P is consistently < 5 mg·L⁻¹. Groves et al. (1998a and b) found that the CRF type (i.e., resin-coated P or polymer-coated P) that produced highest shoot dry weight of *Cotoneaster dammeri* ‘Skogholm’ grown in a pine bark substrate had < 3 mg·L⁻¹ P in pour-through leachate. Similarly, Matysiak (2015) observed that pour-through P concentrations for CRF-fertilized *Euonymus japonicus* Thunb. ‘Ovatus Aureus’ and *Rhododendron* L. ‘Geisha Orange’ associated with highest quality ratings and shoot fresh weight had ≤ 2.6 mg·L⁻¹ P in pour-through leachate when grown in a sphagnum peat substrate.

Few studies have specifically assessed the lowest necessary P content in CRFs and resulting PWP concentration for maximal growth of container-grown nursery crops. Leonard et al. (2007) grew *Codiaeum variegatum* (L.) Blume var. *pictum* (Lodd.) Müll. Arg. ‘Mammy’ and *Chrysobalanus icaco* L. potted in a pine bark-peat substrate and fertilized with CRF containing 18% N, 10% K and 0%, 0.4%, 1.3%, or 2.6% P. In both taxa, growth index values were highest in plants fertilized with CRF granules containing at least 0.4% P in which pour-through leachate concentrations were 0.3 to 0.7 mg·L⁻¹. However, P nutrition of tropical plant taxa may differ from that of temperate species. The objective of this study was to identify the lowest CRF-P content and subsequent substrate PWP concentration that produces maximal shoot growth of two common container-grown nursery crop species, hydrangea and holly, in a pine bark substrate.

2. Materials and Methods

This study was replicated simultaneously in two different ecoregions, the Middle Atlantic Coastal Plain (MACP) and Ridge and Valley (RV). The MACP site was at the Virginia Tech Hampton Roads Agriculture Research and Extension Center in Virginia Beach, Virginia (latitude 36°53'31"N; longitude 76°10'44"W; 8 m elevation; USDA Plant Hardiness Zone 8a) and the RV site was at the Urban Horticulture Center in Blacksburg, VA (latitude 37°12'59"N; longitude 80°27'50"W; 629 m elevation; USDA Plant Hardiness Zone 6b). Daily rainfall and temperature data, obtained from the on-site Virginia Agricultural Experimental Station Mesonet weather station, are illustrated in Fig. 1.

On 14 May 2015, rooted stem cuttings of *Ilex crenata* Thunb. 'Helleri' (holly) were acquired in 18-cell flats (three cuttings per cell) from Saunders Brothers Nursery, Piney River, VA. Rooted stem cuttings of *Hydrangea macrophylla* (Thunb.) Ser. 'P11HM-11' (Bloomstruck®) were received the following day from Bailey Nursery, Yamhill, OR in 45-cell flats (one cutting per cell). Rooted stem cuttings of each taxon were taken to the MACP and RV sites.

2.1. Middle Atlantic Coastal Plain site.

On 1 June 2015 (day 0), rooted stem cuttings within each cell were separated and roots rinsed with a high-pressure water stream to remove substrate and fertilizer. Bare-root plants were then potted individually into 3.8 L black plastic containers (#1, Nursery Supplies, Chambersburg, PA). Substrate used for potting holly and hydrangea consisted of aged pine bark (Sun Gro, Agawam, MA) amended with 0.89 kg·m⁻³ of a granular micronutrient fertilizer (Micromax, Everris, Dublin, OH), 2.08 kg·m⁻³ ground dolomitic limestone [97% calcium carbonate equivalent (CCE), Rockydale Quarries Corporation, Roanoke, VA] and 2.08 kg·m⁻³ pulverized

dolomitic limestone (94% CCE, Old Castle Lawn and Garden, Thomasville, PA). Amendments were thoroughly hand-mixed into substrate to ensure homogeneity.

Each plant received 3.65 ± 0.04 SE g N from either 20 g of one of four low-P CRF treatments (experimental CRFs) or 24 g of a standard CRF used in Virginia nurseries (control; Table 1). Fertilizer was delivered by surface application followed by hand-incorporation of CRF in the top 2.5 cm of substrate. All four experimental CRF treatments were 8- to 9-month heterogeneous formulations (Harrell's LLC, Lakeland, FL), blended specifically for this study. In addition to N, P and K, all four experimental CRFs contained 1.24% magnesium (Mg), 4.38% sulfur (S), 0.11% copper (Cu), 1.26% iron (Fe), 0.12% manganese (Mn), 0.003% molybdenum (Mo) and 0.11% zinc (Zn). The control CRF (8- to 9-month formulation) contained 1.3% Mg, 6.3% S, 0.08% Cu, 0.32% Fe, 0.13% Mn, 0.01% Mo and 0.08% Zn.

All holly were pruned to a width and height of 10.4 cm and hydrangea to a width of 13.8 and height of 17.8 cm. Plants were then placed 0.15 m apart on an outdoor gravel pad in a completely randomized design (CRD) with seven replications per treatment. Due to taxa-specific irrigation requirements, hydrangea and holly were grown under separate irrigation systems. All plants were overhead irrigated with impact sprinklers [Model 2045-PJ SBN-1, 3.18 mm orifice (holly) or 3.97 mm orifice (hydrangea), Rain Bird, Azusa, CA] on 122 cm risers daily or as needed in two cycles, the first at 1200 HR and the second at 1600 HR. On average, hydrangea and holly received 1.1 ± 0.47 SE cm ($n = 15$) and 0.56 ± 0.43 SE cm ($n = 12$) water, respectively, per irrigation cycle; however, irrigation rate was adjusted periodically based on leaching fraction. Irrigation distribution uniformity [i.e., (average of lowest volume quartile \div average volume) $\times 100$] was measured 31 DAI to be 71% and 85% for the hydrangea and holly irrigation systems, respectively. Leaching fraction (volume leached \div volume applied) was measured on three

replications per taxa at 42, 52 and 88 DAI for hydrangea and 30, 52 and 88 DAI for holly. Mean leaching fractions for hydrangea and holly were 0.72 ± 0.07 SE and 0.39 ± 0.11 SE, respectively.

At 16, 28, 52, 88 and 115 DAI, substrate pore-water (i.e., capillary water retained within and between substrate particles) was extracted from four replications per treatment via the pour-through method (Wright, 1986) and analyzed for P concentrations, pH and electrical conductivity (EC). Pour-through extracts were attained by hand-pouring 120 mL deionized water over the surface of the substrate \approx 1 h following a normal irrigation event and collecting at least 50 mL of subsequent leachate for analyses. To measure substrate pore-water and irrigation water nutrient concentrations, an 8-mL aliquot of each sample was filtered (0.2 μ m, 30 mm syringe filter; Thermo Fisher Scientific, Beverly, MA) then stored at -18 °C until ion analysis. Pore-water and irrigation samples were thawed and analyzed for phosphate (PO_4^{3-}) concentration in pore-water samples and NH_4^+ , Ca^{2+} , Mg^{2+} , K^+ , NO_2^- , NO_3^- and PO_4^{3-} concentrations in irrigation water using two ion chromatography (IC) systems. The IC systems used to determine anion concentrations (ICS-2100, Thermo-Fisher Scientific) and cation concentrations (ICS-1600, Thermo-Fisher Scientific) utilized respective 4×250 mm (i.d. \times length) anion- and cation-exchange columns (AS19 and CS12A, respectively, Thermo Fisher Scientific) at 35 °C and an autosampler (AS-AP, Thermo Fisher Scientific) on a 25 μ L sample loop. In addition, the ICS-2100 was equipped with a metal trap column (MFC-1, Thermo Fisher Scientific) to remove metals that may interfere with accurate detection of the aforementioned anions. Pore-water NO_3^- and K^+ concentrations were not assessed because concentrations of these ions were often higher than detection limits (i.e., $136 \text{ mg}\cdot\text{L}^{-1}$) and diluting samples would have compromised PO_4^{3-} data. Irrigation water ($n = 6$; collected 16 DAI) contained $< 0.25 \text{ mg}\cdot\text{L}^{-1}$ NH_4^+ , 14.08 ± 0.44 SE $\text{mg}\cdot\text{L}^{-1}$ Ca^{2+} , 3.93 ± 0.17 SE $\text{mg}\cdot\text{L}^{-1}$ Mg^{2+} , 3.93 ± 0.23 SE $\text{mg}\cdot\text{L}^{-1}$ K^+ , $< 0.25 \text{ mg}\cdot\text{L}^{-1}$ NO_2^- , 13.64

± 1.84 SE $\text{mg}\cdot\text{L}^{-1}$ NO_3^- and < 0.25 $\text{mg}\cdot\text{L}^{-1}$ PO_4^{3-} . Electrical conductivity and pH were measured using a benchtop meter (Orion 4-Star Plus pH/Conductivity Meter, Thermo Fisher Scientific) equipped with a 4-Electrode Conductivity Cell (DuraProbe, Thermo Fisher Scientific) and a double junction pH electrode (9102AP, AquaPro, Orion, Thermo Fisher Scientific). All pH and EC analyses were completed within twelve hours of collecting pore-water samples. Growth index [GI; i.e., (height + widest width + perpendicular width)/3] was measured for all plants 15, 52, 88 and 130 DAI.

At day 130, two replications from each treatment for both taxa were removed from the study due non-treatment-related poor health. Foliar samples were harvested from each remaining plant as described in Bryson et al. (2014) for tissue nutrient analysis. For hydrangea, tissue samples consisted of 12 to 15 leaf blades from recently matured leaves. Holly tissue samples were twelve 3.1-cm terminal stem cuttings. Tissue samples were triple-rinsed with deionized water then visually inspected to ensure samples were clean of possible contaminants (i.e., substrate particles). Foliar samples were then oven-dried (60 °C), weighed and analyzed for N, P and K concentrations (Brookside Laboratories, New Bremen, OH). Tissue sample dry weights were included in final shoot dry weight (SDW) calculations.

At 133 DAI, shoots (i.e., above-substrate level) were severed, oven-dried (60 °C) and weighed for SDW. Substrate was then removed from the roots using a high-pressure water stream, and roots were oven-dried (60 °C) and weighed for root dry weight (RDW). Due to the labor required to remove substrate particles from hydrangea roots, three replications from each treatment were selected at random for hydrangea RDW. All five replications were included to obtain holly RDW.

2.2. Ridge and Valley site.

Materials and methods for the RV site were similar to those used at the MACP site with the follow exceptions. Hydrangea and holly were potted on 4 June 2015 (day 0) and then placed on an outdoor gravel pad under two separate overhead-irrigation systems (137 cm risers, upright mini-Wobblers, 2.78 mm #7 orifice, Senninger, Clermont, FL). On average, hydrangea and holly received 0.43 ± 0.14 SE cm (n = 15) or 0.41 ± 0.13 SE cm (n = 18) irrigation, respectively. Irrigation distribution uniformity was measured 34 DAI to be 93% or 94% for the hydrangea and holly irrigation systems, respectively. Leaching fraction was measured on three replications per taxa at 36, 60 and 91 DAI for hydrangea and 36, 60 and 104 DAI for holly. Mean leaching fraction values for hydrangea and holly were 0.49 ± 0.09 SE and 0.39 ± 0.09 SE, respectively.

Substrate pore-water samples were collected 19, 36, 61, 91 and 109 DAI for analysis of pH, EC and PO_4^{3-} concentrations. Irrigation water (n = 3; collected 110 DAI) contained 0.46 ± 0.001 SE $\text{mg}\cdot\text{L}^{-1}$ NH_4^+ , 18.54 ± 0.33 SE $\text{mg}\cdot\text{L}^{-1}$ Ca^{2+} , 6.86 ± 0.17 SE $\text{mg}\cdot\text{L}^{-1}$ Mg^{2+} , 2.04 ± 0.02 SE $\text{mg}\cdot\text{L}^{-1}$ K^+ , < 0.25 $\text{mg}\cdot\text{L}^{-1}$ NO_2^- , 1.29 ± 0.07 SE $\text{mg}\cdot\text{L}^{-1}$ NO_3^- and 0.39 ± 0.002 SE $\text{mg}\cdot\text{L}^{-1}$ PO_4^{3-} . Electrical conductivity and pH were measured using a pH, EC and TDS meter (HI9811, Hanna Instruments Inc., Woonsocket, RI).

All replications were kept in the study. Foliar samples for tissue nutrient analysis were harvested from each plant 111 DAI, and shoots and roots were harvested 113 DAI. Roots were harvested from four replications per treatment for hydrangea due to difficulty in efficiently separating substrate particles from roots.

2.3. Statistical analysis

To assess treatment effects, including the control CRF, data were subjected to analysis of variance (ANOVA), and *post-hoc* means comparison was accomplished using the Tukey-Kramer Honest Significant Difference test. Linear and quadratic regression were performed to assess the effect of experimental CRF-P rate (i.e., 0.1 to 0.4 g P per container) on response variables measured. The multivariate approach to repeated measures analysis was used to affirm that a time \times CRF-P rate interaction existed within each taxa. Since the time \times CRF-P rate interaction was significant ($P < 0.05$) for each taxa in both ecoregions, ANOVA and subsequent Tukey's HSD means separation was used to assess simple effects of CRF-P rate at each measurement date. Since PWP concentrations, pH and EC were measured on four randomly-selected replications within each treatment, repeated measures analysis was not necessary. When pooled across sampling dates, PWP data were log transformed to meet the constant variance assumption. After ANOVA and regression P-values were attained, mean PWP levels were converted back to original measured values. Correlations were determined using the Pearson correlation coefficient (r). All data were processed using JMP Pro 13 (SAS Institute Inc.).

3. Results and Discussion

3.1. Middle Atlantic Coastal Plain

3.1.1. Pore-water Phosphorus

Pore-water P concentrations were the same across CRF treatments at 16 DAI for hydrangea (0.74 ± 0.20 SE $\text{mg}\cdot\text{L}^{-1}$) and at 16 and 28 DAI for holly (1.74 ± 0.28 SE and 1.13 ± 0.42 SE $\text{mg}\cdot\text{L}^{-1}$, respectively); at all sampling dates thereafter, PWP levels increased linearly for both taxa with increasing CRF-P rate (Table 2). For both taxa, PWP levels of the control CRF were the same as those of the 0.2 g P CRF until 88 DAI, despite three times more P in the control

CRF. At 88 DAI, PWP concentrations of holly fertilized with the control CRF were over three times that of any other treatment, whereas PWP concentrations of hydrangea fertilized with the control were the same as in those given 0.4 g P treatment. These data suggest P in the control CRF had a different release pattern, with a delayed release compared to the experimental CRFs. This assessment is supported by the $\approx 2300\%$ increase in PWP concentration from 52 to 88 DAI for holly fertilized with the control; yet, in all other treatments, PWP was unchanged between 52 and 88 DAI. In addition, pore-water EC (Fig. 2) was consistently lower in holly fertilized with the control CRF compared to the experimental fertilizers. A possible explanation for the differing release between the control and experimental CRFs may be related to their homogenous and heterogenous nature, respectively. In the control CRF, each granule contained a combination of all listed mineral nutrient salts, whereas the experimental fertilizers contained individually-coated prills of monoammonium phosphate ($\text{NH}_4\text{H}_2\text{PO}_4$), urea ($\text{CH}_4\text{N}_2\text{O}$) and potassium sulfate (K_2SO_4). Several studies assessing release of individual nutrients from homogeneous polymer-coated CRF products (similar to our control CRF) have reported a lag in P release (Broschat, 2005; Broschat and Moore, 2007; Du et al., 2006; Newman et al., 2006). However, little evidence exists that individually-coated $\text{NH}_4\text{H}_2\text{PO}_4$, $\text{CH}_4\text{N}_2\text{O}$ and K_2SO_4 prills, all of which are common in a heterogeneous CRF, have belated release.

For all treatments except for hydrangea and holly fertilized with 0.1 g P and holly fertilized with the control, PWP concentrations were the same over time (Table 2). Pore-water P concentrations of hydrangea and holly fertilized with 0.1 g P were highest at 16 DAI, then decreased to levels consistently near or below detection limits (i.e., $\leq 0.08 \text{ mg}\cdot\text{L}^{-1} \text{ PO}_4\text{-P}$). The nearly non-detectable PWP concentrations for the 0.1 g P treatment on all sampling dates after 16 DAI were most likely due to root uptake of P. In other studies, reduction in PWP

concentrations during the equilibration period between irrigation and pour-through extraction was attributed to root uptake when observed for *Hydrangea paniculata* Sieb. ‘Limelight’, *Ilex crenata* ‘Helleri’, *Rhododendron* L. ‘Karen’ (Shreckhise et al., 2018), *Tagetes erecta* L. ‘Inca Gold’ (Tolman et al., 1990) and *Euphorbia pulcherrima* Willd. Ex Klotzch ‘Freedom Red’ (Cavins et al., 2004). Thus, relatively high PWP concentrations at 16 DAI of plants in the 0.1 g P treatment may be attributed to a relatively low P uptake rate by roots since roots were not likely yet established.

3.1.2. Growth index and carbon allocation.

Phosphorus treatment effect on hydrangea and holly GI was first apparent at 130 ($F = 6.59$; $P < 0.0001$) and 88 ($F = 8.47$; $P = 0.0004$) DAI, respectively (Fig. 3). The delayed growth response of hydrangea to the varying P rates and the apparent negative GI values at 52 DAI are attributed to transplant stress that resulted in leaf-drop, which suspended growth as plants recovered. From 52 to 130 DAI, GI of both taxa increased linearly ($P < 0.0001$) for all treatments except 0.1 g P, in which GI responded quadratically. The quadratic growth trend over time and non-detectable PWP concentrations (i.e., $\leq 0.08 \text{ mg}\cdot\text{L}^{-1} \text{ PO}_4\text{-P}$; Table 2) after 52 DAI for holly fertilized with 0.1 g P indicates P was limiting shoot growth.

Hydrangea and holly SDW had strong, positive correlations with respective final GI (GI_f ; GI at harvest) values ($r = 0.89$ and 0.81 , respectively). Hydrangea GI_f and SDW values (Table 3) were highest in those fertilized with 0.3 to 0.4 g P or the control, and holly SDW and GI_f values were maximal in plants given 0.4 g P. The corresponding mean PWP concentrations, pooled across sampling dates (Table 3), that associate with the highest GI_f and SDW values were 0.8 to $2.2 \text{ mg}\cdot\text{L}^{-1}$ P for hydrangea and $2.6 \text{ mg}\cdot\text{L}^{-1}$ P for holly. Shoot dry weight and GI_f values

increased quadratically for hydrangea and linearly for holly with increasing experimental CRF-P rate. The quadratic response of GI_f and SDW to increasing CRF-P rate for hydrangea indicates CRF-P levels were at or approaching sufficiency. The linear increase in holly GI_f and SDW with increasing P rate suggests that > 0.4 g CRF-P or > 2.6 $mg \cdot L^{-1}$ PWP may have resulted in greater shoot growth than that observed in this study. In a liquid feed study performed by Shreckhise et al. (2018), GI of *Ilex crenata* ‘Helleri’ was the same for mean pour-through PWP concentrations between 0.1 and 4.6 $mg \cdot L^{-1}$ (1 to 6 $mg \cdot L^{-1}$ P applied). The apparent discrepancy between these two studies is most likely due to differing nutrient supply methods, solution vs. CRF, and the resulting PWP concentrations and amounts related to those nutrient application methods; hence a comparison of these findings is difficult to interpret. When growing containerized *Spiraea × bumalda* Burven ‘Gold Mound’, Clark and Zheng (2017) showed that the top-dress CRF rate that produced highest quality plants (4.5 g CRF-N per 7.6 L container) had pour-through P concentrations consistently less than ≈ 3 $mg \cdot L^{-1}$ P. Groves et al. (1998a and b) reported similar findings for container-grown *Cotoneaster dammeri* ‘Skogholm’ fertilized with substrate-incorporated CRF. Interestingly, GI_f and SDW values of holly fertilized with 0.4 g CRF-P were higher than those of the control, despite the 50% higher P content in the control CRF. As previously discussed, higher GI_f and SDW of holly given 0.4 g P compared to the control CRF could be a consequence of the delayed nutrient release of the control CRF as indicated by consistently lower pore-water EC values (Fig. 2) and, prior to 88 DAI, PWP concentrations (Table 2).

Hydrangea RDW (Table 3) increased linearly as applied P increased from 0.1 to 0.4 g; however, RDW of those fertilized with the control CRF was the same as in those given 0.1 to 0.4 g CRF-P. Reduced RDW in response to low-P fertility has been reported in *Hydrangea*

paniculata ‘Limelight’ (Shreckhise et al., 2018), *Eucalyptus dunnii* Maiden and *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson (Niu et al., 2015). Root dry weight of holly was the same across all four experimental CRF treatments and was lower in plants given the control than in those that received 0.2 g P. Studies that have reported the effect of P fertilizer levels on RDW of *Ilex crenata* ‘Helleri’ are inconsistent. In agreement with our findings, Shreckhise et al. (2018) observed no differences in RDW of *Ilex crenata* ‘Helleri’ across fertilizer treatments containing 0.5 to 6 mg·L⁻¹ P. Yeager and Wright also (1982) reported no differences in *Ilex crenata* ‘Helleri’ RDW in one experiment in which fertilizer-P was increased in 5 mg·L⁻¹ increments from 0 to 20 mg·L⁻¹; however, RDW decreased in a subsequent experiment in which P was incrementally increased from 0 to 30 mg·L⁻¹.

Root : shoot ratio of hydrangea and holly (Table 3) decreased quadratically and linearly, respectively, with increasing experimental CRF-P rate. Root : shoot ratio values of hydrangea and holly fertilized with the control were the same as in those given 0.2 to 0.4 g P. Increased root : shoot ratio values in response to growth-limiting P levels suggest that as P fertility shifts from growth-limiting to sufficient, shoot growth is preferentially stimulated over root growth. This negative relationship between fertilizer-P and root : shoot ratio has been reported in several nursery and greenhouse taxa [e.g., *Betula alnoides* Buch.-Ham. ex D. Don (Chen et al., 2010), *Betula pendula* Roth. (Ericsson and Ingestad, 1988) and *Lantana camara* L ‘New Gold’ (Kim and Li, 2016)].

3.1.3. Foliar nutrient concentrations.

Hydrangea foliar P concentrations (Table 3) increased linearly with increasing experimental CRF-P rate, and foliar P concentrations of the control plants were higher than in

plants that received an experimental CRF. Luxury consumption of P was evident in hydrangea since SDW and GI_f values were the same as foliar P concentrations increased from 0.19 to 0.34 $mg \cdot g^{-1}$ dry weight (DW). Luxury P consumption has been reported for numerous woody and herbaceous plant taxa (e.g., Dickey et al., 1967; Havis and Baker, 1985; Kraus et al., 2011; Kim and Li, 2016; Ristvey et al., 2007; Yeager and Wright, 1982). Hydrangea foliar P concentrations associated with maximal GI_f and SDW values were in the range of 0.17 to 0.34 $mg \cdot g^{-1}$ DW. This observed foliar P sufficiency range is below that reported by Bryson et al. (2014) for *Hydrangea macrophylla* (0.23 to 0.67 $mg \cdot g^{-1}$ DW); however, optimal foliar nutrient ranges are known to be cultivar-dependent (Sonneveld and Voogt, 2009). Furthermore, foliar nutrient sufficiency ranges reported by Bryson et al. (2014) are based on survey data and therefore may not indicate actual threshold nutrient levels associated with maximal growth. The 400% increase in experimental CRF-P (0.1 to 0.4 g P) resulted in a respective 36% increase in hydrangea foliar P; however, the relatively minor increase in CRF-P from 0.4 to 0.6 g P (50% increase in CRF-P) resulted in a 0.79% increase in foliar P. The reason the increase in CRF-P from 0.4 to 0.6 g (control) for hydrangea resulted in a disproportional increase in tissue P concentration cannot be ascertained from data collected in this study. However, the authors speculate that in addition to P, concentrations of other nutrients and nutrient ions in pore-water throughout the study may have differed for the control CRF compared to the experimental CRFs, as indicated by EC data (Fig. 2). Consequently, the presence and extent of possible mineral nutrient synergistic effects on P uptake (e.g., NO_3^- to NH_4^+ ratio; Jiménez et al., 2007) may have been different for the control CRF compared to the experimental CRFs. Holly foliar P increased quadratically with increasing CRF-P level within experimental CRFs. According to Bryson et al. (2014) and Shreckhise et al. (2018), *Ilex crenata* ‘Helleri’ tissue P concentrations of 0.08 to 0.11 $mg \cdot g^{-1}$ DW and 0.10 to 0.22

mg·g⁻¹ DW, respectively, indicate P sufficiency. In our study, tissue P concentrations of 0.08 to 0.18 mg·g⁻¹ DW (i.e., 0.1 to 0.4 g CRF-P treatments) corresponded with submaximal SDW, suggesting the critical tissue-P concentration for holly is > 0.18 mg·g⁻¹ DW when grown in climactic conditions observed in this study. However, further research is needed to better understand this discrepancy. Despite having 36% less SDW, holly fertilized with the control CRF had equivalent foliar P concentrations to those given 0.4 g P. Bi et al. (2007) reported that biomass of two *Rhododendron* taxa increased two to four weeks after tissue-N accumulation in response to N fertilization. Thus, a possible explanation for the relatively high tissue P concentrations of holly fertilized with the control CRF is that the delayed P release of the control CRF may have enabled plants to take up and translocate P to leaves and growing tips, but tissue samples were harvested prior to shoot growth response to increased P availability.

In response to increasing CRF-P from 0.1 to 0.4 g, foliar N concentrations (Table 3) decreased linearly in hydrangea but increased quadratically in holly. The decrease in foliar N of hydrangea with increasing CRF-P treatment is likely a dilution effect due to greater leaf size of plants that received higher CRF-P rates. The dilution effect is the dilution or concentration of a nutrient in plant tissue relative to the level of biomass accumulated (Jarrell and Beverly 1981). Although leaf area was not measured in this study, hydrangea leaves were noted as being smaller in plants fertilized with 0.1 g P compared to all other CRF-P rates. Relatively low N concentrations of holly fertilized with 0.1 g P is likely a reflection of the phenotypic stage of the tissue sampled. Gilliam and Wright (1979) showed that nutrient-limited *Ilex crenata* 'Helleri', a taxon with episodic shoot growth, required more time to complete a growth flush than those with an ample nutrient supply. In the current study, holly fertilized with 0.1 g P contained no newly expanded shoots at the time of tissue harvest, whereas tissue samples of holly fertilized with 0.2

to 0.4 g P or the control CRF were taken from expanding or recently expanded shoot tips. Since plant tissue N concentration tends to decrease with tissue age (Marschner, 2011), the mature shoot tips of holly given 0.1 g CRF-P had less N than the relatively young shoot tips harvested from all other treatments. Thus, caution should be used when interpreting nutrient levels of stem tip samples, particularly when taken from taxa that have multiple growth flushes within a growing season. Shreckhise et al. (2018), who used the same method employed in this study for obtaining holly tissue samples, also observed increasing tissue N of *Ilex crenata* ‘Helleri’ as P-fertilizer increased from 0.5 to 6.0 mg·L⁻¹ P. Control plants achieved similar foliar N levels as those fertilized with 0.1 to 0.2 g P in hydrangea and 0.3 g P in holly.

In response to increasing CRF-P supply from 0.1 to 0.4 g, foliar K concentrations (Table 3) for hydrangea and holly followed the same decreasing and increasing trends, respectively, as observed for foliar N concentration. Foliar K levels of hydrangea fertilized with the control CRF were the same as in those that received an experimental CRF. Holly fertilized with the control CRF had higher foliar K levels than in all other treatments except 0.4 g P. Similar to tissue N, the dilution effect and tissue age may explain the decreasing K levels with increasing CRF-P for hydrangea and holly, respectively.

3.1.4. pH

Fertilizer-P treatment did not affect substrate pH ($P > 0.05$); thus, values were pooled across fertilizer treatments within each taxon. Hydrangea substrate pH values increased from 5.3 at 1 DAI to 6.2 at 16 DAI, then stayed between 6.2 and 6.5 for the remainder of the study. Substrate pH values for holly increased from 5.1 at 1 DAI to 6.2 at 28 DAI; thereafter, pH values stayed the same until 114 DAI, at which pH was 6.5. The increase in substrate pH in the first 16 to 28 days was most likely a result of dolomite dissolution and subsequent decrease in hydrogen

ion (H^+) concentration as H^+ reacted with hydroxide products to form water. Hydrangea pH values were 0.4 units higher than those of holly at 16 and 88 DAI; however, since pH values were the same across taxa at all other sampling dates, the 0.4-unit pH difference at 16 and 88 DAI has minor horticultural importance.

3.2. Ridge and valley site

3.2.1. Pore-water phosphorus.

At 36 and 91 DAI, hydrangea PWP concentrations (Table 4) increased linearly with increasing P in experimental CRF treatments. Pore-water P concentrations for holly increased linearly with increasing P in experimental CRFs at all sampling dates except 19 DAI in which PWP concentrations responded quadratically. Absence of a treatment effect on PWP concentrations at 61 DAI for hydrangea was due to unusually high variance in measured P concentrations across replications for the 0.2, 0.3 and 0.4 g P rates ($SE = \pm 2.3, 3.0$ and $4.1 \text{ mg}\cdot\text{L}^{-1} \text{ P}$, respectively). With the exception of hydrangea at 91 DAI, PWP concentrations within each sampling date of plants fertilized with the control CRF were repeatedly the same as those given 0.1 g P for both taxa. Additionally, for hydrangea fertilized with the control CRF, PWP concentrations peaked at 91 DAI, whereas PWP of the experimental CRFs were constant over time. Parallel to results observed for the MACP site, these data suggest that the P release pattern of the control CRF was inconsistent with that of the experimental CRFs. Holly fertilized with 0.1 g P had higher PWP levels at 19 DAI than at any other sampling date, indicating PWP concentrations may have been diminished by root uptake after root establishment.

3.2.2. Growth index and carbon allocation.

Hydrangea GI (Fig. 3) increased quadratically with increasing P in experimental CRFs at 60 ($F = 4.55$; $P = 0.0207$), 90 ($F = 25.5$; $P < 0.0001$) and 109 DAI ($F = 37.4$; $P < 0.0001$). Although GI values of hydrangea fertilized with 0.2 to 0.4 or the control CRF were consistently the same, by 90 DAI, GI values of plants given 0.1 g P were 64% less than those of plants in any other treatment. From 19 to 90 DAI, the average PWP concentrations for hydrangea fertilized with 0.1 and 0.2 g P were 0.7 ± 0.32 SE and 2.7 ± 1.00 SE $\text{mg}\cdot\text{L}^{-1}$, respectively; thus, the critical PWP concentration for hydrangea is most likely between 0.7 and 2.7 $\text{mg}\cdot\text{L}^{-1}$. Growth index values of holly fertilized with 0.1 to 0.4 g P were the same at all measurement dates, suggesting mean PWP concentrations between 0.5 and 5.1 $\text{mg}\cdot\text{L}^{-1}$ were adequate for maximal shoot growth. Relative to holly in the control CRF treatment, GI values of plants fertilized with 0.2 g P were 530% higher at 60 DAI and $\approx 108\%$ higher at 90 and 109 DAI. Despite having submaximal GI, holly given the control CRF had mean PWP concentrations within the adequate range according to PWP data from those fertilized with experimental CRFs (Table 5). Since control CRF PWP values of holly were constant over time and the same as in plants fertilized with 0.3 or 0.4 g P, P was not likely limiting for holly given the control CRF. At 19, 36 and 61 DAI, EC values (Fig. 2) of holly fertilized with the control CRF were 42% to 65% lower than those of all other treatments ($P < 0.01$), indicating N or K^+ or both may have been initially limiting growth.

Shoot dry weight and GI_f had a strong, positive correlation in both taxa ($r = 0.87$ for hydrangea; $r = 0.80$ for holly). Consistent with results observed at the MACP site, hydrangea GI_f and SDW (Table 5) increased quadratically with increasing experimental CRF-P and was highest in plants fertilized with the control CRF or 0.3 to 0.4 g P. Hydrangea growth response pattern to increasing experimental CRF-P rate mimics the curvilinear response of mean PWP concentrations (Table 5) to CRF-P rate, suggesting P is the primary variable influencing growth

differences between experimental CRF treatments. Maximal GI_f and SDW of holly was observed in plants fertilized with 0.2 to 0.4 g P. The reason holly seemingly required a higher CRF-P rate at the MACP site than at the RV site is unclear and may be related to environmental differences between ecoregions. Shoot dry weight and GI_f values of holly given the control CRF were about half that of plants fertilized with 0.4 g P. As previously discussed, relatively low GI_f and SDW values of holly fertilized with the control CRF is likely a consequence of delayed N and K^+ release from the CRF as indicated by low EC values (Fig. 2).

Hydrangea and holly RDW (Table 5) response to increasing CRF-P was similar to that of respective GI_f values. Root dry weight of hydrangea increased quadratically with increasing experimental CRF-P and was highest in those fertilized with 0.2 to 0.4 g P or the control CRF. Root dry weight of holly was the same across experimental CRFs, all of which had higher RDW than the control, except for those given 0.3 g P. Root dry weight results for both taxa grown at the RV site generally correspond with results observed at the MACP site. Root : shoot ratio values of both taxa declined quadratically with increasing P among experimental CRFs. Root : shoot values of hydrangea given the control CRF were the same as those fertilized with 0.2 to 0.4 g P, and in holly, root : shoot values of those given the control CRF were the same as those of all other treatments. In hydrangea, CRF-P rate had the greatest effect on root : shoot values as P levels shifted from shoot and root growth-limiting (0.1 g P) to sufficient (≥ 0.2 g P). This finding supports results observed at the MACP site in that P deficiency had a greater effect on shoot growth than root growth.

3.2.3. Foliar nutrient concentrations

Foliar P concentrations (Table 5) in both taxa increased linearly with increasing experimental CRF-P rate. Although the control CRF resulted in highest tissue-P concentrations in hydrangea, tissue-P concentrations as low as $0.11 \text{ mg} \cdot \text{g}^{-1} \text{ DW}$ (0.3 g CRF-P) were sufficient for maximal SDW which indicates luxury consumption of P by hydrangea given 0.4 g P or the control CRF. The tissue-P concentration of P-deficient hydrangea (i.e., those given 0.1 g P) was $0.09 \text{ mg} \cdot \text{g}^{-1} \text{ DW}$, suggesting the critical tissue-P concentration is between 0.09 and $0.11 \text{ mg} \cdot \text{g}^{-1} \text{ DW}$. Similar to hydrangea, highest foliar P levels in holly were observed in those fertilized with the control CRF; however, unlike in hydrangea, relatively high foliar-P concentrations did not coincide with maximal SDW. As previously discussed, P limitation was not likely the cause of relatively low SDW and GI_f values of holly fertilized with the control CRF. Relatively low EC values prior to 91 DAI for holly given the control CRF indicate other nutrients in the CRF (e.g., N and K^+) may have been initially growth-limiting.

Foliar N and K (Table 5) decreased quadratically in hydrangea with increasing P in experimental fertilizer. Foliar N and K levels in hydrangea fertilized with the control CRF were the same as in those fertilized with 0.2 to 0.4 g P . Although P fertilizer level had a moderately strong negative correlation with foliar N ($r = -0.64$) in hydrangea, the negative correlation between SDW and foliar N was stronger ($r = -0.80$). Accordingly, SDW likely had a greater effect on foliar N levels than did CRF-P treatments. The negative relationship between SDW and foliar N level can be explained by the dilution effect as noted for the MACP site. In holly, foliar N and K increased quadratically and linearly, respectively, with increasing P. Foliar N levels in holly fertilized with the control CRF were higher than those in all other treatments, whereas foliar K levels in plants given the control CRF were similar to in those given 0.3 to 0.4 g P .

3.2.4. pH

Controlled-release fertilizer-P treatment did not affect substrate pH ($P < 0.05$); thus, values were pooled across fertilizer treatments within each taxon. In both taxa, substrate pH (Fig. 4) increased from ≈ 5.4 to ≈ 6.5 in the first 19 days after potting, then decreased to ≈ 5.6 between 19 and 36 DAI. After 36 DAI and for the remainder of the study, pH of both taxa increased to a final value of ≈ 6.5 . As previously discussed for the MACP site, reactions following dolomite dissolution likely caused the increase in substrate pH between days 1 and 19. The decrease in pH of both taxa between 19 and 36 DAI may have been caused by plant, microbial, fertilizer and/or other related factors.

4. Conclusions

Hydrangea GI_f and SDW results were consistent across experiment sites, indicating that CRFs containing 1.3% to 2.6% P (3% to 6% P_2O_5), applied at a rate of 0.3 to 0.6 g P per 3.8-L container, are sufficient for maximal growth. Accordingly, compared to the P content in conventional CRF formulations for container-grown nursery crops (i.e., 2.6% P), CRF-P for hydrangea fertilization may be reduced by 50% without affecting plant growth. Given that hydrangea is the second leading deciduous shrub produced in the U.S. (USDA, 2014), a 50% reduction in P fertilization for *Hydrangea macrophylla* could have major implications for reducing P runoff from container-nursery sites. However, since P requirements are often cultivar-specific, growers should experiment with using low-P CRFs on the cultivars they are growing before large-scale adoption.

Our findings also suggest that a constant PWP concentration in pour-through leachate of $5 \text{ mg} \cdot \text{L}^{-1}$, the minimum recommended concentration for CRF-fertilized containerized nursery-

crops (Bilderback et al., 2013), is higher than necessary for producing the hydrangea clone used in this study. At the MACP site, hydrangea PWP concentrations were consistently less than 2 mg·L⁻¹ for CRF treatments that were sufficient for maximal growth. However, since PWP levels are dynamic relative to time, taxa and possibly plant growth stage, measuring PWP concentrations of CRF-fertilized containerized nursery crops is often an ineffective method for assessing P fertility. Results also indicate that P release pattern may differ for heterogeneous compared homogeneous CRFs, despite both having the same nutrient release longevity and coating material. Growth response of holly to CRF-P rate at the MACP site was inconsistent with results observed at the RV site and findings in scientific literature; hence, further research is needed to determine the optimal CRF-P rate for this taxon.

Acknowledgements

The authors thank Julie Brindley, Velva Groover and Anna Birnbaum for technical assistance, as well as Saunders Brothers Nursery and Bailey Nursery for donating plants for this research. Funding was provided by the Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture (SCRI 2014-51181-22372), U.S. Department of Agriculture, the Horticultural Research Institute and the Virginia Nursery and Landscape Association.

References

- Bi, G., C.F. Scagel, L.H. Fuchigami, and R.P. Regan. 2007. Differences in growth, and nitrogen uptake and storage between two container-grown cultivars of *Rhododendron*. *J. Environ. Hort.* 25:13–20.
- Bilderback, T., C. Boyer, M. Chappell, G. Fain, D. Fare, C. Gilliam, B.E. Jackson, J. Lea-Cox, A.V. LeBude, A. Niemiera, J. Owen, J. Ruter, K. Tilt, S. Warren, S. White, T. Whitwell, R. Wright, and T. Yeager. 2013. Best management practices: Guide for producing nursery crops. 3rd ed. Southern Nursery Association, Acworth, GA.
- Bowser, D. 2017. Fertilization and irrigation considerations for container-grown nursery crop production. Univ. Guelph, Guelph, Ontario, MS Thesis
- Broschat, T.K. and K.K. Moore. 2007. Release rates of ammonium-nitrogen, nitrate-nitrogen, phosphorus, potassium, magnesium, iron, and manganese from seven controlled-release fertilizers. *Commun. Soil Sci. Plant Anal.* 38:843–850
- Broschat, T. 1995. Nitrate, phosphate, and potassium leaching from container-grown plants fertilized by several methods. *HortScience* 30:74–77.
- Broschat, T. 2005. Rates of ammonium-nitrogen, nitrate-nitrogen, phosphorus, and potassium from two controlled-release fertilizers under different substrate environments. *HortTechnology* 15:332–335.
- Bryson, G.M., H.A. Mills, N.D. Sasseville, J.B. Jones, Jr., and A.B. Barker. 2014. Plant analysis handbook III: A guide to sampling, preparation, analysis, interpretation and use of results of agronomic and horticultural crop plant tissue. Macro-Micro Publishing, Inc., Athens, GA.

- Cavins, T.J., B.E. Whipker, and W.C. Fonteno. 2004. Establishment of calibration curves for comparing pour-through and saturated media extract nutrient values. *HortScience* 39:1635–1639.
- Chen, L., J. Zeng, D.P. Xu, Z.G. Zhao, and J.J. Guo. 2010. Macronutrient deficiency symptoms in *Betula alnoides* seedlings. *J. Trop. For. Sci.* 22:403–413.
- Clark, M.J. and Y. Zheng. 2017. Effect of topdressed controlled-release fertilizer rates on nursery crop quality and growth and growing substrate nutrient status in the Niagara region, Ontario, Canada. *HortScience* 52:167–173.
- Diara, C., L. Incrocci, G. Incrocci, G. Carmassi, A. Pardossi, P. Marzalletti, P. Cozzi, F. Fibbi, and R. Clemens. 2014. Reduction of nutrient run-off by the use of coated slow-release fertilizers on two container-grown nursery crops. *Acta Hort.* 1034:85–92.
- Dickey, R.D., R.T. Poole and J.N. Joiner. 1967. Effect of nitrogen, phosphorus and potassium levels on growth and chemical composition of *Rhododendron indicum* 'Formosa' and *Viburnum suspensum*. *Proc. Amer. Soc. Hort. Sci.* 91:762–770.
- Du, C., J. Zhou, and A. Shaviv. 2006. Release characteristics of nutrients from polymer-coated compound controlled release fertilizers. *J. Polym Environ.* 14:223–230.
- Ericsson, T and T. Ingestad. 1988. Nutrition and growth of birch seedlings at varied relative phosphorus addition rates. *Physiol. Plant.* 72:227–235.
- Fain, G.B., C.H. Gilliam, K.M. Tilt, J.W. Olive, and B. Wallace. 2000. Survey of best management practices in container production nurseries. *J. Environ. Hort.* 18:142–144.
- Fonteno, W.C., C.T. Hardin, and J.P. Brewster. 1995. Procedures for determining physical properties of horticultural substrates using the NCSU Porometer. Horticultural Substrates Laboratory. NC State University, Raleigh, NC.

- Foy, R.H., R.V. Smith, C. Jordan, and S.D. Lennox. 1994. Upward trend in soluble phosphorus loadings to Lough Neagh despite phosphorus reduction at sewage treatment works. *Wat. Res.* 29:1051–1063.
- Gilliam, C.H. and R.D. Wright. 1978. Effects of three nitrogen levels on tissue nitrogen fluctuation during a flush of growth on ‘Helleri’ holly (*Ilex crenata* Thumb.). *HortScience* 13:301–302.
- Groves, K.M., S.L. Warren, and T.E. Bilderback. 1998. Irrigation volume, application, and controlled-release fertilizer. I. Effect on plant growth and mineral nutrient content in containerized plant production. *J. Environ. Hort.* 16:176–181.
- Groves, K.M., S.L. Warren, and T.E. Bilderback. 1998. Irrigation volume, application, and controlled-release fertilizer. II. Effect on substrate solution nutrient concentration and water efficiency in containerized plant production. *J. Environ. Hort.* 16:182–188.
- Havis, J.R. and J.H. Baker. 1985. Phosphorus requirement of *Rhododendron* 'Victor' and *Cotoneaster adpressa praecox* grown in a perlite-peat medium. *J. Environ. Hort.* 3:63–64.
- Hermans, C., J.P. Hammond, P.J. White, and N. Verbruggen. 2006. How do plants respond to nutrient shortage by biomass allocation? *Trends Plant Sci.* 11:610–617.
- Hoskins, T.C., J.S. Owen, Jr., J.S. Fields, J.E. Altland, Z.M. Easton, and A.X. Niemiera. 2014b. Solute transport through a pine bark-based substrate under saturated and unsaturated conditions. *J. Amer. Soc. Hort. Sci.* 139:634–641.
- Jarrell, W.M. and R.B. Beverly. 1981. The dilution effect in plant nutrition studies. *Adv. Agron.* 34:197–224.

- Jiménez, S., M. Pérez, B.M. Plaza, R. Salinas, and M. T. Lao. 2007. Empirical models of phosphorus uptake under different nitrogen sources in *Dieffenbachia amoena* ‘Tropic Snow’. *HortScience* 42:412–416.
- Kim, H and X. Li. 2016. Effects of phosphorus on shoot and root growth, partitioning, and phosphorus utilization efficiency in lantana. *HortScience* 51:1001–1009.
- Kraus, H.T., S.L. Warren, G.J. Bjorkquist, A.W. Lowder, C.M. Tchir, and K.N. Walton. 2011. Nitrogen : phosphorus : potassium ratios affect production of two herbaceous perennials. *HortScience* 46:776–783.
- Lea-Cox, J.D. and D.S. Ross. 2001. Water Quality Improvement Act: The rationale for developing a water and nutrient management planning process for container nursery and greenhouse operations. *J. Environ. Hort.* 19:226–229.
- Lea-Cox, J.D., D.S. Ross, and K.M. Teffeu. 2001. A Water and nutrient management planning process for container nursery and greenhouse production systems in Maryland. *J. Environ. Hort.* 19:230–236.
- Leonard, M., M. Orfanedes, and T. Yeager. 2007. Response of croton and coco plum to phosphorus. *Proc. South. Nurs. Assoc. Res. Conf.* 52:531–534.
- Mack, R., J.S. Owen, A.X. Niemiera, and J. Latimer. 2017. Virginia nursery and greenhouse grower survey of best management practices. *HortTechnology* 27:386–392.
- Majsztrik, J.C. and J.D. Lea-Cox. 2013. Water quality regulations in the Chesapeake Bay: Working to more precisely estimate nutrient loading rates and incentivize best management practices in the nursery and greenhouse industry. *HortScience* 48:1097–1102.
- Marschner, H. 2012. Mineral nutrition of higher plants. 3rd ed. Academic Press, San Diego, CA.

- Matysiak, B. 2015. Growth response of container grown Japanese azalea and euonymus and concentration of nitrates and phosphates in the runoff water under different irrigation and fertilization. *J. Hort. Res.* 23:21–32.
- Naik, M.R., B.K. Kumar and K. Manasa. 2017. Polymer coated fertilizers as advance technique in nutrient management. *Asian J. Soil Sci.* 12:228–232.
- Newman, J.P., J.P. Albano, D.J. Merhaut, and E.K. Blythe. 2006. Nutrient release from controlled-release fertilizers in a neutral-pH substrate in an outdoor environment: I. Leachate electrical conductivity, pH, and nitrogen, phosphorus, and potassium concentrations. *HortScience* 41:1674–1682.
- Niu, F., D. Zhang, Z. Li, M.W. Van Iersel, and P. Alem. 2015. Morphological response of eucalypts seedlings to phosphorus supply through hydroponic system. *Sci. Hort.* 194:295–303.
- Owen, J.S., S.L. Warren, T.E. Bilderback, and J.P. Albano. 2008. Phosphorus rate, leaching fraction, and substrate influence on influent quantity, effluent nutrient content, and response of a containerized woody ornamental crop. *HortScience* 43:906–912.
- Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2004. Nutrient uptake, partitioning and leaching losses from container-nursery production systems. *Acta Hort.* 630:321–328.
- Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2007. Nitrogen and phosphorus uptake efficiency and partitioning of container-grown azalea during spring growth. *J. Amer. Soc. Hort. Sci.* 132:563–572.
- Sharma, J. and A. Bolques. 2007. Runoff quality at container plant nurseries. *Proc. South. Nurs. Assoc. Res. Conf.* 52:138–142.

- Shreckhise, J.H., J.S. Owen, and A.X. Niemiera. 2018. Growth response of three containerized woody plant taxa to varying low phosphorus fertilizer concentrations. *HortScience* (In press).
- Sonneveld, C. and W. Voogt. 2009. *Plant nutrition of greenhouse crops*. Springer, Dordrecht, The Netherlands.
- Tolman, D.A., A.X. Niemiera, and R.D. Wright. 1990. Influence of plant age on nutrient absorption for marigold seedlings. *HortScience* 25:1612–1613.
- Tyler, H.H., S.L. Warren, and T.E. Bilderback. 1996. Reduced leaching fractions improve irrigation use efficiency and nutrient efficacy. *J. Environ. Hort.* 14:199–204.
- U.S. Dept. of Ag. 2014. 2012 Census of Agriculture: Census of horticultural specialties (2014). p. 27.
- U.S. Environmental Protection Agency. 2010a. Chesapeake Bay total maximum daily load for nitrogen, phosphorus and sediment, Section 4. Sources of nitrogen, phosphorus and sediment to the Chesapeake Bay. 4 Dec. 2017. <https://www.epa.gov/sites/production/files/2014-12/documents/cbay_final_tmdl_section_4_final_0.pdf>.
- U.S. Environmental Protection Agency. 2010b. Chesapeake Bay Phase 5.3 Community Watershed Model. EPA 903S10002 - CBP/TRS-303-10. U.S. Environmental Protection Agency, Chesapeake Bay Program Office, Annapolis MD.
- U.S. Environmental Protection Agency. 2017. Nutrient pollution: Sources and solutions. 15 May 2018. <<https://www.epa.gov/nutrientpollution/sources-and-solutions>>.
- Warren, S.L., T.E. Bilderback, and H.H. Kraus. 2001. Method of fertilizer application affects nutrient losses of controlled-release fertilizer. *Acta Hort.* 548:349–355.

- Warren, S.L., T.E. Bilderback, and H.H. Tyler. 1995. Efficacy of three nitrogen and phosphorus sources in container-grown azalea production. *J. Environ. Hort.* 13:147–151.
- Wright, R.D. 1986. The pour-through nutrient extraction procedure. *HortScience* 21:227–229.
- Yeager, T.H. and J.E. Barrett. 1984. Phosphorus leaching from ³²P-superphosphate-amended soilless container media. *HortScience* 19:216–217.
- Yeager, T.H. and J.E. Barrett. 1985. Influence of incubation time on phosphorus leaching from a container medium. *J. Environ. Hort.* 3:186–187.
- Yeager, T.H. and R.D. Wright. 1982. Phosphorus requirement of *Ilex crenata* Thunb. cv. *Helleri* grown in a pine bark medium. *J. Amer. Soc. Hort. Sci.* 107:558–562.
- Yeager, T.H. and R.D. Wright. 1982. Pine bark – phosphorus relationships. *Commun. Soil Sci. Plant Anal.* 13:57–66.

Table 1. Sources and amount (g) of nitrogen (N), phosphorus (P) and potassium (K) supplied as polymer-coated controlled-release fertilizer (CRF) applied to each hydrangea and holly grown in 3.8-L containers in a pine bark substrate.

Fertilizer treatment	NH ₄ -N	NO ₃ -N	Urea-N	Total N	P	K
15N–2.6P–10K (control) ^y	1.98	1.62	0.00	3.60	0.63	2.39
18N–1.7P–10K ^z	0.30	0.25	3.14	3.69	0.10	2.04
18N–1.3P–10K	0.34	0.25	3.06	3.64	0.18	2.04
18N–0.9P–10K	0.39	0.25	3.06	3.69	0.27	2.04
18N–0.4P–10K	0.43	0.25	2.97	3.65	0.36	2.04

^zNitrogen, phosphorus and potassium in the four experimental fertilizers were derived from ammonium nitrate, ammonium phosphate, urea and potassium sulfate.

^yNitrogen, phosphorus and potassium in the control are derived from ammonium nitrate, ammonium phosphate, and potassium sulfate.

Table 2. Effect of sampling date [days after experiment initiation; (DAI)] and controlled-release fertilizer (CRF)-phosphorus (P) level within each sampling date on pour-through-extracted pore-water P concentration (n = 4) of hydrangea and holly grown at the Middle Atlantic Coastal Plain site (Virginia Beach, VA).

CRF-P (g)	Pore-water P conc. (mg·L ⁻¹)					P-value ^x	HSD
	16 DAI	28 DAI	52 DAI	88 DAI	114 DAI		
hydrangea							
control	0.37	0.08	0.79 ab ^z	3.70 a	5.99	0.0559	-
0.1	0.26	0.08	0.08 c	0.08 b	0.10	0.0092	0.22
0.2	0.67	0.41	0.21 bc	0.12 b	0.18	0.0785	-
0.3	0.77	0.54	0.76 ab	0.85 b	1.14	0.8665	-
0.4	1.61	0.73	1.01 a	1.75 ab	0.65	0.4683	-
<i>P</i> -values							
ANOVA	0.2188	0.0636	0.0027	0.0021	0.0977		
L _{0.1-0.4} ^y	0.0514	0.0232	0.0002	0.0063	0.0495		
Q _{0.1-0.4}	0.1445	0.0749	0.0013	0.0183	0.0923		
holly							
control	1.25	0.33	0.41 b	9.99 a	7.55 a	0.0002	5.83
0.1	1.22	0.10	0.08 b	0.08 b	0.09 b	<0.0001	0.25
0.2	1.92	1.08	0.42 b	1.05 b	0.42 b	0.2694	-
0.3	1.27	1.27	0.65 b	0.49 b	1.74 b	0.2988	-
0.4	3.02	2.87	2.12 a	3.12 b	1.74 b	0.9196	-
<i>P</i> -values							
ANOVA	0.1874	0.2725	0.0015	<0.0001	0.0041		
L _{0.1-0.4}	0.1269	0.0670	0.0008	0.0247	0.0021		
Q _{0.1-0.4}	0.2398	0.1897	0.0014	0.054	0.0097		

^z Letters within columns separate means by Tukey's Honest Significant Difference at P ≤ 0.05.

^y Only the experimental fertilizers were included in linear and quadratic regression analysis.

^x P-values were attained via ANOVA to assess the changes in PWP concentrations over time within CRF treatments. Tukey's Honest Significant Difference (HSD) value is provided for means comparisons within rows.

Table 3. Effect of controlled-release fertilizer (CRF)-phosphorus (P) level per container on mean pore-water P (PWP) concentration (n = 20); growth index at harvest (GI_f; n = 5); shoot dry weight (n = 5); root dry weight (n = 3 for hydrangea; n = 5 for holly); root : shoot ratio (n = 3 for hydrangea; n = 5 for holly); and foliar nitrogen (N), P, and potassium (K) concentrations (n = 5) of hydrangea and holly grown at the Middle Atlantic Coastal Plain site (Virginia Beach, VA) for 130 days.

CRF-P (g)	PWP ^z (mg·L ⁻¹)	GI _f (cm)	Dry wt (g)			Foliar nutrients (mg·g ⁻¹ dry wt)		
			Shoot	Root	Root : shoot	N	P	K
hydrangea								
control	2.2 a ^y	21.8 a	21.2 a	8.1	0.34 b	3.23 a	0.34 a	1.83 ab
0.1	0.1 b	7.2 b	5.1 b	4.1	0.67 a	3.04 ab	0.14 b	2.35 a
0.2	0.3 b	13.6 ab	9.4 b	5.4	0.46 b	2.75 abc	0.15 b	1.89 ab
0.3	0.8 a	17.5 a	15.0 ab	6.7	0.40 b	2.59 bc	0.17 b	1.67 b
0.4	1.1 a	17.5 a	14.1 ab	7.2	0.44 b	2.29 c	0.19 b	1.70 b
<i>P</i> -value								
ANOVA	0.0002	0.0015	0.0027	0.1441	0.0039	0.0019	<0.0001	0.0272
L _{0.1-0.4} ^x	<0.0001	0.0019	0.0029	0.0328	0.0172	0.0002	0.0015	0.0054
Q _{0.1-0.4}	<0.0001	0.0030	0.0066	0.1048	0.0049	0.0014	0.0064	0.0065
holly								
control	3.9 a	16.8 b	11.2 b	3.3 b	0.29 b	2.40 a	0.24 a	1.38 a
0.1	0.3 c	6.9 c	4.7 c	4.3 ab	0.92 a	1.64 c	0.08 c	0.98 c
0.2	1.0 bc	15.1 b	11.1 b	6.1 a	0.63 ab	1.97 bc	0.15 b	1.07 bc
0.3	1.1 bc	16.9 b	11.5 b	4.9 ab	0.43 b	2.08 ab	0.18 b	1.19 b
0.4	2.6 ab	23.3 a	17.6 a	5.7 ab	0.33 b	1.94 bc	0.20 ab	1.23 ab
<i>P</i> -value								
ANOVA	<0.0001	<0.0001	<0.0001	0.0238	0.0002	<0.0001	<0.0001	<0.0001
L _{0.1-0.4}	<0.0001	<0.0001	<0.0001	0.3168	0.0001	0.0156	<0.0001	<0.0001
Q _{0.1-0.4}	<0.0001	<0.0001	<0.0001	0.4658	0.0005	0.0006	<0.0001	0.0003

^z Pore-water P data were pooled over time then log transformed to meet the constant variance

assumption. After ANOVA (and means separation) and linear and quadratic regression P-values

were attained, mean PWP levels were converted back to original measured values.

^y Letters within columns separate means by Tukey's Honest Significant Difference at $P \leq 0.05$.

^x Only the experimental fertilizers were included in linear and quadratic regression analysis.

Table 4. Effect of sampling date [days after experiment initiation (DAI)] and controlled-release fertilizer (CRF)-phosphorus (P) level within each sampling date on pour-through-extracted pore-water P concentration (n = 4) of hydrangea and holly grown at the Ridge and Valley site (Blacksburg, VA).

CRF-P (g)	Pore-water P conc. (mg·L ⁻¹)					P-value ^x	HSD
	19 DAI	36 DAI	61 DAI	91 DAI	110 DAI		
	hydrangea						
control	3.67	1.20 b ^z	2.78	5.20 a	1.08	0.0039	3.05
0.1	1.32	0.44 b	0.28	0.62 b	0.13	0.6836	-
0.2	4.17	2.16 ab	3.33	1.07 ab	0.42	0.6253	-
0.3	2.78	2.89 ab	5.58	3.39 ab	1.06	0.5168	-
0.4	2.26	5.23 a	7.12	2.76 ab	1.34	0.2993	-
<i>P</i> -value							
ANOVA	0.8293	0.0194	0.3922	0.0253	0.3236		
L _{0.1-0.4} ^y	0.8752	0.0033	0.0706	0.0288	0.0531		
Q _{0.1-0.4}	0.7056	0.0152	0.1996	0.0812	0.1655		
	holly						
control	2.91 ab	0.74 b	1.39	2.65 ab	6.52 ab	0.1239	-
0.1	2.08 ab	0.16 b	0.08	0.15 b	0.10 b	<0.0001	0.59
0.2	1.76 b	0.70 b	0.64	1.45 ab	1.50 ab	0.3139	-
0.3	2.34 ab	1.62 ab	1.28	1.55 ab	2.97 ab	0.1979	-
0.4	3.27 a	4.01 a	5.07	5.41 a	7.64 a	0.4910	-
<i>P</i> -value							
ANOVA	0.0385	0.0160	0.0714	0.0276	0.0259		
L _{0.1-0.4}	0.0236	0.0036	0.0162	0.0075	<0.0001		
Q _{0.1-0.4}	0.0189	0.0090	0.0288	0.0175	<0.0001		

^z Letters within columns separate means by Tukey's Honest Significant Difference at P ≤ 0.05

(control not included).

^y Only the experimental fertilizers were included in linear and quadratic regression analysis.

^x P-values were attained via ANOVA to assess the changes in PWP concentrations over time within CRF treatments. Tukey's Honest Significant Difference (HSD) value is provided for means comparisons within rows.

Table 5. Effect of controlled-release fertilizer (CRF)-phosphorus (P) level per container on mean pore-water P (PWP) concentration (n = 20); growth index at harvest (GI_f; n = 7); shoot dry weight (n = 7); root dry weight (n = 4 for hydrangea; n = 7 for holly); root : shoot ratio (n = 4 for hydrangea; n = 7 for holly); and foliar nitrogen (N), P, and potassium (K) concentrations (n = 7) of hydrangea and holly grown at Ridge and Valley site (Blacksburg, VA) for 109 days.

CRF-P (g)	PWP ^z (mg·L ⁻¹)	GI _f (cm)	Dry wt (g)		Root : shoot	Foliar nutrients (mg·g ⁻¹ dry wt)		
			Shoot	Root		N	P	K
hydrangea								
control	2.8 a ^y	18.2 a	30.3 ab	7.49 ab	0.27 b	2.46 b	0.20 a	1.42 b
0.1	0.6 b	05.6 b	10.9 c	5.09 b	0.47 a	3.33 a	0.09 d	1.95 a
0.2	2.2 ab	17.5 a	26.2 b	7.31 ab	0.28 b	2.68 b	0.12 c	1.51 b
0.3	3.1 a	18.4 a	34.0 ab	8.81 a	0.27 b	2.52 b	0.11 cd	1.26 b
0.4	3.7 a	18.8 a	35.9 a	9.39 a	0.25 b	2.60 b	0.16 b	1.25 b
<i>P</i> -value								
ANOVA	<0.0001	<0.0001	<0.0001	0.0206	0.0002	<0.0001	<0.0001	<0.0001
L _{0.1-0.4} ^x	<0.0001	<0.0001	<0.0001	0.0013	0.0010	<0.0001	<0.0001	<0.0001
Q _{0.1-0.4}	<0.0001	<0.0001	<0.0001	0.0040	0.0002	<0.0001	<0.0001	<0.0001
holly								
control	2.8 ab	6.8 b	10.6 b	3.57 b	0.36	2.36 a	0.20 a	1.17 a
0.1	0.5 c	8.2 ab	12.2 b	5.10 a	0.42	1.75 c	0.09 c	0.96 b
0.2	1.2 b	13.8 a	17.5 a	5.83 a	0.34	1.90 bc	0.13 b	0.92 b
0.3	1.9 b	11.5 ab	13.0 ab	4.65 ab	0.37	2.04 b	0.14 b	1.01 ab
0.4	5.1 a	13.1 a	15.5 ab	4.96 a	0.32	1.98 bc	0.17 b	1.05 ab
<i>P</i> -value								
ANOVA	<0.0001	0.0048	0.0027	0.0006	0.0797	<0.0001	<0.0001	0.0031
L _{0.1-0.4}	<0.0001	0.0925	0.3916	0.3151	0.0109	0.0040	<0.0001	0.0233
Q _{0.1-0.4}	<0.0001	0.1155	0.4245	0.5159	0.0310	0.0036	<0.0001	0.0382

^z Pore-water P data were pooled over time then log transformed to meet the constant variance

assumption. After ANOVA (and means separation) and linear and quadratic regression P-values were attained, mean PWP levels were converted back to original measured values.

^y Letters within columns separate means by Tukey's Honest Significant Difference at P ≤ 0.05.

^x Only the experimental fertilizers were included in linear and quadratic regression analysis.

Figure captions

Fig. 1. Daily rainfall (bars) and maximum (dotted line), mean (solid line) and minimum (dotted line) daily temperature at the Middle Atlantic Coastal Plain (MACP) and Ridge and Valley (RV) sites over the course of the experiment. For the MACP site, days 0 and 133 were on 6 June 2015 and 10 October 2015, respectively, and for the RV site, days 0 and 113 were on 4 June 2015 and 25 September 2015, respectively

Fig. 2. Effect of controlled-release fertilizer (CRF) treatment [g phosphorus (P) per container] on substrate pore-water electrical conductivity (EC) over time for hydrangea and holly grown at the Middle Atlantic Coastal Plain (n = 4) and Ridge and Valley (n = 4) sites for 133 and 113 days, respectively. Vertical bars indicate SE.

Fig. 3. Effect of controlled-release fertilizer (CRF) treatment [g phosphorus (P) per container] on growth index [i.e., (widest width + perpendicular width + height)/3] over time for hydrangea and holly grown at the Middle Atlantic Coastal Plain (n = 5) and Ridge and Valley (n = 7) sites for 133 and 113 days, respectively. Vertical bars indicate SE

Fig. 4. Substrate pore-water pH of hydrangea and holly grown at the Middle Atlantic Coastal Plain and Ridge and Valley sites for 133 and 113 days, respectively. Values were pooled across controlled-release fertilizer treatments at each sampling date (n = 20) since treatment did not affect pH ($P < 0.05$). Vertical bars indicate SE.

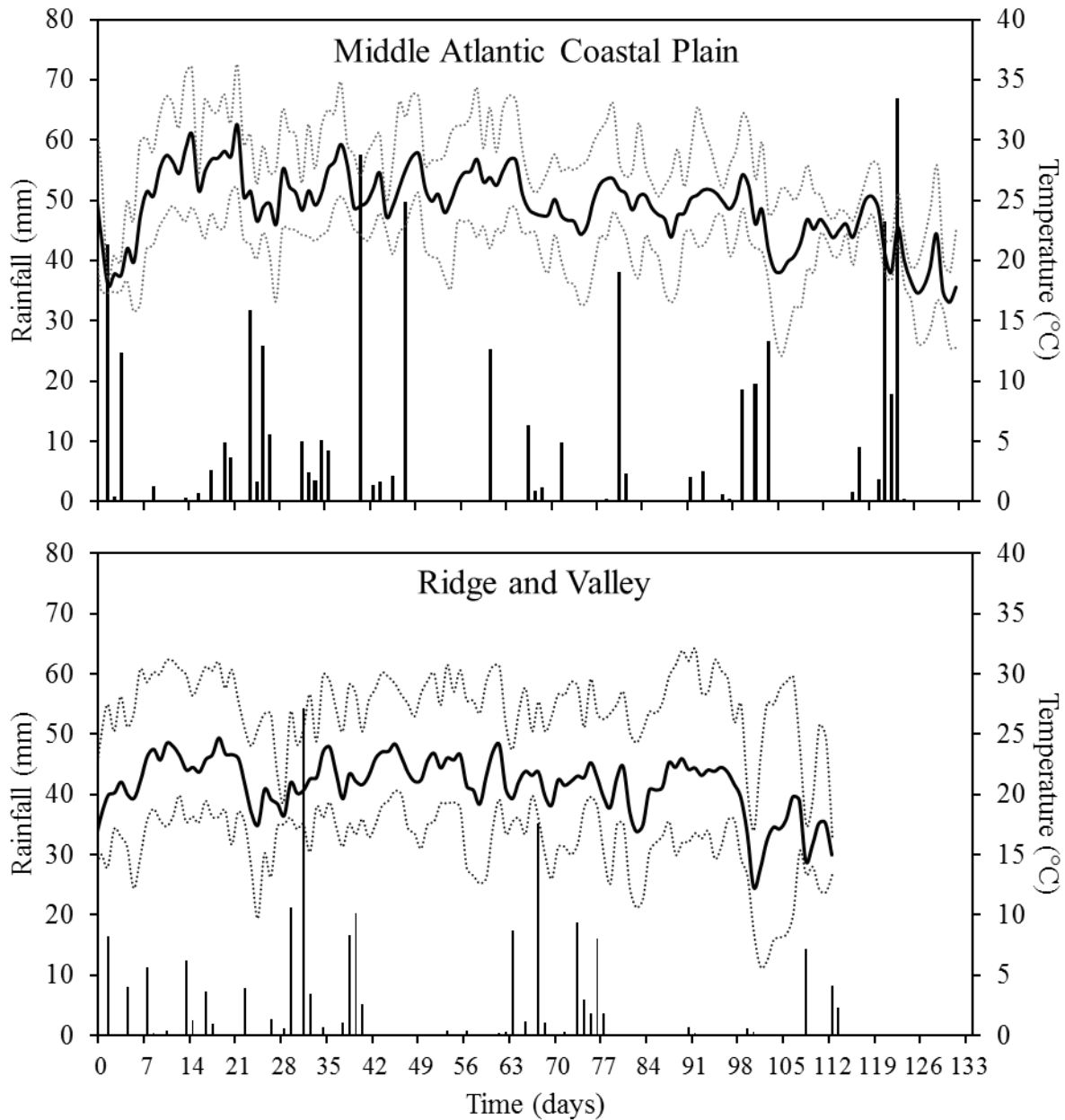


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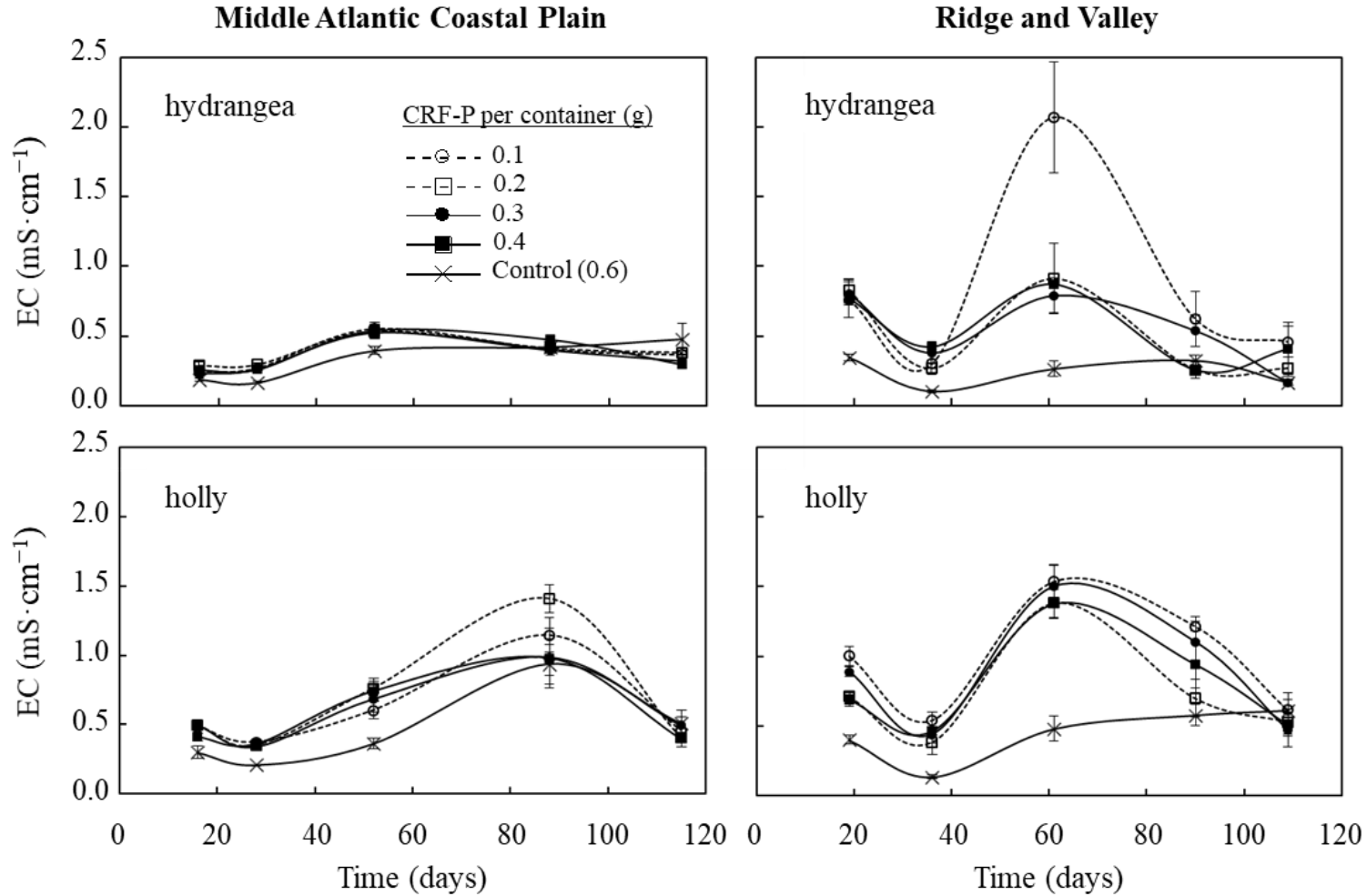


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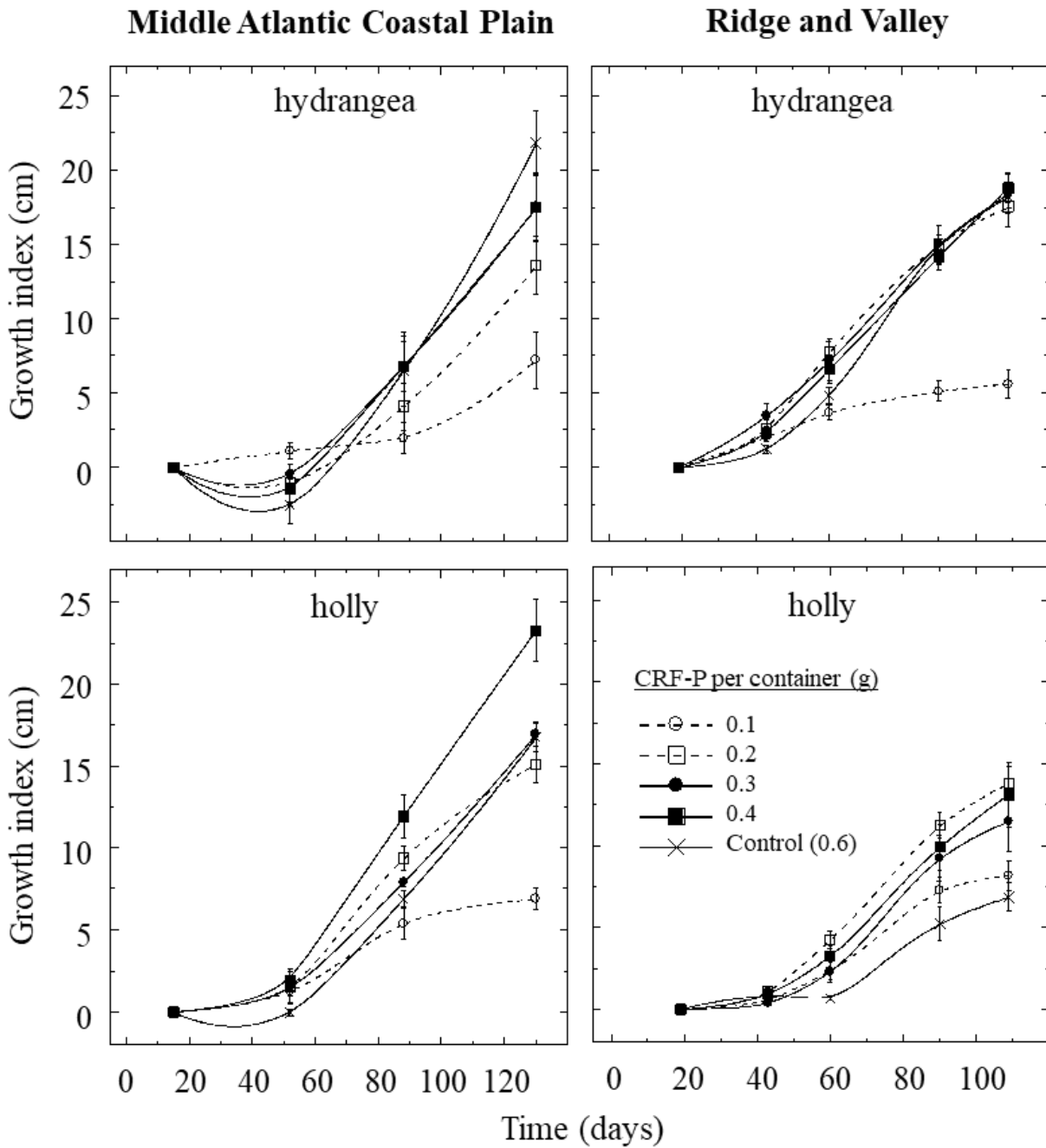


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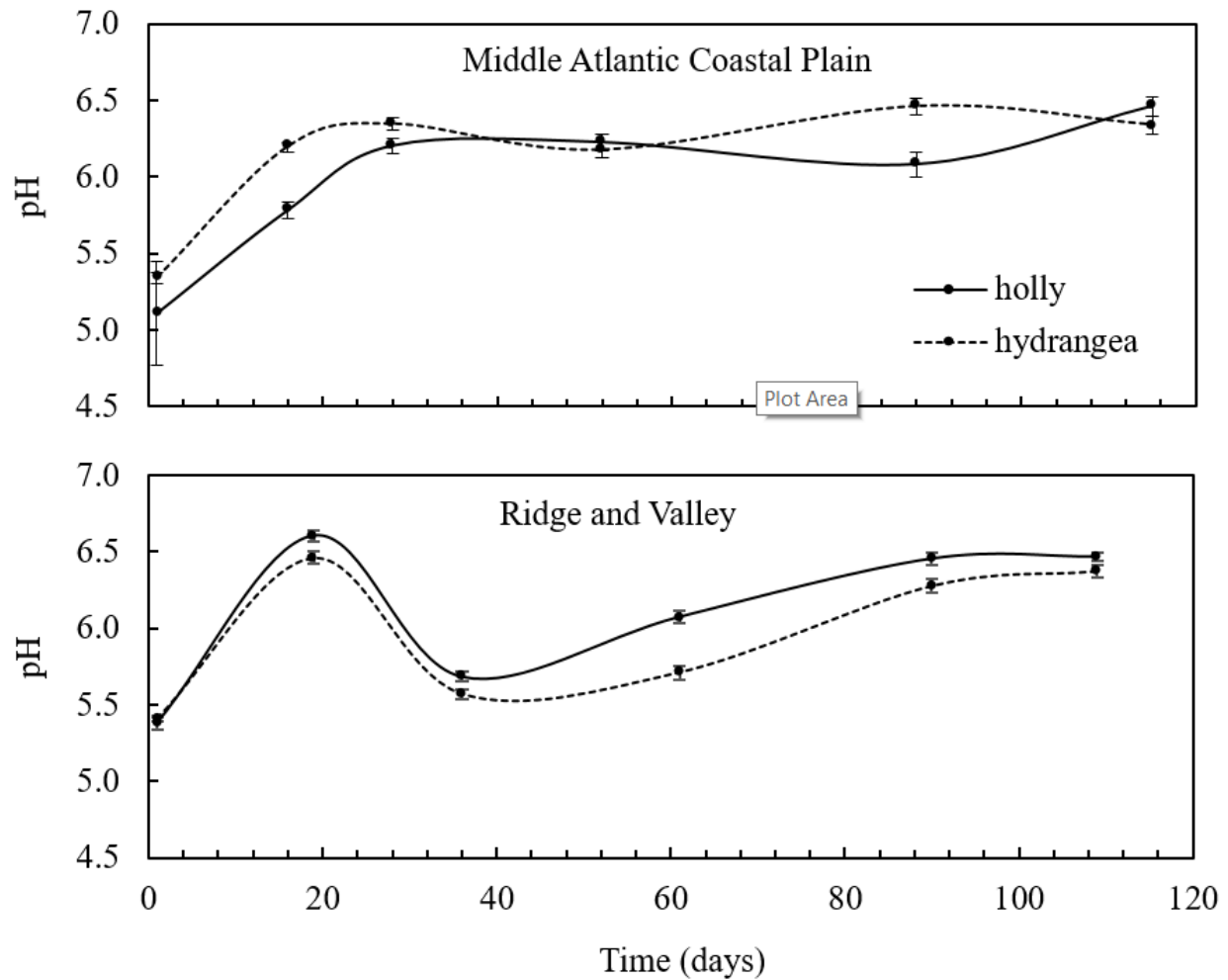


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Chapter 4

Dolomite and Micronutrient Fertilizer Effect on Phosphorus Fate in Pine

Bark I: Laboratory Study

Abstract

Retention of phosphorus (P), a contaminant in aquatic ecosystems, is poor in pine bark-based substrates commonly utilized for production of containerized nursery crops. Dolomitic limestone (DL) and micronutrient fertilizers (MFs) are routinely added to pine bark substrates to improve fertility, yet the effect of these amendments on P in pore-water of pine bark substrate is not well understood. The objectives of this research were to 1) determine the effect of DL and MF amendments on P partitioning among four P fractions (i.e., orthophosphate-P [OP-P], non-orthophosphate dissolved P [NODP], total dissolved P [TDP] and particulate P [PP] in pore-water of pine bark substrate, and 2) simulate P speciation in pine bark pore-water using Visual MINTEQ, a computer-based chemical equilibrium model. Milled pine bark was packed into 30-cm PVC columns and irrigated daily for 48 d with enough tap water to displace (i.e., leach) pine bark pore-water from columns while minimally diluting the effluent. Amendment treatments incorporated into bark at experiment initiation included 1) a control (no controlled-release fertilizer [CRF], DL or MF), 2) CRF, 3) CRF and DL, 4) CRF and MF or 5) CRF, DL and MF. Phosphorus fractions in pore-water were determined at eight sampling intervals over the course of the study. The combined DL and MF treatment reduced OP-P concentrations by 70% when averaged across the sampling dates. These effects were primarily due to retention of OP-P in the substrate by DL. The NODP fraction was unaffected by amendments, and the response of TDP was similar to that of OP-P, but reductions were of lesser magnitude. Particulate P was present throughout the study and was highest in substrates containing DL at days 31 and 48 DAI. Visual MINTEQ modeling indicated $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ was saturated with respect to the solid phase at all sampling dates in substrates containing DL. However, kinetics and observed high dissolved organic carbon (DOC) concentrations may have limited formation of Ca precipitates.

Introduction

Understanding chemical fate of phosphorus (P) fertilizers is critical for determining P bioavailability and potential effect on down-stream ecosystems. Eutrophication and subsequent toxic algae blooms induced by elevated P concentrations in surface waters is a global issue affecting drinking water, irrigation water, fisheries and water-related recreation (Carpenter et al., 1998). Agriculture is the leading source of P contamination in U.S. surface waters (USEPA, 2017). In mineral soils, interactions between fertilizer-P and soil components have been thoroughly examined; however, little research has assessed the chemical fate of fertilizer-P in organic substrates, specifically milled pine bark, used to produce container-grown plants.

In the U.S., containerized woody plants contribute 63% of all nursery stock sales (USDA, 2014), and the predominate substrate utilized in Eastern U.S. for container-grown nursery crops is pine bark (*Pinus taeda*; Bilderback et al., 2013; Lu et al., 2006). Pine bark has limited ability to sorb P (Marconi and Nelson, 1984; Paradelo et al., 2017; Yeager and Wright, 1982); consequently, soluble P fertilizers rapidly leach from pine bark-filled containers during irrigation (Cole and Dole, 1997; Godoy and Cole, 2000; Yeager and Barrett, 1984, 1985a, 1985b). Phosphorus leaching from containers is exacerbated since container-grown crops are typically irrigated daily during the growing season. The use of polymer- or resin-coated controlled-release fertilizers (CRFs), a best management practice (Bilderback et al., 2013) that reduces P leaching relative to soluble fertilizers (Broschat, 1995; Diara, 2014), has been widely adopted by the US nursery industry according to survey studies (Dennis et al., 2010; Fain et al., 2000; Mack et al., 2017). However, P uptake efficiency (PUE, percent of applied P taken up by plant roots) of container-grown nursery crops has been reported to remain between 7% and 62% (McGinnis et

al., 2009; Owen et al., 2008; Tyler et al., 1996; Warren et al., 1995; Warren et al. 2001) when using CRFs.

Pine bark has an initial pH range of 4.0 to 4.3. Optimal plant nutrient availability in organic substrates occurs when pH is 5.0 to 5.5 (Bunt, 1988), and growers routinely amend pine bark with a liming agent to increase pH and supply potentially growth-limiting base cations. Dolomitic limestone [DL; $\text{CaMg}(\text{CO}_3)_2$] is a common liming agent incorporated into substrates for nursery crop production that supplies plants with both Ca and Mg. However, the possible effects of DL on P solubility in pine bark substrate has not been assessed. Multiple studies have reported DL sorption of orthophosphate (OP) in aqueous solution (Karaca et al., 2004; Karaca et al., 2006; Mangwandi et al., 2014; Xu et al., 2014; Yuan et al., 2014; Yuan et al., 2015). For example, in batch sorption experiments, Yuan et al. (2014, 2015) found that 10 g DL removed up to 98% P from 1 L solution containing $50 \text{ mg}\cdot\text{L}^{-1}$ P. Karaca et al., (2006) reported rapid sorption of OP by DL in solution, with equilibration occurring within 15 to 30 min depending on initial OP concentration (Karaca et al., 2006). Several mechanisms have been proposed to explain OP removal by DL, including chemisorption (Karaca et al., 2004; Yuan et al., 2015), intraparticle diffusion (Karaca et al., 2004), physical adsorption (Karaca et al., 2006), outer-sphere complexation and surface precipitation (Mangwandi et al., 2014). Xu et al. (2014) utilized attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy, X-ray absorption near edge structure (XANES) spectroscopy, and diffuse layer modeling to investigate P speciation on calcite and DL. When solution pH values were 5.0 to 7.0 and orthophosphate-P (OP-P) concentrations were 2 or $10 \text{ mg}\cdot\text{L}^{-1}$, which are common conditions in pore-water of DL-amended pine bark substrate, the authors determined that sorbed OP on the surface of DL was primarily in the form of Mg and Ca surface complexes and Ca-P secondary minerals.

Evidence of OP sorption by liming agents has also been observed in organic container-substrates. Amending peat-based substrates with varying rates of $\text{Ca}(\text{OH})_2$ was shown to reduce P leaching from containers from 20% (Haynes, 1982) to 88% (Havis and Baker, 1985) compared to substrates without $\text{Ca}(\text{OH})_2$. Two studies by Argo and Biernbaum (1996a and 1996b) reported that the amount of soluble OP in a peat-based substrate amended with various forms of DL was inversely related to the substrate pH. For example, Argo and Biernbaum (1996a) measured OP-P concentrations in saturated media extracts (Warncke, 1986) after growing *Impatiens walleriana* fertilized with $20 \text{ mg}\cdot\text{L}^{-1}$ P for 16 weeks in a Sphagnum peat-based substrate containing $1.5 \text{ kg}\cdot\text{m}^{-3}$ microfine dolomitic hydrated lime [$\text{Ca}(\text{OH})_2\cdot\text{MgO}$] or $8.4 \text{ kg}\cdot\text{m}^{-3}$ dolomitic carbonate lime ($\text{CaCO}_3\cdot\text{MgCO}_3$). The authors found that extract OP-P concentrations for both lime treatments were ≈ 21 and $3 \text{ mg}\cdot\text{L}^{-1}$ for substrates with pH values of ≈ 5.0 and 8.1 , respectively, and speculated that the reduction in soluble OP-P was due to precipitation of P as CaHPO_4 or $\text{CaH}_5\text{O}_6\text{P}$. However, reports on the effect of liming agents, specifically DL, on OP leaching from pine bark substrate are limited. One week after potting *Buddleia davidii* in a pine bark-based substrate, Altland et al. (2015) observed a linear decrease in pour-through-extracted pore-water P as DL amendment treatment incrementally increased from 0.0 to $14.3 \text{ kg}\cdot\text{m}^{-3}$. However, DL had no effect on pore-water P in substrate extracts collected at 4 or 16 weeks after potting.

In addition to a liming agent, growers of containerized crops routinely amend organic substrates with a granular fertilizer containing micronutrients (MFs). Iron, which is a primary constituent of many MFs, is often supplied in chelated form or as FeSO_4 . Ferrous sulfate has been shown to reduce OP solubility due to Fe-PO_4 precipitation (Moore and Miller, 1994; Rich, 2005; Tasistro and Kissel, 2006) and OP sorption to humic substances via Fe bridges (Gerke and Hermann, 1992; Weir and Soper, 1963). Iron-phosphate precipitation is dependent on pH and

P:Fe ratio. Hsu (1976) determined that decreasing the P:Fe molar ratio resulted in greater P removal from solution, and when the P:Fe molar ratio was 0.2 to 0.5, the optimal pH for precipitation was 4.7 to 7.1. At a pH of 4.5, Fytianos et al. (1998) found that ferrous iron removed 63% of P in aqueous samples when added at a 1P:1Fe molar ratio. Rich (2005) concluded that ferrous and ferric sulfate effectively removed OP from natural waters when buffered pH was between 7.0 and 8.0 and determined the mechanism of OP removal was formation of the insoluble precipitates, $\text{Fe}_2(\text{HPO}_4)_3$, $\text{Fe}(\text{H}_2\text{PO}_4)_3$, and $\text{Fe}_3(\text{PO}_4)_2$.

Phosphate- FeSO_4 interactions have also been observed in pine bark. Findings from multiple studies performed by Handreck (1991a, 1991b, 1992, 1996) have revealed that increasing the quantity of ferrous sulfate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$) in pine bark-based substrates decreases the amount of diethylenetriaminepentaacetic acid (DTPA)-extractable P. However, Fe-P chemical speciation in pore-water of irrigated pine bark substrate has not been thoroughly investigated. Determining Fe-P species in effluent of irrigated pine bark would have environmental implications since many Fe-P species are water-insoluble (Sanyal and De Datta, 1991).

In natural waters, P fractionation is commonly utilized to assess the threat of P to water quality (Worsfold et al., 2016). In soil-water extracts, fractionating P provides insight into P lability for plant uptake. Orthophosphate (H_3PO_4 , H_2PO_4^- , HPO_4^{2-} or PO_4^{3-}) is the most bioavailable P species for uptake by plants, planktonic algae and bacteria (Boström et al., 1988). In water samples, orthophosphate is routinely determined via colorimetric methods (e.g., molybdate blue method) or ion chromatography (IC; Worsfold et al., 2016) after samples are filtered through a 0.22 or 0.45 μm membrane (Broberg and Persson, 1988). However, Hens and Merckx (2002) and Maruo et al. (2016) reported release of OP from filterable colloids (< 0.45

μm) during colorimetric analysis ($\text{pH} < 1$) resulting in overestimation of OP concentrations. Hence, IC may be a more effective instrument for estimating actual OP concentrations compared to traditional colorimetric methods. The total dissolved P (TDP) fraction is operationally defined as that which passes through a $0.45 \mu\text{m}$ filter and includes orthophosphate and condensed, organic, and colloidal phosphates (McKelvie et al., 1995). If filtrate is first digested to convert all P into OP, TDP can be determined colorimetrically; however, analyzing TDP concentrations with inductively coupled plasma atomic emission spectroscopy (ICP-AES) may be a simpler approach since digestion is not required (Rowland and Haygarth, 1997; Van Moorlehem et al., 2011). Orthophosphate-P and TDP values have been shown to diverge as dissolved organic carbon (DOC) content of the analyzed matrix increases (Zhang et al., 2004), purportedly due to the increased presence of dissolved organic P compounds. Hence, OP-P and TDP should not be interpreted interchangeably in solutions with relatively high DOC, which would be expected in water extracts or leachate from pine bark substrate. Particulate P (PP) fraction is determined by subtracting TDP from total P (TP) analyzed via ICP-AES after digestion of non-filtered sample. Components in PP, operationally defined as the P retained by a $0.45 \mu\text{m}$ filter (McKelvie et al., 1995), include biological sources (e.g., material from plants, animals and bacteria), weathering products (e.g., primary and secondary minerals), inorganic P precipitates, coprecipitates and organic aggregates (Broberg and Persson, 1988).

Despite P fractions' varying degree of relevance to plant availability and surface water quality, few studies have measured P fractions in container-substrate extracts or leachate from containerized crop production. Yeager and Barrett (1984, 1985a) found that TP and TDP concentrations were the same in effluent of superphosphate-amended pine bark columns. In a study assessing DTPA extracts of pine bark from potted *Hakea francisiana* and *Hakea laurina*

amended with crushed bone, rock phosphate, calcined rock phosphate or sewage sludge, Handreck (1996) observed minor differences between TP and OP-P concentrations in the range of 0 to 25 mg·L⁻¹ P. Million et al. (2007) analyzed for TP and OP-P in runoff when producing containerized *Viburnum odoratissimum* grown in DL- and MF-amended substrate composed of 2 pine bark : 1 Sphagnum peat : 1 coarse sand (by volume). The authors found that 89% to 92% of TP in runoff was in the OP-P fraction. To our knowledge, PP, TDP, OP-P and non-orthophosphate dissolved P (NODP) concentrations and the impact of DL and MF on these P fractions has not been studied in effluent or water-extracts of pine bark substrate with incorporated CRF. Accordingly, the objectives of our study were to 1) determine the effect of DL and MF amendments on OP-P, NODP, TDP and PP concentrations in pore-water of pine bark substrate, and 2) simulate P speciation in pine bark pore-water using Visual MINTEQ, a computer-based chemical equilibrium model.

Materials and methods

Stabilized pine bark (milled through a 12.7 mm screen) was attained from Pacific Organics (Henderson, NC) on 15 Jan. 2016. Measured air space, container capacity and available water (by volume) of the substrate were 39.8%, 39.7% and 27.4%, respectively, and bulk density was 0.15 g·cm⁻¹ (NCSU porometer method; Fonteno et al., 1995). Substrate particle size distribution (by weight) was 72% coarse (> 2 mm), 16% medium (2.0 to 0.5 mm) and 12% fine (< 0.5 mm). Pine bark was sent to Brookside Laboratories (New Bremen, OH) to be digested (microwave-assisted, nitric acid) and analyzed for mineral nutrient levels (Peters et al., 2004) using a Thermal 6500 Duo inductively coupled plasma optical emission spectrometer (ICP-OES). Pine bark was 0.29% N, 0.01% P, 0.14% K, 0.29% Ca, 0.05% Mg, 0.03% S, 6.2 mg·kg⁻¹

B, 1321 mg·kg⁻¹ Fe, 117.4 mg·kg⁻¹ Mn, 6.3 mg·kg⁻¹ Cu and 27.5 mg·kg⁻¹ Zn. The C:N ratio of the pine bark was 176.

One day prior to experiment initiation, pine bark was amended with 4.75 kg·m⁻³ of a homogeneous, 5 to 6 mo (release based on a 27 °C) CRF (Polyon 19N–2.6P–10K, Harrell's, Lakeland, FL) and one of four amendment treatments: 1) 4.15 kg·m⁻³ DL (FL), 2) 0.89 kg·m⁻³ granular MF (FM), 3) both DL and MF (FLM), or 4) neither DL nor MF (F; i.e., CRF-only). In addition, non-amended pine bark without CRF was included as a control. The CRF was composed of NH₄NO₃, NH₄H₂PO₄ and K₂SO₄. For the FL and FLM substrate treatments, DL was supplied as 50% pulverized DL (94% CaCO₃ equivalent [CCE], Old Castle Lawn and Garden, Thomasville, PA) and 50% ground DL (97% CCE, Rockydale Quarries Corporation, Roanoke, VA). The pulverized DL had 100%, 95%, 72% and 54% and the ground DL 100%, 90%, 50% and 35% passing through 10, 20, 60 and 100 mesh screens, respectively. The granular MF (Micromax, Everris, Dublin, OH) contained 6.00% Ca, 3.00% Mg, 12.00% S, 0.10% B, 1.00% Cu, 17.00% Fe, 2.50% Mn, 0.05% Mo and 1.00% Zn derived from CaMg(CO₃)₂, FeSO₄·1H₂O, MnSO₄, ZnSO₄, CuSO₄·5H₂O, Na₂B₄O₇ and Na₂MoO₄·2H₂O. The CRF and substrate amendments were incorporated into the pine bark by hand-mixing for 2 min to ensure homogeneity without damaging CRF granules.

Twenty columns were constructed of 30-cm sections of polyvinyl chloride (PVC) pipe (7.8 cm i.d.; 8.9 cm o.d.), and the bottom of each column was fastened with a PVC flat cap with 20 evenly distributed, 8-mm, circular holes. Precision-woven, monofilament, 500 µm mesh fabric (Sefar Propyltex 05-500/36, Heiden, Switzerland) was placed between the bottom end-cap and column to prevent substrate from falling through or clogging the 8-mm holes. The top of each column was affixed to a PVC coupler which was used to elevate diffusers 5 cm above the

substrate surface. Diffusers were PVC flat caps with 16 evenly distributed, 2-mm holes and were used to evenly disperse irrigation water over the substrate surface, minimizing preferential flow. A diagram of the column assembly can be found in Hoskins et al. (2014), labeled “unsaturated column”. A high-density polyethylene (HDPE) funnel was positioned beneath each column to direct leachate into individual 250-mL HDPE bottles.

For each treatment and the control, four columns were packed with ≈ 1.4 L substrate to achieve a mean bulk density of $0.145 \text{ g}\cdot\text{cm}^{-3}$ and then mounted vertically in a custom-made wooden rack. The difference in bulk density between the top and bottom thirds of each column was $\approx 0.02 \text{ g}\cdot\text{cm}^{-1}$. The four single-column replicates of the four treatments and control were arranged in a randomized complete block design (RCBD), with two rows of ten columns. Within each row, the first and last five consecutive columns each made up a block. The experiment was blocked in case microclimates in the laboratory caused temperature differences which could affect CRF release of nutrients. In addition, since leachate samples were filtered in order of block and filtering leachate from one sampling date often require multiple days to complete, assessing the block effect accounted for time until filtration.

Polyethylene tubing (127 mm) with a pressure compensating emitter ($2.1 \text{ mL}\cdot\text{s}^{-1}$, 01WPCLL8, Netafim, Fresno, CA) for each column was fastened to the column rack ≈ 2 cm above the diffusers. Polyethylene tubing was connected to a tap water faucet, and irrigation timing and duration was controlled using a GEVA 75 irrigation window controller with a latch solenoid and hydraulic valve (G75-C-1W-61, Baccara Automation Control, Bayswater, Victoria, AU). To ensure a flow rate of $2.1 \text{ mL}\cdot\text{s}^{-1}$, water volume delivered from each emitter was measured after a 15 s cycle. The observed flow rate that was used to calculate target irrigation volumes was $2.2 \pm 0.01 \text{ SE mL}\cdot\text{s}^{-1}$. Irrigation water was collected at experiment termination and

analyzed by Brookside Laboratories (New Bremen, OH). Irrigation water contained ($n = 3$) 11.9 ± 0.12 SE $\text{mg}\cdot\text{L}^{-1}$ Ca, 4.82 ± 0.04 SE $\text{mg}\cdot\text{L}^{-1}$ Mg, 4.79 ± 0.19 SE $\text{mg}\cdot\text{L}^{-1}$ K, 9.33 ± 0.08 SE $\text{mg}\cdot\text{L}^{-1}$ Na, < 0.1 $\text{mg}\cdot\text{L}^{-1}$ Fe, < 0.20 $\text{mg}\cdot\text{L}^{-1}$ Al, 5.22 ± 0.37 SE $\text{mg}\cdot\text{L}^{-1}$ Cl, 5.51 ± 0.23 SE $\text{mg}\cdot\text{L}^{-1}$ $\text{SO}_4\text{-S}$, 0.18 ± 0.04 SE $\text{mg}\cdot\text{L}^{-1}$ Zn and < 0.05 $\text{mg}\cdot\text{L}^{-1}$ B, Mn, and Cu. Total alkalinity of irrigation water was 55.9 ± 0.79 SE $\text{mg}\cdot\text{L}^{-1}$.

At experiment initiation (day 0), all columns were irrigated for 228 s through the diffusers to deliver ≈ 500 mL tap water per column; for 48 days thereafter, columns were irrigated every 24 h for 57 s to deliver 125 mL (2.6 cm) tap water. The intent of the initial high-volume irrigation event was to settle substrate particles and mimic standard nursery practices following pot-up of a crop. The daily irrigation volume of 125 mL was determined after conducting a preliminary study which showed 125 mL displaced enough substrate pore water for subsequent analyses while minimally diluting leachate with the fresh tap water. Mean air temperature throughout the course of the study was 22.4 ± 0.33 SE $^{\circ}\text{C}$.

Sample collection and preparation

Leachate volume from each column was recorded approximately 12 h after irrigation throughout the study (48 d) to ensure leaching was consistent across treatments. On days 1, 2, 5, 6, 9, 10, 15, 16, 23, 24, 31, 32, 41, 42, 47 and 48, a 20-mL aliquot of leachate from each column and the irrigation water was analyzed for pH and electrical conductivity (EC) using a benchtop pH and EC meter (HI5521-01, Hanna Instruments, Woonsocket, RI) within 4 h of sample collection (i.e., within 16 h of irrigation). The pH and EC meter was equipped with a double junction pH electrode (HI1131B, Hanna Instruments) and conductivity probe (HI76312, Hanna Instruments). All subsequent analyses were performed on 2-d composite samples in which

leachate from each column was collected each sampling day then combined before storing at -30 °C.

The first half of each composite sample was collected on days 1, 5, 9, 15, 23, 31, 41, and 47, and were prepared as follows. For samples in which leachate ion concentrations (i.e., PO_4^{3-} , NO_3^- , NO_2^- , Cl^- and NH_4^+) were determined, 3 mL leachate were syringe-filtered within 12 h of collection into a 10-mL glass vial using a 60-mL Luer Lok syringe (26300, Exel International Medical Products, St. Petersburg, FL) and 0.2- μm hydrophilic polyvinylidene fluoride (PVDF) disposable syringe-filters. Filters of 0.2 μm pore size were used to reduce possible sorption of OP to Fe colloids in the 0.45 to 0.2 μm size range which have been found to affect accurate measurement of PO_4^{3-} when using IC (Sinaj et al., 1998). The 0.2- μm filters were then replaced with 25 mm Swinnex filter holders (SX0002500, Millipore Sigma, Burlington, MS), each containing a 0.45 μm PVDF membrane (HVLP02500, Millipore Sigma, Burlington, MS), to filter 13.5 mL and 10 mL leachate into respective 60-mL HDPE bottles. The 13.5 mL filtered sample (27 mL composite), which was ultimately analyzed for dissolved organic carbon (DOC) concentration, was acidified to a pH of < 3 with two drops of 2 M HCl to remove inorganic carbon from samples via volatilization. The filtered 10-mL sample (20-mL composite) was later analyzed for dissolved element concentrations (i.e., B, Ca, Cu, Fe, K, Mg, Mn, Mo, Na, Ni, P, S and Zn). Ten mL of non-filtered leachate was dispensed into a 60-mL HDPE bottle within 12 h of collection for eventual determination of total concentrations of elements, B, Ca, Cu, Fe, Mg, Mn, Mo, Ni, P and Zn. Samples were refrigerated at 8 °C until the remainder of the composite sample was added the following day (days 2, 6, 10, 16, 24, 32, 42 or 48). The 6-mL, 27-mL and two 20-mL composite samples were then stored at -30 °C. Non-filtered samples were later thawed and digested according to the method described in QuikChem Method 10-115-01-4-B,

except sample volume and amount of digestion reagent added to samples was reduced by half. After digestion, white sediment was present in some samples; thus, digested samples were filtered through a 0.2 μm PVDF filter before being stored at 8 $^{\circ}\text{C}$.

Sample analysis

Analysis of leachate ion concentrations was accomplished using two ion chromatography (IC) systems. The IC systems used to determine anion concentrations (ICS-2100, Thermo-Fisher Scientific) and cation concentrations (ICS-1600, Thermo-Fisher Scientific) utilized respective 4 \times 250 mm (i.d. \times length) anion- and cation-exchange columns (AS19 and CS12A, respectively, Thermo Fisher Scientific) at 35 $^{\circ}\text{C}$ and an autosampler (AS-AP, Thermo Fisher Scientific) on a 25 μL sample loop. In addition, the ICS-2100 was equipped with a metal trap column (MFC-1, Thermo Fisher Scientific) to facilitate detection of the anions. Total and dissolved element concentrations were determined with optical emission spectrometry (iCAP 6300 Duo View ICP-OES Spectrometer; Thermo Fisher Scientific). Dissolved organic carbon (non-purgeable organic carbon from organic sources that is available for microbial metabolic functions) concentrations were analyzed via NPOC/TN analysis using a Shimadzu TOC-V_{CPH} total organic carbon analyzer with TNM-1 total nitrogen measuring unit (Shimadzu Scientific Instruments, Kyoto, Japan).

Theoretical chemical P speciation in leachate was modeled using Visual MINTEQ (Gustafsson, 2010). Input parameters included pH, DOC (NICA-Donnan model), PO_4^{3-} , NH_4^+ , NO_2^- , NO_3^- , B(III), Ca^{2+} , Cl^- , Cu^{2+} , Fe^{3+} , K^+ , Mg^{2+} , Mn^{2+} , Mo(VI), Na^+ , Ni^{2+} , SO_4^{2-} and Zn^{2+} concentrations. Element input values were based on concentrations measured in previously described filtered samples. Metals were assumed to be in their oxidized state. Carbonate

concentrations were estimated based on measured irrigation water alkalinity as well as Ca and Mg concentrations in leachate of substrates containing DL and/or MF. Saturation indices provided in output were used to interpret degree of saturation in solutions with respect to solid phases.

Statistics

Phosphorus fraction partitioning in the particulate versus dissolved phases was analyzed via three-way analysis of variance (ANOVA) with a repeated measures factor (time) and two between subjects factors, substrate (F, FL, FM and FLM) and fraction (PP [i.e., TP – TDP] and TDP). Dissolved Fe (D-Fe; i.e., $< 0.45 \mu\text{m}$) and particulate Fe (P-Fe; i.e., total Fe – D-Fe) concentrations were analyzed in the same manner. The dissolved P phase was further assessed in a separate, but similar, analysis that included fractions, OP-P and NODP (i.e., TDP – OP-P) instead of PP and TDP. For all three-way ANOVA, the three-way interaction (time \times P fraction \times substrate), three two-way interactions (time \times fraction, time \times substrate and fraction \times substrate) and three main effects were assessed. Concentrations of other elements were equivalent between filtered and non-filtered samples; thus, analyses were performed using dissolved (i.e., filterable) concentration values. Effects of substrate, time and the substrate \times time interaction on Ca, Mg, EC, DOC and pH were analyzed via two-way RM ANOVA. The block effect (random) was non-significant ($P > 0.05$) in all ANOVA and was therefore removed from analysis to reduce the model.

Prior to analysis, data were transformed to correct for heteroskedasticity and non-normality. The log-transformation was used for Ca, Mg, EC and DOC values, and the normalized Johnson's transformation (Johnson, 1949) was used for all Fe and P data. Repeated

measures analysis was executed by modeling covariance structures (Wolfinger, 1993). The most appropriate covariance structure was selected by fitting data to various homogeneous and heterogeneous covariance structures available in JMP Pro 14 (SAS Institute Inc., Cary, NC) and comparing corrected Akaike information criterion (AIC_c) values. According to AIC_c values, the following covariance structures were selected for RM analyses: homogeneous antidependent for P (PP vs. TDP fractions), Fe, Ca and Mg; heterogeneous antidependent for P (OP-P vs. NODP fractions); first-order autoregressive (AR[1]) for EC and DOC; and unequal variance for pH data.

When interactions were significant ($P < 0.05$), simple effects were analyzed using Tukey's Honest Significant Difference. Dunnett's test was used to compare values of the control to those of each substrate treatment. Correlations were assessed using the Pearson correlation coefficients (r). Percent reduction in element concentrations by amended substrates, discussed hereafter, were calculated on the basis of concentrations in the F unless otherwise noted. All data were processed using JMP Pro 14 (SAS Institute Inc., Cary, NC). Figures were created using KaleidaGraph 4.5.3 (Synergy Software, Reading, PA).

Results and Discussion

Calcium and magnesium

Calcium concentrations were affected by a substrate \times time interaction (Table 1); thus, substrate effects were analyzed at each sampling date (Fig. 1). Solution Ca concentrations in FM were $90 \text{ mg}\cdot\text{L}^{-1}$ at 1 DAI, over twice as high as concentrations in FL (Fig. 1). Thereafter, Ca concentrations in FM sharply decreased to $14 \text{ mg}\cdot\text{L}^{-1}$ by 9 DAI before gradually decreasing to a minimum of $5.1 \text{ mg}\cdot\text{L}^{-1}$ at 23 DAI. From 31 to 48 DAI, Ca concentrations in FM were equivalent to those in F. Calcium concentrations in FL decreased from $39 \text{ mg}\cdot\text{L}^{-1}$ at 1 DAI to a

minimum of $15 \text{ mg}\cdot\text{L}^{-1}$ at 9 DAI, then gradually increased to $22 \text{ mg}\cdot\text{L}^{-1}$ between 15 and 49 DAI. The relatively high initial Ca concentrations in FM compared to FL suggests that the DL component in the MF was highly soluble relative to the separately added DL amendment in FL and FLM. Rapid dissolution of the DL component in MF is likely partially attributed to the acidic pH of the FM substrate at 1 to 9 DAI (pH = 3.8 to 4.3) relative to that in FL (pH = 6.4 to 7.3; Fig. 2). Solubility of $\text{CaMg}(\text{CO}_3)_2$, a carbonate mineral, is well known to increase with decreasing pH (Gautelier et al., 1999; Lindsay, 1979). In FLM, Ca concentrations were equivalent to those in FM at 1 DAI and to those in FL from 15 to 48 DAI. Calcium concentrations in the control and F responded similarly from 1 to 23 DAI, decreasing from 22 to $2.5 \text{ mg}\cdot\text{L}^{-1}$ in both substrates. From 23 to 48 DAI, Ca concentration values in F increased to $13 \text{ mg}\cdot\text{L}^{-1}$, whereas those in the control remained constant. The increase in Ca concentration in F between 23 and 41 DAI corresponded with increased leachate K concentrations due to CRF release (data not shown). Displacement of Ca from exchange sites in pine bark by K^+ ions has been reported and may explain the increasing leachate Ca concentration between 23 and 48 DAI in F (Hoskins et al., 2014).

Magnesium concentrations were also influenced by a substrate \times time interaction (Table 1), and Mg data closely resembled Ca data (Fig. 3). At 1 DAI, Mg concentrations in FLM were 54% higher than those in FM, indicating both amendments contributed to initial Mg concentrations in the FLM substrate. Similar to Ca, Mg concentrations in substrates containing MF rapidly decreased between 1 and 9 DAI. Magnesium concentrations in FM were no higher than those in F by 23 DAI, whereas Mg in FLM were the same as those in FL at 15 to 48 DAI. As was observed for Ca, Mg concentrations in F increased $\approx 7 \text{ mg}\cdot\text{L}^{-1}$ between 15 and 48 DAI, whereas Mg in the control remained the same during this period. As was discussed for Ca, this

delayed increase in Mg concentrations in F was likely due to displacement of Mg from pine bark exchange sites by K⁺ released by the CRF (Hoskins et al., 2014).

Iron

Iron concentrations were affected by a fraction × substrate × time interaction (Table 2). The simple interaction of fraction × substrate was present at 1, 5 and 9 DAI, but not at sampling dates thereafter. Among substrates, highest D-Fe concentrations were found in FM at 1 to 15 DAI and in the control from 31 to 48 DAI (Table 3). Dissolved Fe concentrations in FLM were lower than those in FM until 31 DAI. From 31 to 48 DAI, D-Fe concentrations were ≤ 0.10 mg·L⁻¹. The observed relatively low D-Fe concentrations in MF-amended pine bark corresponds with Wright and Hinesley (1991) who reported that granular MF amendment rates of up to 1.5 kg·m⁻³ in a pine bark substrate had no effect on water-extractable Fe concentrations when measured 60 d after potting *Juniperus virginiana*. The authors also reported that liming the substrate reduced Fe in water extracts, regardless of the presence of MF. Similarly, Abreu et al. (2006) found that water-extractable Fe concentrations were similar in limed pine bark with and without added micronutrients. In a study that used the same MF product used in the current study, Handreck (1989) found that < 1% of 111 mg Fe added to pine bark was recovered in effluent after 50 days of daily leaching, most of which was found within the first six days. Our results agree with these studies in that liming agents and the subsequent increase in pH reduces Fe solubility in pine bark.

Particulate-Fe concentrations were highest in the control, F and FL at 1 DAI and in the control and F at 5 DAI (Table 3). From 9 to 48 DAI, substrates generally had a similar effect on P-Fe as that described for D-Fe. Higher P-Fe concentrations in substrates containing no

supplemental Fe from MF may be explained by coagulation of organic compounds with Fe within columns, which subsequently reduced movement of coagulated, Fe-containing particles through the columns and the 500 μm mesh endcap. Iron sulfate, the Fe component in MF, has been shown to be an effective coagulant in waters containing high DOC levels (Sinsabaugh et al., 1986).

pH

Leachate pH values in all five substrate treatments increased by $\approx 1.8 \pm 0.07$ SE units over the course of the study, reaching pH values of ≈ 8.0 at 41 and 48 DAI in substrates containing DL (Fig. 2). Final pH values in FL and FLM were higher than values reported in studies in which a comparable DL rate was used. In a comprehensive review on the effects of DL additions in pine bark, Altland and Jeong (2016) proposed that DL can increase pH up to ≈ 6.5 before pH limits its solubility and concomitant effects on pH. A possible explanation for the observed abnormally high pH values is that the inherent acidity of the pine bark was leached due to daily irrigation with ≈ 2.6 cm water; however, this amount of water can be applied in a conventional nursery setting via daily irrigation and rainfall. Assuming the inherent acidity of the pine bark was reduced, substrate pH would have been more responsive to the lime reaction and the pH of the irrigation water, which increased from 6.9 to 7.8 over the course of the experiment (data not shown). Leachate pH values of the control and F were equivalent at all sampling dates except 23 DAI at which pH values of F were 0.4 units higher than those of the control; hence, the fertilizer generally had a minor effect on leachate pH. From 1 to 23 DAI, pH values of FM were 0.38 ± 0.03 SE units lower than those of F suggesting MF was acidifying for the first 23 days. The contention that MF reduces pH in pine bark is supported by the 0.5, 0.3 and 0.2 unit lower pH in

FLM compared to FL at 1, 5 and 9 DAI, respectively. Acidifying effects of the MF used in this study was also reported by Wright et al. (1999), who observed a 0.5 unit decrease in pH when pine bark was amended with MF.

Electrical conductivity

Electrical conductivity was equated to IS for convenient reference using the formula proposed by Alva et al. (1991; i.e., $IS = [EC \times 0.012] - 0.0002$) and presented in conjunction with EC in Fig. 4. Electrical conductivity values in F were the same as those in the control at 1, 5 and 9 DAI during which EC values in both substrates decreased from 0.51 to 0.21 $mS \cdot cm^{-1}$. At 15 DAI, EC values in F were 29% higher than those in the control, and from 15 to 48 DAI, EC values in F and the control increased 178% and decreased 29%, respectively. Accordingly, these data suggest that the fertilizer began releasing salts between 9 and 15 DAI. Electrical conductivity values in FM and FLM were equivalent at 1 and 5 DAI during which EC values in these two treatments decreased $\approx 50\%$. From 1 to 23 DAI, EC values in FL were between 27% and 89% higher than those in F. Observed higher EC values in FL compared to F were likely due to DL dissolution and subsequent presence of Mg^{2+} and Ca^{2+} in leachate.

Dissolved organic carbon

Dissolved organic carbon was influenced by a substrate \times time interaction (Table 1); substrate effects were therefore analyzed at each sampling data (Fig. 5). Dissolved organic carbon concentrations were the same across treatments at 1, 5 and 9 DAI during which DOC decreased from ≈ 265 to ≈ 85 $mg \cdot L^{-1}$. Thereafter, DOC concentrations increased by ≈ 10 to 20 $mg \cdot L^{-1}$ between 15 and 23 DAI then decreased for the remainder of the study. A substrate effect

on DOC concentrations was first apparent at 15 DAI, during which the control or substrates containing DL had highest DOC. This relationship among treatments remained the same until experiment termination, with DOC concentrations 35% to 45% higher in limed treatments or the control compared to F or FM. In organic soils, leaching of DOC is affected by both pH and EC. In a study in which DOC leaching was measured in effluent from peat-sand soil columns, Tiemeyer et al. (2017) found that low pH and high EC (1064 compared to 94 $\mu\text{S}\cdot\text{cm}^{-1}$) greatly reduced DOC leaching. Assessing EC data collected between 15 and 48 DAI (Fig. 4), during which DOC was influenced by substrate treatments, the marginally higher EC values in FL and FLM compared to F and FM at 15, 23 and 48 DAI were not likely great enough to impact DOC leaching. More likely, the lower pH in F and FM compared to those in limed substrates (Fig. 2) was responsible for lower respective DOC concentrations between 15 and 48 DAI. However, DOC in the control substrate did not follow this trend, as pH values of the control were similar to those in FL and FLM. According to findings by Tiemeyer et al. (2017), pH has less impact on DOC when EC values are low (i.e., 94 $\mu\text{S}\cdot\text{cm}^{-1}$). Hence, the low EC values in the control between 15 and 48 DAI may have nullified the effect of relatively low pH values on DOC.

Phosphorus

The time \times substrate \times treatment interaction was significant for both P-fraction analyses (i.e., PP versus TDP and OP-P versus. NODP; Table 2). Thus, simple two-way interactions at each level of the remaining factor were assessed. For the PP versus TDP analysis, all simple interactions were significant except the substrate \times fraction interaction at 9 and 15 DAI.

Particulate P concentrations were affected by substrate treatments at all sampling dates except 9, 15 and 41 DAI (Table 4). Particulate P concentrations were highest at 1 DAI in all

substrates and concentrations decreased rapidly between 1 and 5 DAI, then remained < 1.5 $\text{mg}\cdot\text{L}^{-1}$. At 1 DAI, highest PP was found in the control, F and FL, whereas at 5 DAI, PP concentrations were highest in the control, F and FM. According to Visual MINTEQ modeling, thermodynamics indicate that both MnHPO_4 and $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ were saturated with respect to their solid phases in all substrates at 1 and 5 DAI (Table 5). The response of PP concentrations to substrate treatments paralleled that of P-Fe (Table 3). When pooled across all substrates and sampling dates, PP had a strong, positive correlation with P-Fe ($r = 0.94$) and DOC ($r = 0.86$). These parallel changes in PP, P-Fe and DOC suggest possible formation of humic-Fe- PO_4 ternary complexes (Gerke, 2010; Gerke and Hermann, 1992). This fate of P is supported by Visual MINTEQ aqueous speciation output which predicted between 95% and 99% of Fe in solution was associated organic acids (data not shown). However, further investigation is needed to confirm the existence of humic-Fe(Al)- PO_4 complexes in pine bark substrates. At 31 and 48 DAI, highest PP concentrations were in the control and substrates containing DL (Table 4). Relatively high PP concentrations in substrates containing DL at 31 and 48 DAI, during which pH values were favorable for Ca- PO_4 precipitation, may have been due to formation of Ca- PO_4 precipitates. Formation of Ca- PO_4 precipitates is supported by data from Visual MINTEQ modeling which indicated leachate solutions from FL and FLM were saturated with respect to the $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ solid phase at 41 and 48 DAI (Table 5). The solid phase of $\text{Ca}_3(\text{PO}_4)_2$ was also saturated in FL and FLM at 48 DAI; however, saturation index (SI) values of $\text{Ca}_3(\text{PO}_4)_2$ were low relative to those of $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$. Kinetics, metastable fast formers and the presence of DOC can limit precipitation and thus interpretation of this simulation output. Relatively high DOC concentrations, such as those observed in the current study make interpretation of Ca- PO_4

precipitate formation particularly challenging, as DOC has been shown to impede nucleation of Ca-PO₄ precipitates (Song, 2006).

Total dissolved P concentrations in F were similar to those in the control until 41 DAI (Table 4), suggesting indigenous P in the pine bark was the primary source of TDP until the CRF began releasing P between 31 and 41 DAI. Delayed P release from CRF is commonly reported in nursery crop production research (Broschat, 2005; Broschat and Moore, 2007; Du et al., 2006; Newman et al., 2006). Total dissolved P concentrations in the control decreased 75% between 1 and 15 DAI. Yeager and Wright (1982) also reported relatively high amounts of soluble P in a pine bark substrate. At 1, 5, 41 and 48 DAI, TDP concentrations were lower in FL and FLM than in F. Total dissolved P concentrations were lowest in FLM at 1 and 5 DAI, whereas at 41 and 48 DAI, FL and FLM had equivalent TDP concentrations. The FM substrate reduced TDP at 1 and 5 DAI in similar amounts as FL; however, TDP concentrations in FM were the same as those in F at 9 to 48 DAI. These data suggest that while MF reduced TDP concentrations by 30% and 40% at 1 and 5 DAI, respectively, this effect was short-lived. The greater effectiveness of FLM at reducing TDP concentrations at the beginning of the study, relative to the end of the study, can therefore be explained by initial TDP reductions by MF. Decreases in TDP by DL and MF did not necessarily correspond with a proportional increase in PP, suggesting DL and MF amendments improved total P retention in the substrate. To further investigate the effect of substrate amendments on P retention in pine bark, an additional RM ANOVA was performed on total P (TDP + PP; data not shown). Assessment of total P concentrations revealed a similar response to substrate treatments as was described for TDP. The effect of substrate on TP was significant at 1 ($P < 0.0001$), 5 ($P < 0.0001$), 9 ($P = 0.0004$), 31 ($P = 0.0050$) and 41 ($P = 0.0076$) DAI. The FLM substrate reduced TP concentrations by, on average, 51% at 1, 5 and 9 DAI and

by 45% at 41 DAI. The FL substrate reduced TP concentrations by 19% and 36% at 1 and 5 DAI, respectively, and by $\approx 52\%$ at 31 and 41 DAI. In contrast, effects of FM on TP concentrations were apparent only at 1 and 5 DAI, during which TP concentrations were $\approx 36\%$ lower than those in F. Thus, TP retention in FLM was initially controlled by the combination of DL and MF and later by DL only. The short-term effect of MF on P sorption may be related to its solubility. The rapid decrease in Ca and Mg to concentrations equivalent to or nearly equivalent to those in F by 23 DAI suggests that most of the DL in MF had dissolved by 31 DAI. The component in MF controlling P sorption is unclear, however, since both the DL and Fe constituents in MF may have had a role. Spectroscopic evidence has shown that retention of P by DL at relatively low P concentrations (i.e., $< 2 \text{ mg}\cdot\text{L}^{-1}$) is primarily due to PO_4 sorption to Ca and Mg sorption complexes (Xu et al., 2014).

The substrate \times time simple interaction was significant for OP-P, but not for NODP (Table 4). The main effect of substrate on NODP was also non-significant ($P > 0.05$), but the main effect of time on NODP was highly significant ($P < 0.0001$), with NODP decreasing until 31 DAI before plateauing. Both the substrate \times fraction interaction and simple effect of substrate on OP-P concentrations were significant on the same sampling dates. The substrate \times time interaction for OP-P can be explained by the decreasing effect of MF on OP-P concentrations. At 1 and 5 DAI, OP-P concentrations were lower in FM than in FL, and lowest concentrations were in FLM. In contrast, at 31 to 48 DAI, OP-P concentrations in FM were the same as those in F, and lowest concentrations were found in $\text{FL} \approx \text{FLM}$. Hence, the response of OP-P to substrate amendments was generally similar to that described for TDP; however, percent reductions in OP-P in response to amendments was generally greater than those of TDP. Comparing the proportion of TDP contributed by OP-P, OP-P was 23% to 55% of TDP in all substrates at 1 and 5 DAI,

with generally lower proportions found in FLM. At sampling dates during which CRF was releasing P (i.e., 31 to 48 DAI), OP-P contributed 74% to 86% of TDP in F, FL, FM and FLM with no perceivable differences among these substrates. This trend is due to relatively high initial NODP concentrations (i.e., $3.9 \text{ mg}\cdot\text{L}^{-1}$) that decreased until 31 DAI, then remained relatively low compared to OP-P. As was presumed to be a source of PP, the NODP fraction may have been associated with (dissolved) metal-humic complexes. Van Moorlehem et al. (2011) determined that organic Fe colloids present in filtered solutions ($< 0.45 \mu\text{m}$) reduced detection of OP-P by ion chromatography by as much as 51%, whereas TDP (determined via ICP-OES) was unaffected. In the current study, the positive correlation between NODP and DOC ($r = 0.90$) was stronger than that between OP-P and DOC ($r = 0.58$), which supports the possibility of an association between NODP and organic substances. Additional research is needed to investigate the OP-P:TDP ratio in pine bark once CRF release increases OP-P concentrations to the range of 5 to $10 \text{ mg}\cdot\text{L}^{-1}$, the current recommendation according to best management practices for container-grown crops (Bilderback et al., 2013)

Conclusions

Results presented herein demonstrate that DL and MF improve P retention, and therefore reduce P leaching from a pine bark substrate commonly used for production of containerized ornamentals. Compared to the fertilized substrate containing no DL or MF, the pine bark with DL and MF additions reduced OP-P concentrations by 70% when averaged across the sampling dates. The effects of MF on P retention were diminished by the ninth leaching event, whereas DL reduced OP-P leaching throughout the 48-d study. Further investigation is needed to determine the longevity of these P-retention effects of DL and the extent of P retention when baseline P

concentrations are $> 5 \text{ mg}\cdot\text{L}^{-1}$. If DL additions to pine bark can be shown to reduce P leaching throughout a growing season, DL amendments may be considered a best management practice for containerized crop production. The results of this study suggest that MF and DL amendments may reduce plant-availability of P, at least for the first 48 d after potting and fertilizing with CRF. However, lower concentrations of water-extractable OP-P does not necessarily translate to reduced plant-availability. A possibility is that the P retained by the DL-amended substrate may serve as a labile source of P in pine bark, a substrate that otherwise has no inherent labile P. Further evidence is needed to support this speculation.

Due to the convenience of simultaneously analyzing multiple nutrient ions, ICP-OES is a popular method for determining P concentrations in horticulture research. Results of the current study showed that TDP (i.e., filtered P measured via ICP-OES) correlated well with the plant-available P species, OP-P ($r = +0.92$). Thus, under the conditions observed in our study, TDP seem to be an adequate proxy for relative OP-P levels. However, since up to 45% of TDP was NODP, TDP cannot be assumed to equivalent to OP-P in leachate of pine bark substrate. Visual MINTEQ modeling indicated MnHPO_4 in all treatments and $\text{CaMg}(\text{CO}_3)_2$ in limed treatments were saturated with respect to their solid phases in leachate throughout the study.

Acknowledgements

The authors thank Julie Brindley, Velva Groover, Anna Birnbaum and Doug Sturtz for technical assistance, as well as Pacific Organics for donating pine bark for this research. Funding was provided by the Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture (SCRI 2014-51181-22372), U.S. Department of

Agriculture, the Horticultural Research Institute and the Virginia Nursery and Landscape Association.

Literature Cited

Abreu, C.A., A.M.C. Furlani, P.R. Furlani, M.F. Abreu, O.C. Bataglia, and A. Paz-Gonzalez.

2006. Quest of water extract analysis of micronutrients in soilless organic substrates.

Commun. Soil Sci. Plant. Anal. 37:2327–2338.

Altland, J.E. and K.Y. Jeong. 2016. Dolomitic lime amendment affects pine bark substrate pH, nutrient availability, and plant growth: A review. HortTechnology 26:565–573.

Altland, J.E., W. Zellner, and J.C. Locke. 2015. Substrate pH and butterfly bush response to dolomitic lime or steel slag amendment. J. Environ. Hort. 33:89–95.

Alva A.K., M.E. Sumner, and W.P. Miller. 1991. Relationship between ionic strength and electrical conductivity for soil solutions. Soil Sci. 152(4):239–242.

Argo, W.R. and J.A. Biernbaum. 1996a. The effect of lime, irrigation-water source, and water-soluble fertilizer on root-zone pH, electrical conductivity, and macronutrient management of container root media with impatiens. J. Amer. Soc. Hort. Sci. 121:442–452.

Argo, W.R. and J.A. Biernbaum. 1996b. Availability and persistence of macronutrients from lime and preplant nutrient charge fertilizers in peat-based root media. J. Amer. Soc. Hort. Sci. 121:453–460.

Bilderback, T., C. Boyer, M. Chappell, G. Fain, D. Fare, C. Gilliam, B.E. Jackson, J. Lea-Cox, A.V. LeBude, A. Niemiera, J. Owen, J. Ruter, K. Tilt, S. Warren, S. White, T. Whitwell, R. Wright, and T. Yeager. 2013. Best management practices: Guide for producing nursery crops. 3rd ed. Southern Nursery Association, Acworth, GA.

- Bilderback, T.E., E.D. Riley, B.E. Jackson, H.T. Kraus, W.C. Fonteno, J.S. Owen, Jr., J.E. Altland, G.B. Fain. 2013. Strategies for developing sustainable substrates in nursery crop production. *Acta Hort.* 1013:43–56.
- Boström B., G. Persson, and B. Broberg. 1988. Bioavailability of different phosphorous forms in freshwater systems. *Hydrobiologia.*170:133–155.
- Broberg, O, and G. Persson. 1988. Particulate and dissolved phosphorus forms in freshwater: composition and analysis. *Hydrobiologia* 170:61–90.
- Broschat, T.K. and K.K. Moore. 2007. Release rates of ammonium-nitrogen, nitrate-nitrogen, phosphorus, potassium, magnesium, iron, and manganese from seven controlled-release fertilizers. *Commun. Soil Sci. Plant Anal.* 38:843–850.
- Broschat, T. 1995. Nitrate, phosphate, and potassium leaching from container-grown plants fertilized by several methods. *HortScience* 30:74–77.
- Broschat, T. 2005. Rates of ammonium-nitrogen, nitrate-nitrogen, phosphorus, and potassium from two controlled-release fertilizers under different substrate environments. *HortTechnology* 15:332–335.
- Carpernter, S.R., N.F. Caraco, D.L, Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint pollution of surface waters with phosphorus and nitrogen. *Ecol. Appl.* 8:559–568.
- Cole, J.C., and J.M. Dole. 1997. Temperature and phosphorus source affect phosphorous retention by pine bark- based container medium. *HortScience.* 32:236–240.
- Dennis, J.H., R.G. Lopez, B.K. Behe, C.R. Hall, C. Yue, B.L. Campbell. 2010. Sustainable production practices adopted by greenhouse and nursery plant growers. *HortScience* 45:1232–1237.

- Diara, C., L. Incrocci, G. Incrocci, G. Carmassi, A. Pardossi, P. Marzialesi, P. Cozzi, F. Fibbi, and R. Clemens. 2014. Reduction of nutrient run-off by the use of coated slow-release fertilizers on two container-grown nursery crops. *Acta Hort.* 1034:85–92.
- Du, C., J. Zhou, and A. Shaviv. 2006. Release characteristics of nutrients from polymer-coated compound controlled release fertilizers. *J. Polym. Environ.* 14:223–230.
- Fain, G.B., C.H. Gilliam, K.M. Tilt, J.W. Olive, and B. Wallace. 2000. Survey of best management practices in container production nurseries. *J. Environ. Hort.* 18:142–144.
- Fonteno, W.C., C.T. Hardin, and J.P. Brewster. 1995. Procedures for determining physical properties of horticultural substrates using the NCSU Porometer. Horticultural Substrates Laboratory. NC State University, Raleigh, NC.
- Fytianos, K., E. Voudrias, and N. Raikos. 1998. Modeling of phosphorous removal from aqueous and wastewater samples using ferric iron. *Environmental Pollution.* 101:123–130.
- Gautelier, M., E.H. Oelkers, and J. Schott. 1999. An experimental study of dolomite dissolution rates as a function of pH from –0.5 to 5 and temperature from 25 to 80°C. *Chem. Geol.* 157:13–26.
- Gerke, J. 2010. Humic (organic matter)-Al(Fe)-phosphate complexes: An underestimated phosphate form in soils and source of plant-available phosphate. *Soil Sci* 175:417–425.
- Gerke, J., and R. Hermann. 1992. Adsorption of orthophosphate to Humic-Fe-Complexes and to amorphous Fe-Oxide. *Z. Pflanzenernähr. Bodenk.* 155:233–236.
- Godoy, A., and J.C. Cole. 2000. Phosphorous source affects phosphorous leaching and growth of containerized spirea. *HortScience.* 35:1249–1252.
- Handreck, K.A. 1989. Assessment of iron availability in soilless potting media. *Commun. Soil Sci. Plant Anal.* 20:1297–1320.

- Handreck, K.A. 1991a. Interactions between iron and phosphorus in the nutrition of *Banksia ericifolia* L.f. var. *ericifolia* (Proteaceae) in soil-less potting media. *Aust. J. Bot.* 39:373–84.
- Handreck, K.A. 1991b. Available phosphorus in potting media: Extractants and interpretation of extract data. *Commun. Soil Sci. Plant Anal.* 22:529–557.
- Handreck, K.A. 1992. Iron phosphorous interactions in in the nutrition of seedling macadamia in organic potting media. *Australian journal of experimental horticulture.* 32:773–779.
- Handreck, K.A. 1996. Comparison of phosphate-phosphorous and total phosphorus in DTPA extracts for assessing plant-available phosphorus in soilless potting media. *Commun. Soil Sci. Plant Anal.* 27:2125–2135.
- Havis, J.R., and J.H. Baker. 1985. Influence of liming rate and phosphorus leaching from a peat-sand medium. *J. Environ. Hort.* 3:74–76.
- Haynes, J.R. 1982. Leaching losses of nutrients and yield and nutrient uptake by container-grown begonia as affected by lime and fertilizer applications to a peat medium. *J. Sci. Food Agric.* 33:407–413.
- Hens, M., and R. Merckx. 2002. The role of colloidal particles in the speciation and analysis “dissolved” phosphorous. *Water Res.* 36:1483–1492.
- Hoskins, T.C., J.S. Owen, Jr., and A.X. Niemiera. 2014. Controlled-release fertilizer placement affects the leaching pattern of nutrients from nursery containers during irrigation. *HortScience* 49:1341–1345.
- Hsu, P.H. 1976. Comparison of iron(III) and aluminum in precipitation of phosphate from solution. *Water Research.* 10: 903–907.

- Johnson, N. L. 1949. Systems of frequency curves generated by methods of translation. *Biometrika* 36:149–176.
- Karaca, S., A. Gürses, M. Edjer, and M. Açıkyıldız. 2004. Kinetic modeling of liquid-phase adsorption and phosphate on dolomite. *J. Colloid Interface Sci.* 277:257–263.
- Karaca, S., A. Gürses, M. Edjer, and M. Açıkyıldız. 2006. Adsorptive removal of phosphate from aqueous solutions using raw and calcinated dolomite. *J. Hazard. Mater.* B128: 273–279.
- Lindsay, W.L. 1979. *Chemical Equilibria in Soils*. John Wiley, New York.
- Lu, W., J.L. Sibley, C.H. Gilliam, J.S. Bannon, and Y. Zhang. 2006. Estimation of U.S. bark generation and implications for horticultural industries. *J. Environ. Hort.* 24:29–34.
- Mack, R., J.S. Owen, A.X. Niemiera, and J. Latimer. 2017. Virginia nursery and greenhouse grower survey of best management practices. *HortTechnology* 27:386–392.
- Mangwandi, C., A. B. Albadarin, Y. Glocheux, G. M. Walker. Removal of ortho-phosphate from aqueous solution by adsorption onto dolomite. *J. Environ. Chem. Eng.* 2:1123–1130.
- Marconi, D.J. and P.V. Nelson. 1984. Leaching of applied phosphorous in container media. *Sci. Hort.* 22:275–285.
- Maruo, M. M. Ishimaru, Y. Azumi, Y. Kawasumi, O. Nagafuchi, and H. Obata. 2016. Comparison of soluble reactive phosphorous and orthophosphate concentrations in river waters. *Limnology.* 17:7–12.
- McGinnis, M.S., S.L. Warren, and T.E. Bilderback. 2009. Replacing conventional nursery crop nutrient inputs with vermicompost for container production of *Hibiscus moscheutos* L. ‘Luna Blush’. *HortScience* 44:1690–1703.

- McKelvie, I.D., D. M.W. Peat, and P.J. Worsfold. 1995. Techniques for the quantification and speciation of phosphorous in natural waters. *Anal. Proc. incl. Anal. Commun.* 32:437–445.
- Million, J., T. Yeager, and J. Albano. 2007. Consequences of excessive overhead irrigation on runoff during container production of sweet viburnum. *J. Environ. Hort.* 25:117–125.
- Moore, P.A., Jr., and D.M. Miller. 1994. Decreasing phosphorus solubility in poultry litter with aluminum, calcium, and iron amendments. *J. Environ. Qual.* 23:325–330.
- Newman, J.P., J.P. Albano, D.J. Merhaut, and E.K. Blythe. 2006. Nutrient release from controlled-release fertilizers in a neutral-pH substrate in an outdoor environment: I. Leachate electrical conductivity, pH, and nitrogen, phosphorus, and potassium concentrations. *HortScience* 41:1674–1682.
- Owen, J.S., S.L. Warren, T.E. Bilderback, and J.P. Albano. 2008. Phosphorus rate, leaching fraction, and substrate influence on influent quantity, effluent nutrient content, and response of a containerized woody ornamental crop. *HortScience* 43:906–912.
- Paradelo, R., M. Conde-Cid, M. Arias-Estévez, J.C. Nóvoa-Muñoz, E. Álvarez-Rodríguez, M.J. Fernández-Sanjurjo, A. Núñez-Delgado. 2017. Removal of anionic pollutants by pine bark influenced by the mechanism of retention. *Chemosphere* 167:139–145.
- Peters, J., S. Combs, B. Hoskins, J. Jarman, J. Kovar, M. Watson, A. Wolf, and N. Wolf. 2003. Recommended methods of manure analysis. University of Wisconsin Coop. Ext. Publication A3769. Coop. Ext. Publishing, Madison, WI.
- Rich, D. 2005. The removal of total phosphorus from natural waters by precipitation. Ph.D. diss., Northwestern International Univ., Evanston, IL.

- Rowland, A.P. and P.M. Haygarth. 1997. Determination of total dissolved phosphorous in soil solutions. *J. Environ. Qual.* 26:410–415.
- Sanyal, S.K., and S.K. De Datta. 1991. Chemistry of phosphorous transformations in soil. *Adv. Soil Sci.* 16:1–94.
- Sinaj, S., F. Mächler, E. Frossard, C. Fäisse, A. Oberson, and C. Morel. 1998. Interference of colloidal particles in the determination of orthophosphate concentrations in soil water extracts. *Commun. in Soil Sci. and Plant Anal.* 29:9–10.
- Sinsabaugh, R.L., III, R.C. Hoehn, W.R. Knocke, and A.E. Linkins, III. 1986. Removal of dissolved organic carbon by coagulation with iron sulfate. *J. Am. Water Works Assoc.* 78(5):74–82.
- Song, Y., H.H. Hahn, E. Hoffmann, and P.G. Weidler. 2006. Effect of humic substances on the precipitation of calcium phosphate. *J. Environ. Sci.* 18:852–857.
- Tasistro, A. S., and D.E. Kissel. 2006. Composition of aqueous extracts of broiler litter treated with aluminum sulfate, ferrous sulfate, ferric chloride and gypsum. *J. Environ. Sci. Health B* 41:989–1000.
- Tiemeyer, B., N. Pfaffner, S. Frank, K. Kaiser, S. Fieldler. 2017. Pore water velocity and ionic strength effects on DOC release from peat-sand mixtures: Results from laboratory and field experiments. *Geoderma* 296:86–97.
- Tyler, H.H., S.L. Warren, and T.E. Bilderback. 1996. Reduced leaching fractions improve irrigation use efficiency and nutrient efficacy. *J. Environ. Hort.* 14:199–204.
- U.S. Department of Agriculture. 2014. 2012 Census of Agriculture: Census of horticultural specialties (2014). p. 24.

- U.S. Environmental Protection Agency. 2017. Nutrient pollution: Sources and solutions. <https://www.epa.gov/nutrientpollution/sources-and-solutions> (accessed 15 May 2018).
- Van Moorlehem, C., L. Six, F. Degryse, E. Smolders, and R. Merckx. 2011. Effect of organic P forms and P present in inorganic colloids on the determination of dissolved P in environmental samples by the diffusive gradient in thin films technique, ion chromatography, and colorimetry. *Anal. Chem.* 83:5317–5323. doi:10.1021/ac200748e.
- Warncke, D.D. 1986. Analyzing greenhouse growth media by the saturation extraction method. *HortScience* 21:223–225.
- Warren, S.L., T.E. Bilderback, and H.H. Kraus. 2001. Method of fertilizer application affects nutrient losses of controlled-release fertilizer. *Acta Hort.* 548:349–355.
- Warren, S.L., T.E. Bilderback, and H.H. Tyler. 1995. Efficacy of three nitrogen and phosphorus sources in container-grown azalea production. *J. Environ. Hort.* 13:147–151.
- Weir, C.C., and R. J. Soper. 1963. Interaction of phosphates with ferric organic complexes. *Can. J. Soil Sci.* 43:393–399.
- Wolfinger, R. 1993. Covariance structure selection in general mixed models. *Commun. Statist.-Simula.* 22:1079–1106.
- Worsfold, P., I. McKelvie, and P. Monbet. 2016. Determination of phosphorous in natural waters: a historical review. *Anal. Chim. Acta* 918:8–20.
- Wright, A.N., A.X. Niemiera, J.R. Harris, and R.D. Wright. 1999. Micronutrient fertilization of woody seedlings essential regardless of pine bark pH. *J. Environ. Hort.* 17:69–72.
- Wright, R.D. and L.E. Hinesley. 1991. Growth of containerized Eastern redcedar amended with dolomitic limestone and micronutrients. *HortScience* 26:143–145.

- Xu, N., H. Yin, Z. Chen, S. Liu, M. Chen, and J. Zhang. 2014. Mechanisms of phosphate retention by calcite: effects of magnesium and pH. *J. Soils Sediments* 14:495–503.
- Yeager, T. H., and J.E. Barrett. 1984. Phosphorus leaching from ³²P-superphosphate-amended soilless container media. *HortScience* 19:216–217.
- Yeager, T. H., and J.E. Barrett. 1985a. Phosphorous and sulfur leaching from an incubated superphosphate-amended soilless container medium. *HortScience* 20:671–672.
- Yeager, T. H., and J.E. Barrett. 1985b. Influence of incubation time on phosphorous leaching from a container medium. *J. Environ. Hort.* 3:186–187.
- Yeager, T.H., and R.D. Wright. 1982. Pine bark-phosphorus relationships. *Commun. Soil Sci. Plant Anal.* 13:57–66.
- Yuan, X., W. Xia, J. An, W. Yang, and J. Yin. 2014. Removal of phosphate anions from aqueous solutions using dolomite as adsorbent. *Adv. Mater. Res.* 864–867:1454–1457.
- Yuan, X., X. Wentag, J. An, J. Yin, X. Zhou, and W. Yang. 2015. Kinetic and thermodynamic studies on the phosphate adsorption removal by dolomite mineral. *J. Chem.* 1–8.
- Zhang, M., R. Wright, D. Heaney, and D. Vanderwel. 2004. Comparison of different phosphorus extraction and determination methods used manured soils. *Can. J. Soil Sci.* 84: 469–475.

Table 1. Degrees of freedom (df), F-values and P-values for analysis of variance (ANOVA) to determine significant effects of substrate treatment, time (1 to 48 DAI) and the substrate \times time interaction on calcium (Ca), magnesium (Mg), dissolved organic carbon (DOC), pH and electrical conductivity (EC).

ANOVA source	df	F-value	P-value
Ca			
Substrate	3	165.9	<0.0001
Time	7	265.5	<0.0001
Substrate \times Time	21	17.8	<0.0001
Mg			
Substrate	3	398.1	<0.0001
Time	7	236.8	<0.0001
Substrate \times Time	21	22.5	<0.0001
DOC			
Substrate	3	54.6	<0.0001
Time	7	539.4	<0.0001
Substrate \times Time	21	2.3	0.0043
pH			
Substrate	3	4715.9	<0.0001
Time	7	1121.3	<0.0001
Substrate \times Time	21	19.2	<0.0001
EC			
Substrate	3	47.0	<0.0001
Time	7	404.8	<0.0001
Substrate \times Time	21	23.2	<0.0001

Table 2. Degrees of freedom (df), F-values and P-values for three-way analysis of variance

(ANOVA) to determine significant effects of substrate treatment, fraction, time and their interactions on phosphorus (P) and iron (Fe) concentrations in leachate of irrigated pine bark.

Two analyses were performed for P, one included total P fractions (particulate P [PP] and total dissolved P [TDP]), and the other included dissolved P fractions (orthophosphate-P [OP-P] and non-orthophosphate dissolved P [NODP]). Fractions in Fe analysis included particulate Fe (P-Fe) and dissolved Fe (D-Fe).

ANOVA source	df	Total P fractions		Dissolved P fractions		Total Fe fractions	
		F-value	P-value	F-value	P-value	F-value	P-value
3-way ANOVA							
Time (T)	7	273.4	<0.0001	309.8	<0.0001	735.5	<0.0001
Substrate (Sub) [†]	3	9.9	<0.0001	28.9	<0.0001	93.3	<0.0001
Fraction (Frac)	1	355.2	<0.0001	34.9	<0.0001	1032.4	<0.0001
T × Sub	21	5.8	<0.0001	6.5	<0.0001	13.0	<0.0001
T × Frac	7	45.5	<0.0001	45.8	<0.0001	74.4	<0.0001
Sub × Frac	3	11.6	<0.0001	14.2	<0.0001	26.1	<0.0001
T × Sub × Frac	21	3.0	0.0002	4.3	<0.0001	16.6	<0.0001
Simple interactions[‡]							
T × Sub (in each frac)							
PP, NODP, P-Fe	21	4.8	<0.0001	1.2	0.3186	13.0	<0.0001
TDP, OP-P, D-Fe	21	4.3	<0.0001	10.7	<0.0001	11.8	<0.0001
T × Frac (in each Sub)							
F	7	34.8	<0.0001	10.1	0.0026	58.9	<0.0001
FL	7	14.0	<0.0001	3.8	0.0480	46.7	<0.0001
FM	7	3.3	0.0370	10.9	0.0032	12.6	<0.0001
FLM	7	18.0	<0.0001	60.9	<0.0001	36.8	<0.0001
Sub × Frac (at each T)							
1	3	6.5	0.0025	27.5	<0.0001	120.4	<0.0001
5	3	5.7	0.0042	18.6	<0.0001	37.3	<0.0001
9	3	2.1	0.1336	3.5	0.0296	5.0	0.0081
15	3	2.5	0.0812	4.4	0.0130	1.2	0.3340
23	3	3.0	0.0496	0.2	0.8897	2.0	0.1481
31	3	15.3	<0.0001	4.0	0.0193	2.8	0.0596
41	3	8.7	0.0004	3.3	0.0369	1.1	0.3795

48

3

8.6

0.0005

2.6

0.0740

1.0

0.4272

†Four substrate amendment treatments each contained $4.75 \text{ kg} \cdot \text{m}^{-3}$ controlled-release fertilizer (CRF; 19N–2.6P–10K) and either $4.15 \text{ kg} \cdot \text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg} \cdot \text{m}^{-3}$ micronutrient fertilizer (FM), both amendments (FLM) or no amendments (F).

‡Since 3-way interactions were significant, 2-way interactions were analyzed at each level of the third factor (i.e., simple interactions).

Table 3. The effect of substrate amendments (n = 4) on iron (Fe) concentrations ($\text{mg}\cdot\text{L}^{-1}$) and partitioning of Fe in the particulate (P-Fe; i.e., total Fe – dissolved Fe) and dissolved (D-Fe; < 0.45 μm) fractions over time in leachate of daily-irrigated pine bark columns containing 4.75 $\text{kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of 4.15 $\text{kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), 0.89 $\text{kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or pine bark with no fertilizer or amendments (control).

Time (days)	Substrate	D-Fe	P-Fe	P-value†
1	control	1.30	22.84	<0.0001
	F	1.10 b‡	23.64 a	<0.0001
	FL	0.46 c*	20.96 a	<0.0001
	FM	8.30 a*	7.00 b*	0.3070
	FLM	0.47 c*	9.33 b*	<0.0001
	P-value	<0.0001	<0.0001	
5	control	0.85	7.67	<0.0001
	F	0.79 b	8.00 a	<0.0001
	FL	0.23 c*	3.70 b*	<0.0001
	FM	3.15 a*	4.00 b*	0.3533
	FLM	0.20 c*	2.53 b*	<0.0001
	P-value	<0.0001	0.0006	
9	control	0.56	4.33	0.0002
	F	0.38 b	4.11 b	<0.0001
	FL	0.19 b*	2.56 c*	<0.0001
	FM	1.30 a*	6.73 a*	<0.0001
	FLM	0.20 b*	3.17 bc	<0.0001
	P-value	<0.0001	<0.0001	
15	control	0.27	1.06	0.0033
	F	0.19 b	0.87 bc	<0.0001
	FL	0.07 c*	0.50 c*	0.0014
	FM	0.63 a*	2.29 a*	0.0002
	FLM	0.13 bc*	1.07 b	0.0002
	P-value	<0.0001	0.0002	
23	control	0.22	0.71	0.0107
	F	0.05 b*	0.16 b*	0.0185
	FL	0.04 b*	0.16 b*	0.1416

	FM	0.23 a	0.92 a	0.0009
	FLM	0.08 b*	0.35 b	0.0004
	P-value	<0.0001	0.0005	
31	control	0.22	0.52	0.0068
	F	0.04 b*	0.08 b*	0.0049
	FL	0.06 b*	0.10 b*	0.0009
	FM	0.10 a*	0.22 a*	0.0164
	FLM	0.10 a*	0.30 a*	0.0074
	P-value	0.0005	0.0005	
41	control	0.12	0.43	0.0007
	F	0.02 b*	0.08 *	0.0022
	FL	0.03 ab*	0.06 *	0.0024
	FM	0.04 a*	0.10 *	0.0934
	FLM	0.05 a*	0.14 *	0.0210
	P-value	0.0059	0.1016	
48	control	0.12	0.27	0.0091
	F	0.01 b*	0.04 *	0.0088
	FL	0.01 b*	0.06 *	0.1392
	FM	0.04 a*	0.07 *	0.0454
	FLM	0.02 b*	0.08 *	0.0161
	P-value	0.0047	0.5428	

* Significantly different from control (S₀) according to Dunnett's test at the 0.05 probability level.

† P-values < 0.05 indicate P-Fe and D-Fe concentrations are significantly different

‡ Within columns for each sampling date, means followed by the same letter are not significantly different according to Tukey's HSD (0.05).

Table 4. The effect of substrate amendments (n = 4) on phosphorus (P) concentrations ($\text{mg}\cdot\text{L}^{-1}$ P) and distribution of P concentration across fractions over time in leachate of daily-irrigated pine bark columns containing $4.75\text{ kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15\text{ kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), $0.89\text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or pine bark with no fertilizer or amendments (control). Total P (TP) was fractioned into total dissolved P (TDP; $< 0.45\ \mu\text{m}$) and particulate P (PP; $\text{TP} - \text{TDP}$), and TDP was further divided into orthophosphate-P (OP-P) and non-orthophosphate dissolved P (NODP; $\text{TDP} - \text{OP-P}$) for separate statistical analysis.

Time (days)	Substrate	PP	TDP	P-value†	NODP	OP-P	P-value‡
1	control	6.34	8.60	0.0235	4.08	4.52	0.3479
	F	6.67 a§	8.86 a	0.0097	4.28	4.58 a	0.3554
	FL	5.94 ab	6.57 b*	0.2384	3.54	3.03 b*	0.1264
	FM	3.07 c*	6.24 b*	0.0072	4.29	1.96 c*	0.0053
	FLM	4.04 bc*	4.48 c*	0.2400	3.45	1.03 d*	<0.0001
	P-value	0.0003	<0.0001		0.0911	<0.0001	
5	control	0.97	7.53	<0.0001	3.57	3.97	0.0486
	F	1.28 a	8.06 a	<0.0001	3.65 a	4.42 a	0.0810
	FL	0.56 b	5.46 b*	<0.0001	2.44 b*	3.02 b*	0.0662
	FM	1.04 ab	4.80 b*	0.0003	2.95 ab	1.85 c*	0.0240
	FLM	0.45 b	3.30 c*	<0.0001	2.38 b*	0.92 d*	<0.0001
	P-value	0.0125	<0.0001		0.0131	<0.0001	
9	control	1.21	5.15	<0.0001	1.68	3.47	0.0002
	F	1.28	4.20 a	0.0004	1.31	2.89 a	0.0043
	FL	1.15	3.33 a*	0.0023	1.10 *	2.23 a*	0.0156
	FM	1.37	3.24 a*	0.0021	1.30	1.94 ab*	0.1034
	FLM	0.82	1.98 b*	0.0025	0.90 *	1.08 b*	0.0548
	P-value	0.0895	0.0026		0.1631	0.0028	
15	control	0.25	2.17	0.0008	0.86	1.31	0.5276
	F	0.53	1.15 *	0.0002	0.32	0.84	0.2086
	FL	0.58	1.06 *	0.1272	0.63	0.43	0.4733
	FM	0.43	1.64	0.0014	0.37	1.27	0.1596
	FLM	0.65	0.91 *	0.2607	0.84	0.07	<0.0001

	P-value	0.7876	0.0513		0.2437	0.0750	
23	control	0.26	1.20	<0.0001	0.90	0.30	0.1024
	F	0.09 b	0.60	0.0017	0.20	0.40	0.1732
	FL	0.20 ab	0.69	0.1923	0.37	0.32	0.9330
	FM	0.18 ab	1.11	0.0011	0.45	0.66	0.5723
	FLM	0.39 a	0.50	0.2906	0.21	0.29	0.5760
	P-value	0.0279	0.1952		0.8770	0.4832	
31	control	0.27	0.56	0.0123	0.26	0.30	0.7567
	F	0.06 b*	0.99 ab	0.0008	0.17 ab	0.82 a*	0.0086
	FL	0.22 a	0.27 c	0.7162	0.07 b	0.20 b	0.1191
	FM	0.07 b*	1.25 a*	0.0002	0.23 a	1.02 a*	0.0036
	FLM	0.28 a	0.53 bc	0.0923	0.10 ab	0.43 ab	0.0117
	P-value	0.0025	0.0018		0.0453	0.0036	
41	control	0.20	0.30	0.0740	0.09	0.21	0.0291
	F	0.08 *	1.77 a*	<0.0001	0.27 *	1.50 a*	0.0002
	FL	0.13	0.81 bc*	0.0003	0.12	0.69 c*	0.0016
	FM	0.10	1.57 ab*	<0.0001	0.22	1.35 ab*	0.0003
	FLM	0.19	0.83 c*	0.0089	0.13	0.70 bc*	0.0123
	P-value	0.1781	0.0046		0.0910	0.0050	
48	control	0.34	0.23	0.8684	0.08	0.15	0.0921
	F	0.16 bc	2.47 a*	<0.0001	0.36 *	2.11 a*	<0.0001
	FL	0.36 ab	1.20 b*	0.2441	0.14	1.06 b*	0.0011
	FM	0.09 c	1.98 ab*	0.0003	0.32	1.66 ab*	0.0032
	FLM	0.40 a	1.17 b*	0.0133	0.23	0.94 b*	0.0101
	P-value	0.0022	0.0138		0.2534	0.0069	

* Significantly different from control (S₀) according to Dunnett's test at the 0.05 probability

level.

† P-values < 0.05 indicate TDP and PP concentrations are significantly different

‡ P-values < 0.05 indicate OP-P and NODP concentrations are significantly different

§ Within columns for each sampling date, means followed by the same letter are not significantly different according to Tukey's HSD (0.05).

Table 5. Saturation indices calculated by Visual MINTEQ for phosphorus species saturated with respect to the solid phase in leachate at 1, 5, 40 and 48 days after experiment initiation (DAI) of irrigated pine bark columns containing no amendments (control) or 4.75 kg·m⁻³ of 19N–2.6P–10K controlled-release fertilizer (F) with 4.15 kg·m⁻³ dolomitic limestone (FL), 0.89 kg·m⁻³ micronutrient fertilizer (FM) or the combination of dolomitic limestone and micronutrient fertilizer (FLM).

Substrate	Species	Time (DAI)			
		1	5	41	48
control	MnHPO ₄	1.32	0.90	-1.09	-0.87
F	MnHPO ₄	1.32	0.98	2.21	2.41
FL	Ca ₅ (PO ₄) ₃ (OH)	2.01	4.09	7.00	7.70
	MnHPO ₄	2.95	2.97	2.11	2.09
	Ca ₃ (PO ₄) ₂ (beta)	-2.43	-1.37	-0.12	0.30
FM	MnHPO ₄	1.94	1.65	2.22	2.61
FLM	Ca ₅ (PO ₄) ₃ (OH)	0.23	3.51	6.59	7.26
	MnHPO ₄	3.42	3.53	2.53	2.50
	Ca ₃ (PO ₄) ₂ (beta)	-3.44	-1.70	-0.33	0.10

Figure captions

Fig. 1. The effect of substrate amendments on calcium (Ca) concentrations in leachate over time of daily-irrigated pine bark columns containing 4.75 kg·m⁻³ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of 4.15 kg·m⁻³ dolomitic limestone (FL), 0.89 kg·m⁻³ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no controlled-release fertilizer or amendments (control). Different vertically aligned letters next to means indicate significant difference among substrate treatments (F, FL, FM and FLM) within each sampling date via Tukey's HSD (P < 0.0001). Asterisks (*) indicate significant difference from control using Dunnett's test (P < 0.05). Vertical bars represent SE of the mean.

Fig. 2. The effect of substrate amendments on pH in leachate over time of daily-irrigated pine bark columns containing $4.75 \text{ kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15 \text{ kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no controlled-release fertilizer or amendments (control). Vertical bars represent SE of the mean.

Fig. 3. The effect of substrate amendments on magnesium (Mg) concentrations in leachate over time of daily-irrigated pine bark columns containing $4.75 \text{ kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15 \text{ kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no fertilizer or amendments (control). Different vertically aligned letters next to means indicate significant difference among substrate treatments (F, FL, FM and FLM) within each sampling date via Tukey's HSD ($P < 0.0001$). Asterisks (*) indicate significant difference from control within each sampling date using Dunnett's test ($P < 0.05$). Vertical bars represent SE of the mean.

Fig. 4. The effect of substrate amendments on electrical conductivity (EC) and concomitant ionic strength (IS) in leachate over time of daily-irrigated pine bark columns containing $4.75 \text{ kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15 \text{ kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no F or amendments (S; control). Ionic strength was calculated as $(\text{EC} \times 0.012) - 0.0002$ based on Alva et al. (1991). Vertical bars represent SE of the mean.

Fig. 5. The effect of substrate amendments on dissolved organic carbon (DOC) concentrations in leachate over time of daily-irrigated pine bark columns containing $4.75 \text{ kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15 \text{ kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no controlled-release fertilizer or amendments (control). Different vertically aligned letters next to means indicate significant difference among substrate treatments (F, FL, FM and FLM) within each sampling date via Tukey's HSD ($P < 0.0001$). Asterisks (*) indicate significant difference from control within each sampling date using Dunnett's test ($P < 0.05$). Vertical bars represent SE of the mean.

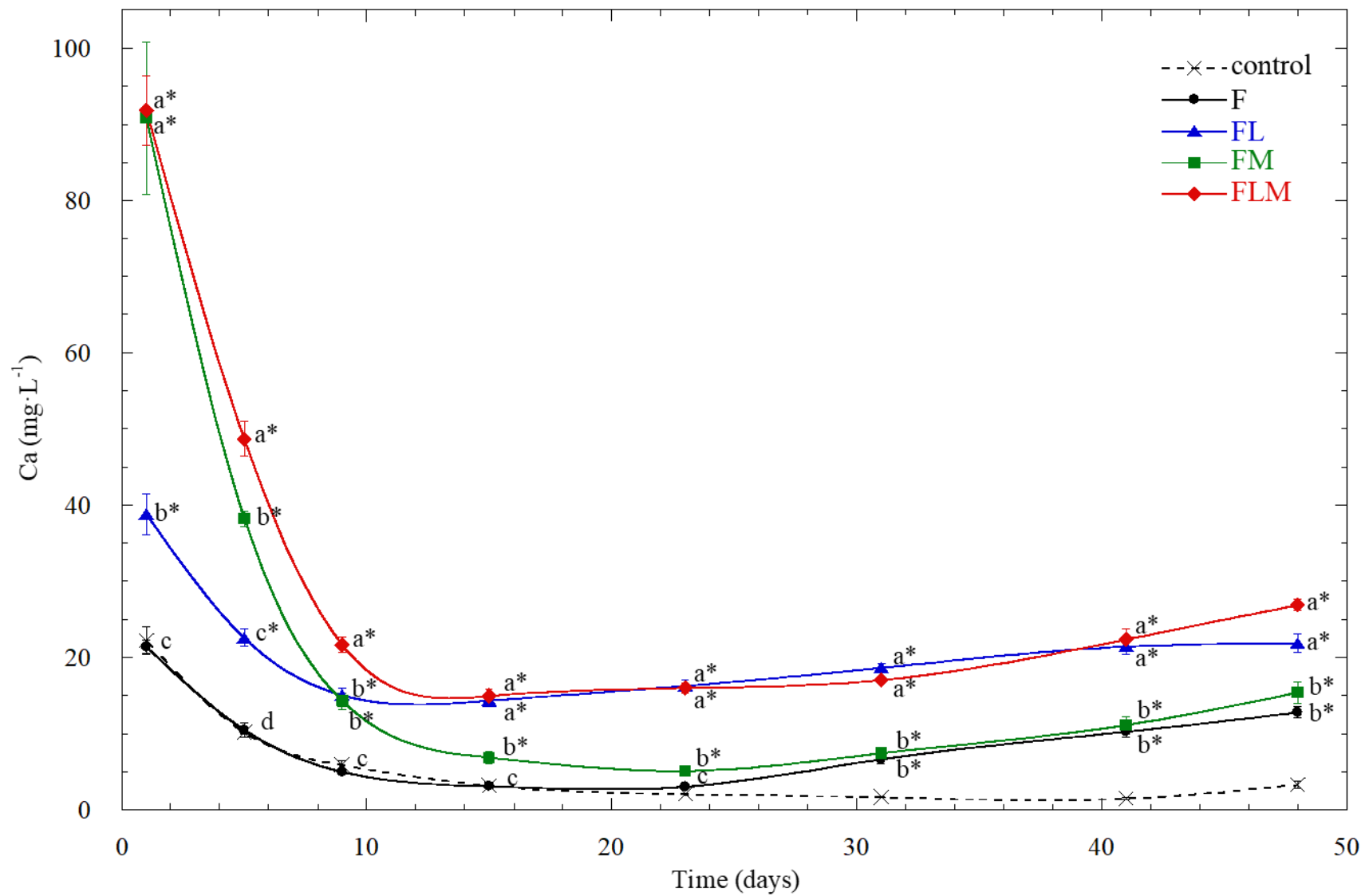


Fig. 1. The effect of substrate amendments on calcium (Ca) concentrations in leachate over time of daily-irrigated pine bark columns containing $4.75 \text{ kg} \cdot \text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15 \text{ kg} \cdot \text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg} \cdot \text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no controlled-release fertilizer or amendments (control). Different vertically aligned letters next to means indicate significant difference among substrate treatments (F, FL, FM and FLM) within each sampling date via Tukey's HSD ($P < 0.0001$). Asterisks (*) indicate significant difference from control using Dunnett's test ($P < 0.05$). Vertical bars represent SE of the mean.

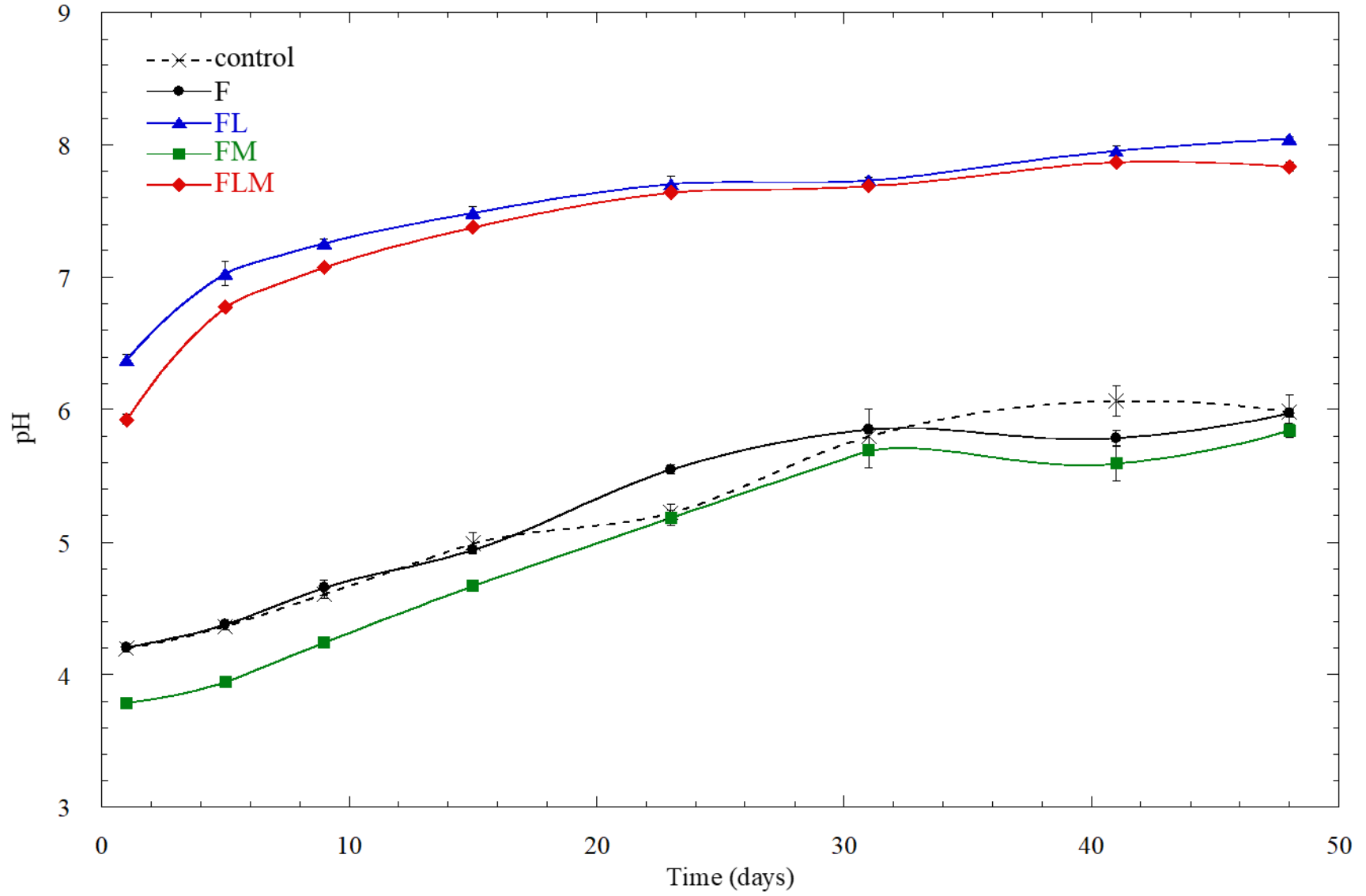


Fig. 2. The effect of substrate amendments on pH in leachate over time of daily-irrigated pine bark columns containing $4.75 \text{ kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15 \text{ kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no controlled-release fertilizer or amendments (control). Vertical bars represent SE of the mean.

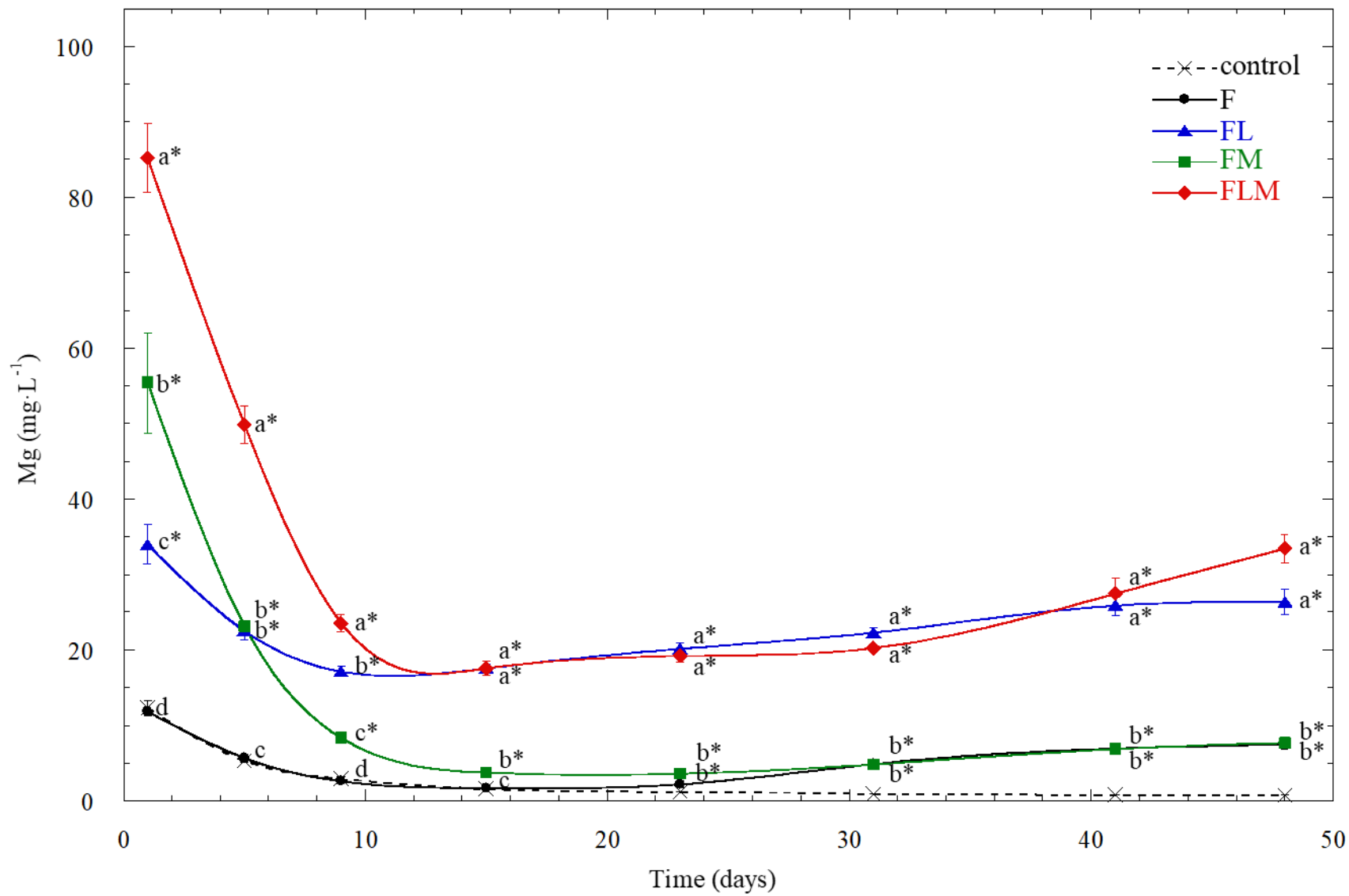


Fig. 3. The effect of substrate amendments on magnesium (Mg) concentrations in leachate over time of daily-irrigated pine bark columns containing $4.75 \text{ kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15 \text{ kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no fertilizer or amendments (control). Different vertically aligned letters next to means indicate significant difference among substrate treatments (F, FL, FM and FLM) within each sampling date via Tukey's HSD ($P < 0.0001$). Asterisks (*) indicate significant difference from control within each sampling date using Dunnett's test ($P < 0.05$). Vertical bars represent SE of the mean.

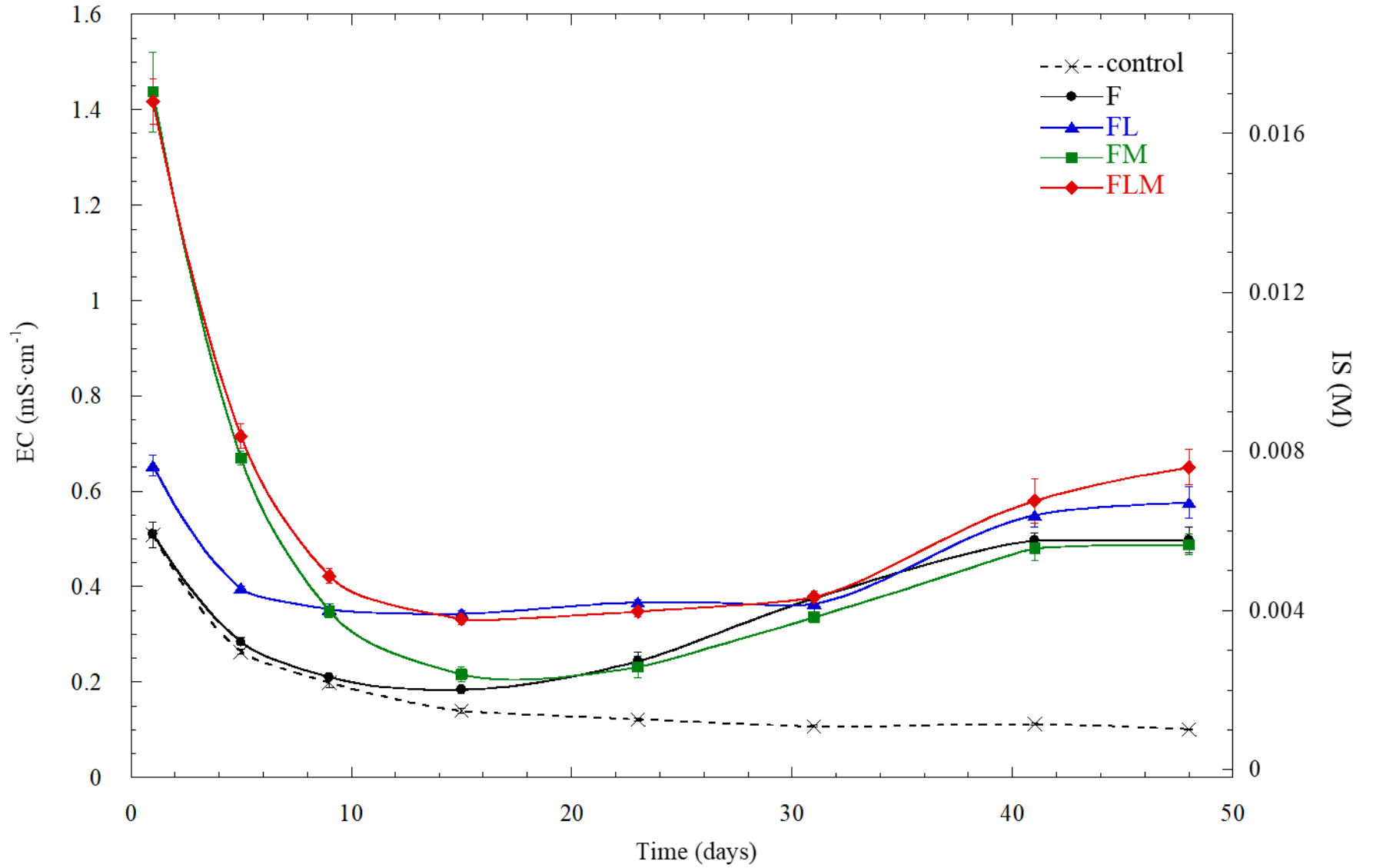


Fig. 4. The effect of substrate amendments on electrical conductivity (EC) and concomitant ionic strength (IS) in leachate over time of daily-irrigated pine bark columns containing $4.75 \text{ kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15 \text{ kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no F or amendments (S; control). Ionic strength was calculated as $(\text{EC} \times 0.012) - 0.0002$ based on Alva et al. (1991). Vertical bars represent SE of the mean.

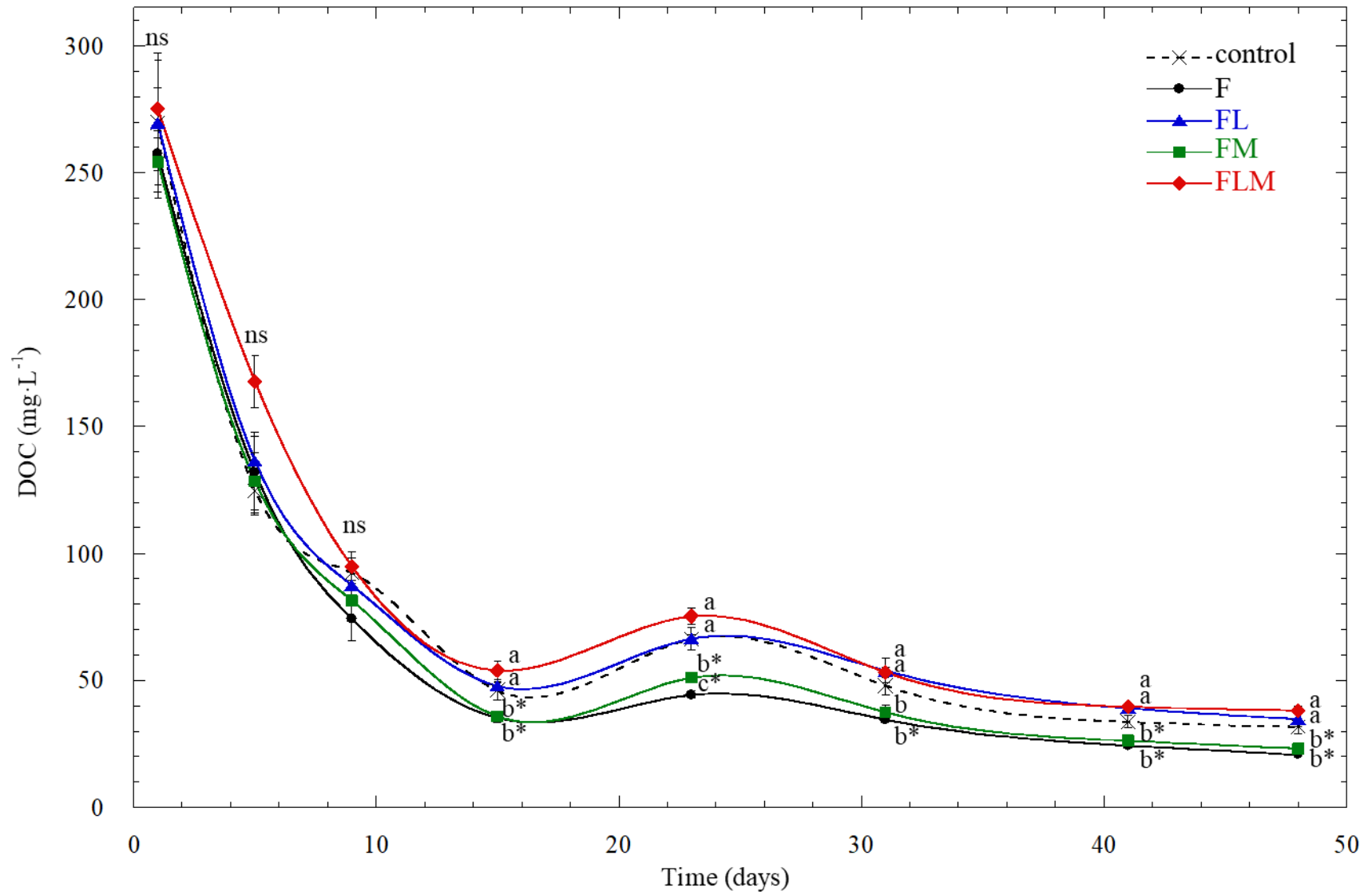


Fig. 5. The effect of substrate amendments on dissolved organic carbon (DOC) concentrations in leachate over time of daily-irrigated pine bark columns containing $4.75 \text{ kg}\cdot\text{m}^{-3}$ controlled-release fertilizer (F; 19N–2.6P–10K). Substrate treatments consisted of $4.15 \text{ kg}\cdot\text{m}^{-3}$ dolomitic limestone (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM), the combination of dolomitic limestone and micronutrient fertilizer (FLM) or substrate with no controlled-release fertilizer or amendments (control). Different vertically aligned letters next to means indicate significant difference among substrate treatments (F, FL, FM and FLM) within each sampling date via Tukey's HSD ($P < 0.0001$). Asterisks (*) indicate significant difference from control within each sampling date using Dunnett's test ($P < 0.05$). Vertical bars represent SE of the mean.

Chapter 5

Dolomite and Micronutrient Fertilizer Effect on Phosphorus Fate in Pine

Bark II: Greenhouse Study

Abstract

Dolomitic limestone (DL) and micronutrient fertilizers (MFs) are routinely added to pine bark substrates to improve fertility, yet the effect of these amendments on P in pore-water of pine bark substrate is not well understood. In part-one of this two-parted series, we showed that amending pine bark with both DL and MF reduced orthophosphate-P (OP-P) concentrations in leachate of fallow columns by 70%, on average. The objective of the current research was to determine the effect of DL and MF amendments on total P (TP), total dissolved P (TDP) and OP-P concentrations in pour-through extracts and their relative influence on subsequent P uptake efficiency (PUE) of *Lagerstroemia* 'Natchez' (crape myrtle). Crape myrtle were grown in a pine bark substrate amended with $2.97 \text{ kg}\cdot\text{m}^{-3}$ of a polymer-coated 19N–2.6P–10.8K controlled-release fertilizer (CRF) for 91 days in a greenhouse. Substrate amendment treatments included the following: no DL or MF (F), $2.97 \text{ kg}\cdot\text{m}^{-3}$ dolomite (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ MF (FM) or both DL and MF (FLM). Pour-through extracts were collected approximately weekly and fractioned to measure TP, TDP and OP-P. At experiment termination plants were harvested to determine total dry weight and total P content in tissue. Amending pine bark with a combination of DL and MF reduced water-extractable OP-P and TP concentrations by, on average, 64% and 58%, respectively. Depending on the substrate treatment, OP-P contributed, on average, 70 to 83 % of TP concentrations, whereas TDP was not different from TP. The F and particularly the FL substrates consistently had highest OP-P concentrations in pour-through extracts among substrate treatments. Relatively high OP-P concentrations in FL were attributed to poor health and lower biomass production of plants grown in this substrate. Total dry weight values of plants grown in FLM or FM were 40% higher than those grown in F; however, tissue P amounts and relative

PUE were the same among plants in these three treatments. Therefore, sorption of OP-P by DL and MF did not limit P uptake by plants.

Introduction

Nutrient enrichment and subsequent eutrophication of receiving waters has profound effects on aquatic resources. Proliferation of primary producers, including toxic algae and cyanobacteria species, induced by elevated nutrient levels in aquatic ecosystems has resulted in species-biodiversity loss, contamination of drinking water and widespread fish kills (Carpenter et al., 1998). For eutrophication to ensue, critical concentrations of both nitrogen (N) and phosphorus (P) must be present; however, P is generally regarded as the limiting nutrient for accelerated growth of photosynthesizing organisms (e.g., phytoplankton, algae, cyanobacteria, plants) in fresh water ecosystems (Correll, 1998; Khan and Mohammad, 2014; Schindler et al., 2008). Boesch et al. (2001) and Michalak et al. (2015) have concluded the P runoff from agricultural operations is a primary contributor to eutrophication in the US.

Substrates used in containerized nursery crop production are predominantly comprised of pine bark (*Pinus taeda*) in Southeastern US (Bilderback et al., 2013; Lu et al., 2006). Pine bark-based substrates have little ability to sorb fertilizer-P, enabling P to readily leach from containers during irrigation (Marconi and Nelson, 1984; Paradelo et al., 2017; Yeager and Wright, 1982). The use of polymer- or resin-coated controlled-release fertilizers (CRFs), a best management practice (Bilderback et al., 2013) that reduces P leaching relative to soluble fertilizers (Broschat, 1995; Diara, 2014), has been widely adopted by the US nursery industry according to survey studies (Dennis et al., 2010; Fain et al., 2000; Mack et al., 2017). However, P uptake efficiency (PUE, percent of applied P taken up by plant roots) of container-grown nursery crops has been reported to remain between 7% and 62% (McGinnis et al., 2009; Owen et al., 2008; Tyler et al., 1996; Warren et al., 1995; Warren et al. 2001) when using CRFs. Phosphorus uptake efficiency in containerized crop production is affected by both cultural practices and substrate amendments.

Studies by Lea-Cox and Ristvey (2003) and Ristvey et al. (2004; 2007) found that decreasing P fertilization amount increased PUE of *Rhododendron* ‘Karen’. Warren et al. (1995) determined that the fertilizer-P source affected PUE when producing *Rhododendron* ‘Sunglow’, with resin-coated P resulting in higher PUE than sulfur-coated P or composted turkey litter. McGinnis et al. (2009) observed higher PUE of *Hibiscus moscheutos* ‘Luna Blush’ when supplying P via vermicompost compared to CRF. When growing containerized *Cotoneaster dammeri* ‘Skogholm’, Owen et al. (2008) observed improved PUE in plants that received a 50% lower CRF-P application rate or when grown in pine bark substrate amended with 11% calcined palygorskite-bentonite clay (by volume). Other studies have demonstrated that various clay products reduce P leaching from containers when mixed into a pine bark substrate (Owen et al., 2007; Ogutu et al., 2009; Ruter, 2004). Adoption of P-sorbing clay products by the US nursery industry has been slow (Bilderback, personal communication), which may be partially attributed to their relatively high cost of shipping and purchase (Ogutu et al., 2009) as well as the fact that growers are unaccustomed to these unconventional amendments.

In contrast to calcined clay amendments, dolomitic limestone (DL) and micronutrient fertilizer (MF) are routinely mixed into container substrates prior to potting. Dolomitic limestone is used to increase substrate pH and supply plants with Ca and Mg. Phosphorus sorption by DL has been well-established in studies examining its use as a P adsorbent for waste-water treatment (Karaca et al., 2004; Karaca et al., 2006; Mangwandi et al., 2014; Xu et al., 2014; Yuan et al., 2014; Yuan et al., 2015). Additionally, the ability of DL to sorb P in peat- or pine bark-based substrates has been reported in containerized crop production research (Argo and Biernbaum, 1996a and 1996b; Havis and Baker, 1985; Haynes, 1982; Shreckhise et al., 2018).

Micronutrient fertilizers provide B, Cl, Cu, Fe, Mn, Mo, Ni and Zn in sulfated or chelated form, and some MFs supply plants with additional Ca and Mg. Shreckhise et al. (2018) found that a sulfate-based MF reduced OP-P concentrations in leachate by > 50% from fallow columns of CRF-fertilized pine bark substrate during the first and fifth 2.6-cm irrigation events. The authors also found that on day 48 of daily irrigation, totaling \approx 125 cm applied tap water, OP-P concentrations in leachate were at least 50% lower in pine bark amended with both DL and MF compared to non-amended pine bark. In addition to reducing P runoff from container nursery sites, conventional DL and MF soilless substrate amendments may improve PUE of container-grown crops since P is retained in the root zone. In contrast to soils, pine bark substrates have no appreciable source of labile P to resupply pore-water with OP-P during root-uptake. However, the effect of DL and MF in pine bark substrate on PUE of containerized crops has not been investigated.

In containerized nursery trials attempting to recover all fertilizer P partitioned in the effluent, plant, substrate and non-dissolved fertilizer, P recovery has been reported to be between 16% and 57% (McGinnis et al., 2009; Owen et al., 2008; Ristvey et al., 2004; Tyler et al., 1996a; Warren et al., 2001). In these studies, a definitive explanation for incomplete P recovery is not reported. We postulate that low P recovery was a factor of the analytical method used to measure P. Phosphorus concentrations in effluent of containerized nursery crops are commonly measured colorimetrically after filtration through a 0.45 μ m membrane as dissolved reactive P, the P fraction available for plant uptake. In all of the aforementioned P budget studies, effluent P was reported as dissolved reactive P or PO₄-P. Thus, effluent P associated with metals supplied by the dissolution of DL and MF (e.g., Ca, Mg, Mn, Fe), would not have been detected and may account for a portion of the non-recovered P. When measuring P fractions in leachate of daily-

irrigated (i.e., $\approx 2.6 \text{ cm} \cdot \text{d}^{-1}$), fallow pine bark columns, Shreckhise et al. (2018) reported that OP-P contributed between 12% and 50% of total P (TP) measured on days 1, 5, 9, 15 and 23, regardless of DL and MF amendments. Comparing relative amounts of TP, total dissolved P (TDP) and orthophosphate-P (OP-P) in pore-water of pine bark substrate containing DL and MF would build on our understanding of P fate in containerized crop production. The objective of this research was to determine the effect of DL and MF amendments on TP, TDP and OP-P in pour-through extracts and their relative influence on subsequent P use efficiency of *Lagerstroemia* ‘Natchez’ potted in pine bark with incorporated CRF.

Materials and methods

On 10 February 2017, 60, dormant *Lagerstroemia* ‘Natchez’ (crape myrtle) liners were acquired in 15-cell trays (1-L cells) from Saunders Brothers Nursery (Piney River, VA). Crape myrtle was chosen due to its popularity in the southeastern nursery industry and relatively fast growth rate that would ensure a noticeable level of nutrient-uptake. Of the 60 liners, the 20 most uniform, single-trunk plants were selected for this study and pruned to a height of 30 cm.

Pine bark (aged at least 8 mo; 15.9 mm screen) was obtained from Carolina Bark Products (Seaboard, NC) on 21 February 2017. Measured air space and container capacity (by volume) of the substrate were 22.3% and 60.7%, respectively, and bulk density was $0.16 \text{ g} \cdot \text{cm}^{-3}$ (NCSU porometer method; Fonteno et al., 1995). Initial pine bark saturated media extracts (SMEs; $n = 3$; Warncke, 1986) contained (in $\text{mg} \cdot \text{L}^{-1} \pm \text{SE}$) $< 0.31 \text{ NH}_4\text{-N}$, $< 0.12 \text{ NO}_2\text{-N}$, $0.12 \pm 0.02 \text{ NO}_3\text{-N}$, $7.67 \pm 0.46 \text{ PO}_4\text{-P}$, $25.4 \pm 1.51 \text{ K}$ and $9.5 \pm 0.90 \text{ Cl}$. Electrical conductivity (EC) and pH values in SMEs were $0.84 \pm 0.041 \text{ mS} \cdot \text{cm}^{-1}$ and 4.91 ± 0.041 , respectively. Methods used to determine ion concentrations, EC and pH are described in Shreckhise et al. (2018). Pine

bark samples were also analyzed for total mineral nutrient levels (percent dry weight) by Brookside Laboratories (compost analysis Z004; New Bremen, OH). Pine bark was 0.31% N, 0.01% P, 0.10% K, 0.23% Ca, 0.05% Mg, 0.04% S, 4.25 mg·kg⁻¹ B, 1184 mg·kg⁻¹ Fe, 79.25 mg·kg⁻¹ Mn, 6.91 mg·kg⁻¹ Cu and 27.48 mg·kg⁻¹ Zn. The carbon to N ratio of the pine bark was 189.

On 22 February 2017, pine bark was either left non-amended (F) or was amended with 2.97 kg·m⁻³ DL (FL; half ground, half pulverized), 0.89 kg·m⁻³ MF (FM; Micromax), or both DL and MF (FLM). The DL and MF products were the same as those reported by Shreckhise et al. (2018). Incorporation of DL and MF into the substrate was accomplished by mixing for 5 min using a small cement mixer (0.14 m³ capacity; ≈ 23 rotations per minute). Five 11.4-L aliquots of each of the four substrates were then amended with 28 g (i.e., 2.97 kg·m⁻³ pine bark) of a polymer-coated 19N–2.6P–10.8K CRF (Harrell's LLC, Lakeland, FL). The CRF was a homogeneous, 3- to 4-month release formulation (at 27 °C) in which N, P and K were derived from NH₄NO₃, NH₄H₂PO₄ and K₂SO₄. To ensure equal distribution of CRF throughout each aliquot of pine bark without damaging granules, the 20 substrate aliquots were each hand-mixed for 2 min. The entirety of each aliquot of CRF-amended pine bark was then added to 20 respective 11.4-L black plastic containers (C1200, Nursery Supplies, Chambersburg, PA) to pot up one crape myrtle liner per container. Existing substrate was left in the liner root-balls to minimize transplant stress. Plants were then hand-watered until leaching.

Plants were grown for 93 days on four 353 × 97 × 76 cm (l × w × h) benches (five plants per bench) that each ran south to north in an environmentally controlled glass greenhouse (80% photosynthetically active radiation transmission) that ran east to west. The experiment was a randomized complete block design (RCBD) with four substrate treatments and five blocks. A

RCBD was utilized to account for possible temperature and shading variation within the greenhouse caused by the cooling pads. Blocks were arranged across benches south to north. Plants on the same benchtop were spaced ≈ 37 cm and benches were ≈ 46 cm apart. Average daily maximum and minimum air temperatures, measured using a digital temperature and humidity sensor (AcuRite 00619HD, Chaney Instrument Co., Lake Geneva, WI), were 25.4 ± 0.46 SE °C and 14.2 ± 0.39 SE °C, respectively. To monitor substrate temperature, a digital meat thermometer (AcuRite 00641W, Chaney Instrument Co.) with a 12.3-cm probe was inserted horizontally through the container wall, ≈ 11 cm above the container bottom, of a randomly selected plant in each of blocks 1, 3 and 5. Thermometers remained in the same three containers for the entirety of the study with the tip of each probe approximately in the center of the horizontal substrate profile. Average substrate temperature, recorded prior to irrigating, over the course of the study was 21.0 ± 0.5 SE °C.

Plants were irrigated with tap water every 4 to 7 d until 63 days after experiment initiation (DAI), then every 2 to 3 d for the remainder of the study based on need. During each irrigation event, water was delivered to plants in two identical cycles, with ≈ 3 min between cycles, using pressure-compensating (PC) spray stakes [01PSDS-PL1-B; Netafim, Fresno, CA ($202 \text{ mL} \cdot \text{min}^{-1}$)] controlled with GEVA 75 irrigation window controllers, each with a latch solenoid and hydraulic valve (G75-C-1W-61, Baccara Automation Control, Bayswater, Victoria, AU). An additional PC spray stake of the same flow rate was added to all containers at 20 DAI to improve moisture distribution uniformity of the substrate. The cycle duration was adjusted throughout the study to maintain a target leaching fraction (volume leached/volume applied) of 0.20. After 47 DAI, due to treatment-specific water requirements, irrigation cycle duration was adjusted on a treatment basis to sustain the target leaching fraction of 0.2. Weekly measured

leaching fraction ($n = 264$) was 0.20 ± 0.009 SE over the course of the study. Irrigation source water samples were collected at ten intervals over the course of the study and analyzed for pH, EC and dissolved (i.e., $< 0.45 \mu\text{m}$) Ca, Mg, Fe and P concentrations. Element concentrations in irrigation water were stable over time, with mean ($n = 10$) values of 13.0 ± 0.38 SE $\text{mg}\cdot\text{L}^{-1}$ Ca, 5.4 ± 0.17 SE $\text{mg}\cdot\text{L}^{-1}$ Mg, 0.1 ± 0.02 SE $\text{mg}\cdot\text{L}^{-1}$ Fe and 0.4 ± 0.01 SE $\text{mg}\cdot\text{L}^{-1}$ P. Mean pH and EC were 7.1 ± 0.04 SE and 0.15 ± 0.002 SE $\text{mS}\cdot\text{cm}^{-1}$, respectively.

Plants were monitored weekly for signs and symptoms of pests, diseases and nutrient deficiency. On day 8, all plants received a ≈ 600 mL drench of cyantraniliprole (Mainspring GNL, $0.793 \text{ mL}\cdot\text{L}^{-1}$) to prevent infestation of common greenhouse pests (e.g., thrips, whitefly, scale). Fungus gnat larvae were controlled at 48 DAI by releasing 75,000 predatory mites (*Hypoaspis miles*; Biobest Sustainable Crop Management, Westerlo, Belgium), evenly divided among the 20 plants, on the substrate surface.

On days 14, 35 and every 7 days thereafter through 91 DAI, substrate pore-water was extracted from each plant via the pour-through method (Wright, 1986). Pour-through extracts were attained by hand-pouring 300 mL deionized water evenly over the substrate surface 1 h following irrigation and collecting the ≈ 110 mL subsequent leachate for analyses. Irrigation prior to pour-through extraction was accomplished by pouring enough tap water through a diffuser to achieve $\approx 20\%$ leaching. The diffuser was similar to that describe in Shreckhise et al. (2018). This irrigation method was adopted prior to pore-water extraction to further improve moisture uniformity of the substrate and ensure a consistent leaching fraction across treatments and reps by individually adjusting irrigation volume when necessary.

An aliquot of each pore-water sample was analyzed for pH and EC within 4 h of pour-through extraction. The remainder of each sample was divided, prepared and analyzed for ion,

filterable elements, dissolved organic carbon (DOC) and total (non-filtered) element concentrations in the same manner as described by Shreckhise et al. (2018), with the following exceptions. Pore-water samples collected on a given date were analyzed individually (i.e., samples were not combined to form composite samples). Samples collected at 14 DAI were stored at 8 °C for 14 d before filtering to allow settling of suspended particles to reduce clogging of filters. A portion of each filtered sample for ion analysis was diluted 50% before being frozen and later analyzed on the ion chromatography system to ensure NO_3^- and K^+ concentrations were within detection limits.

On day 90, plant shoots were severed level with the substrate surface and triple rinsed with each of tap water and distilled water. Approximately 80% of the substrate volume was removed from the root system by hand and set aside for later collection of CRF granules to determine the proportion of initial N, P and K remaining. A tap water stream was used to remove the remaining substrate particles that could not be efficiently removed by hand. Shoots and roots were then oven-dried at 65 °C until weight remained constant. Shoots and roots were weighed, then ground separately to a 0.5 mm particle size using a 3379-K35 Variable Speed Digital ED-5 Wiley Mill (Thomas Scientific, Swedesboro, NJ) set to 900 RPM. Ground samples were sent to Brookside Laboratories for tissue-nutrient analysis (T002 test package). Tissue-element concentrations were multiplied by dry weight values to calculate P, Fe, Ca and Mg content in shoots and roots. Total tissue P content (i.e., the sum P amounts in roots and shoots) was calculated to assess relative PUE in plants among substrate treatments.

To determine the amount of N, P and K remaining in CRF granules, ≈ 2.0 g of CRF from each replication within each treatment (totaling twenty 2-g samples) were collected from the post-experiment substrate that was removed from plant root-balls. Since the CRF granules used

in this study (Polyon) do not swell and therefore maintain a consistent volume over time, post-experiment CRF was compared to fresh CRF on a volume-basis. The volume of each of the 20 post-experiment CRF samples, as well as five 2-g samples of fresh CRF, was determined by submerging granules in 5 mL DI water contained in a 10-mL graduated cylinder and measuring displaced water volume (mL). The DI water and CRF within the graduated cylinder was then poured into a 1-L volumetric flask and brought to volume with DI water. The CRF-DI water mixture was blended for 1-minute using a 12-Speed blender (Oster, 006843-000-NP1; Boca Raton, FL). An aliquot of the blended fertilizer solution was filtered using a 0.2 μm PVDF filter, diluted 90% with DI water, then analyzed for NO_3^- , NO_2^- , NH_4^+ , PO_4^{3-} and K concentrations using the ion chromatography system described by Shreckhise et al. (2018). Amount of each ion remaining in the CRF was calculated by dividing mg ion in post-experiment CRF by mg ion in fresh CRF on an equivalent CRF-volume basis. Based on this method, 4%, 0.4%, 1% and 15% of initial NH_4^+ , NO_3^- , PO_4^{3-} and K amounts, respectively, in fresh CRF remained in CRF granules at experiment termination, and no differences were observed among substrate treatments.

Theoretical chemical P speciation in leachate was modeled using Visual MINTEQ (Gustafsson, 2010). Input parameters included pH, DOC (NICA-Donnan model), PO_4^{3-} , NH_4^+ , NO_2^- , NO_3^- , B(III), Ca^{2+} , Cl^- , Cu^{2+} , Fe^{3+} , K^+ , Mg^{2+} , Mn^{2+} , Mo(VI), Na^+ , Ni^{2+} , SO_4^{2-} and Zn^{2+} concentrations. Element input values were based on concentrations measured in previously described filtered samples. Metals were assumed to be in their oxidized state. Carbonate concentrations were estimated based on measured irrigation water alkalinity as well as Ca and Mg concentrations in leachate of substrates containing DL and/or MF as an indicator of $\text{CaMg}(\text{CO}_3)_2$ dissolution. Saturation indices provided in output were used to interpret degree of saturation in solutions with respect to solid phases.

Statistical analysis

Prior to analysis, data were transformed to correct for heteroskedasticity and non-normality. The log-transformation was used for P, Ca and DOC values, and the normalized Johnson's transformation (Johnson, 1949) was used for Fe data. All data collected in pour-through extracts over time were subjected two-way repeated measures (RM) analysis of variance (ANOVA) with one between-subjects factor, substrate (F, FL, FM and FLM), and repeated measures factor, time (14, 35, 42, 48, 56, 63, 70, 77, 84 and 91 DAI). Repeated measures analysis was accomplished via covariance structure modeling (Wolfinger, 1993). The most appropriate covariance structure was selected by fitting data to various homogeneous and heterogeneous covariance structures available in JMP Pro 14 (SAS Institute Inc., Cary, NC) and subsequently comparing corrected Akaike information criterion (AIC_c) values. According to lowest AIC_c values, the heterogeneous antidependent structure was used for analyzing TP, TDP and OP-P, whereas Ca, Fe, DOC, pH and EC data were fitted to the first-order autoregressive (AR[1]) covariance structure. The random block effect was removed from analysis as it did not improve the model fit. When the substrate \times time interaction was significant, simple effects were analyzed via Tukey's Honest Significant Difference (HSD). Substrate treatment effects on dry weight and tissue nutrient content were analyzed using one-way ANOVA, and post-hoc means separation was accomplished using Tukey's HSD. Correlations were assessed using the Pearson correlation coefficients (r). All data were processed using JMP Pro 14 (SAS Institute Inc., Cary, NC), and figures were created using KaleidaGraph 4.5.3 (Synergy Software, Reading, PA).

Results and Discussion

Main effects of substrate and time and the substrate \times time interaction were significant for TP, OP-P, Ca, Fe, DOC, pH and EC (Table 1). Thus, simple effects of substrate were assessed at each level of time (i.e., sampling date).

Phosphorus

Total dissolved P concentrations were, on average, $0.69 \text{ mg}\cdot\text{L}^{-1}$ lower than TP concentrations over the course of the study, but both fractions had a similar response to substrate treatments. Since TDP and TP trend lines were nearly parallel ($r = 0.986$), only TP and OP-P concentrations are reported (Fig. 1). From 14 to 42 DAI, pore-water TP and OP-P concentrations decreased in F, FL and FLM, but stayed the same in FM. Thereafter, TP and OP-P concentrations increased until peaking between 56 and 70 DAI, then decreased for the remainder of the study. The observed curvilinear increase followed by a decrease in P concentrations between 42 and 91 DAI in all substrate treatments is similar to that described by Du. et al. (2006) who assessed P release from two polyurethane-coated CRFs in silica sand. The relatively high initial pore-water OP-P concentrations at 14 DAI (i.e., $9.5 \pm 0.72 \text{ SE mg}\cdot\text{L}^{-1} \text{ P}$) in the F and FL treatments, can be primarily attributed to indigenous P in the pine bark. Orthophosphate-P concentrations in SMEs of non-amended bark were $7.67 \pm 0.46 \text{ SE mg}\cdot\text{L}^{-1}$, which is equivalent to $\approx 13.7 \text{ mg}\cdot\text{L}^{-1} \text{ OP-P}$ when extracted via the pour-through method according to the calibration equation reported by Cavins et al. (2004). These observed indigenous OP-P concentrations in pine bark are in line with the range of 6.9 to $9.0 \text{ mg}\cdot\text{L}^{-1} \text{ P}$ reported by Ogden et al. (1987) in a review of chemical properties of pine bark substrates. Damaged CRF granules have been shown to release an immediate supply of soluble P (Huett and Morris, 1999); however, this was not likely the case in the current study since damaged CRF granules were avoided when weighing

CRF for each plant. In addition, extra caution was taken when incorporating the CRF into the pine bark to avoid marring the polymer coating. In the F, FL and FM substrates, OP-P contributed >70% of TP at all sampling dates, averaging 83%, 82% and 79% of TP, respectively, when pooled across sampling dates. Orthophosphate-P in FLM was as low as 41% and 54% of TP at 35 and 42 DAI, respectively, and averaged 70% of TP across sampling dates. The apparent lower proportion of TP as OP-P in FLM compared to the other substrates may have been partially due to formation of MnHPO_4 precipitates in FLM. Visual MINTEQ modeling indicated that MnHPO_4 in extracted pore-water was consistently saturated with respect to its solid phase over time, with highest saturation index (SI) values in FLM compared to other substrates (Table 2). Shreckhise et al. (2018) also reported positive SI values for MnHPO_4 in leachate of irrigated pine bark columns amended with DL and MF. Broschat and Donselmann (1985) observed a 90% reduction in NH_4OAc -extractable Mn from a peat-based substrate in response to superphosphate additions and speculated formation of Mn-PO_4 precipitates. Accordingly, Mn-PO_4 precipitates may have also contributed to OP-P retention during water extractions. Visual MINTEQ simulations also indicated $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ was saturated with respect to its solid phase at most sampling dates in FL and at 70, 77 and 84 DAI in FLM. The generally higher SI values for $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ in FL compared to FLM is ascribed to the combined effects of higher pH values (Fig. 2) and higher OP-P concentrations (Fig. 1) in FL (Song et al., 2002). Although thermodynamics suggest pore-water samples were saturated with respect to the solid MnHPO_4 and $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ phases, spectroscopic evidence is needed to prove their formation in pore-water of pine bark substrates.

Among substrate treatments, highest pore-water OP-P concentrations were observed in F, FL and FM from 35 to 63 DAI, and in F or FL from 70 to 84 DAI (Fig. 1). At 91 DAI, OP-P

concentrations in FL were lower than those in F. Orthophosphate-P concentrations in FM were the same as those in F except at 1 DAI. In FLM, OP-P concentrations were, on average, 64% lower than those in F at all sampling dates except 70, 77 and 84 DAI during which OP-P concentrations in F and FLM were equivalent. Since total P was generally >70% OP-P, as previously stated, TP concentrations responded to substrate treatments in a similar manner as that describe for OP-P; e.g., TP concentrations were, on average, 58% lower in FLM than in F. Thus, observed lower OP-P concentrations in FLM compared to F was primarily a result of greater P retention in the substrate, whereas leaching of P-precipitates or erosion of P-sorbed particles had minor contribution to reduced OP-P concentrations. Shreckhise et al. (2018) also concluded that DL and MF improved P retention in pine bark in fallow columns. Reduced solubility of OP-P due to lime additions has also been reported in peat-based substrates (Argo and Biernbaum, 1996a and 1996b; Havis and Baker, 1985). The primary sorption mechanism and amendment component responsible for OP-P retention in FLM could not be discerned in this study. Formation of previously mentioned MnHPO_4 and $\text{Ca}_5(\text{PO}_4)_3(\text{OH})$ precipitates may have contributed to lower OP-P mobility. In addition, sorption of OP-P to DL via Ca and Mg surface complexes and surface precipitation of Ca- PO_4 compounds may also have reduced OP-P solubility (Xu et al., 2014).

Electrical conductivity

Electrical conductivity was equated to ionic strength (IS) for convenient reference using the formula proposed by Alva et al. (1991; i.e., $\text{IS} = [\text{EC} \times 0.012] - 0.0002$) and presented in conjunction with EC in Fig. 2. Electrical conductivity values in F increased between 14 and 42 DAI, plateaued at $\approx 0.52 \text{ mS} \cdot \text{cm}^{-1}$ until 70 DAI, then decreased to values similar to those

observed at 14 DAI. Electrical conductivity values in FL were initially the same as those in F; however, EC in FL continued to increase until peaking at 70 DAI ($0.74 \text{ mS}\cdot\text{cm}^{-1}$), mimicking pore-water Ca concentrations in this substrate (Fig. 3). Presumably due to dissolution of sulfate salts, EC values in substrate containing MF were initially much higher than in those without MF. From 1 to 48 DAI, pore-water EC values in FM and FLM remained at $1.3 \pm 0.05 \text{ SE}$ and $1.0 \pm 0.03 \text{ SE mS}\cdot\text{cm}^{-1}$, respectively. Thereafter, EC values in FM and FLM generally decreased for the remainder of the study. Higher EC values of FM compared to FLM at 1 to 56 DAI was likely due to greater metal solubility in FM resulting from relatively low pH values (i.e., $\text{pH} < 4$).

pH

Substrate pore-water pH was influenced by a substrate \times time interaction (Table 1); however, despite this significant interaction, pore-water pH varied by ≤ 0.3 , 0.6 , 0.5 and 0.7 units in F, FL, FM and FLM, respectively, over the course of the study (Fig. 2). Thus, fluctuations in pore-water pH within each substrate over time likely had minor impact on the results of this study. When averaged over time, pore-water pH values in F, FL, FM and FLM were $4.3 \pm 0.02 \text{ SE}$, $6.4 \pm 0.03 \text{ SE}$, $3.7 \pm 0.02 \text{ SE}$ and $6.2 \pm 0.03 \text{ SE}$, respectively. The ≈ 0.6 unit higher pH in F compared to FM indicates that the MF acidified substrate pore-water. The acidifying effect of the MF was also present in pine bark that contained DL as indicated by the ≈ 0.2 unit lower pH in FLM compared to FL. Acidifying effects of the MF used in this study has been reported in other studies utilizing MF-amended pine bark for containerized crop production (Browder et al., 2005; Wright et al., 1999a and 1999b; Wright and Hinesley, 1991). As was postulated by Wright et al. (1999a), the decrease in pore-water pH in substrates containing sulfated MF was likely due to hydrolysis of metal-SO₄ salts supplied by the MF and subsequent release of H⁺.

Calcium

Pore-water Mg concentrations followed the same trend as pore-water Ca in all treatments, with correlation (*r*) values of 0.926, 0.975, 0.996 and 0.993 for F, FL, FM and FLM, respectively. To avoid redundancy, only Ca data are presented (Fig. 3). Calcium concentrations in F were relatively constant over time, fluctuating between maximum and minimum concentrations of 14 and 6 mg·L⁻¹, respectively. The irrigation water supplied 13.0 ± 0.4 SE mg·L⁻¹ Ca over the course of the study (n = 10) and, thus, was likely the source of Ca in the F substrate. Calcium concentrations in FM were generally equivalent to those in FLM. In both FM and FLM, Ca concentrations decreased from 98 mg·L⁻¹ at 14 DAI to 16 mg·L⁻¹ at 77 DAI, then increased for the remainder of the study. In contrast, Ca concentrations in FL increased from 15 mg·L⁻¹ at 14 DAI to a maximum concentration of 32 mg·L⁻¹ at 70 DAI then decreased to 20 mg·L⁻¹ by 91 DAI. The initially high Ca concentrations in substrates containing MF was similar to the trend observed by Shreckhise et al. (2018). In addition to micronutrients, the MF contained 6% Ca and 3% Mg (by weight) supplied by DL (labeled as insoluble). Apparent rapid dissolution of the DL component in MF may have been due to a highly acidic microenvironment surrounding MF granules due to hydrolysis of metal sulfates. Dolomite solubility increases with decreasing pH and therefore would have dissolved rapidly during acidification (Lindsay, 1979). This contention is supported by the observed acidifying effects of MF in the current study and others (Browder et al., 2005; Wright et al., 1999a and 1999b).

Iron

From 14 to 42 DAI, during which substrate treatment had no effect on pore-water Fe, pore-water Fe concentrations pooled across substrates decreased from 0.69 ± 0.15 SE to 0.11 ± 0.02 SE $\text{mg}\cdot\text{L}^{-1}$ (Fig. 3). From 42 to 91 DAI, pore-water Fe concentrations in F and FLM were the same and remained constant at 0.12 ± 0.005 SE $\text{mg}\cdot\text{L}^{-1}$, whereas Fe concentrations in FM increased to a final value of 0.33 ± 0.04 SE $\text{mg}\cdot\text{L}^{-1}$. In FL, pore-water Fe concentrations continued to decrease until 56 DAI, stabilizing at 0.06 ± 0.005 SE $\text{mg}\cdot\text{L}^{-1}$. From 56 to 91 DAI, Fe concentrations in FL were consistently lower than those in all other substrates. At all sampling dates from 63 to 91 DAI, Fe concentrations in FM were higher than those in F and were generally the same as those in FLM except at 77 and 91 DAI during which Fe concentrations were, respectively, 0.11 and 0.22 $\text{mg}\cdot\text{L}^{-1}$ higher than FM. Relatively low water-extractable levels of Fe from pine bark, particularly in limed substrates, is consistent with findings reported by Wright et al. (1999a, 1999b) and Handreck (1996). Greater degree of Fe sorption by pine bark in the presence of DL, which increases pH, can be explained by the resulting decrease in competition between H^+ and Fe for negatively charged functional groups in the pine bark. Visual MINTEQ modeling of pore-water collected from FM at 14 DAI (i.e., when Fe concentrations were highest) and 91 DAI, (i.e., when DOC concentrations were lowest [Fig. 3]) indicated aqueous Fe species were entirely associated with dissolved organic compounds at both sampling dates. Thus, Fe-DOC interactions may partially explain the absence of saturated Fe- PO_4 solid phases in Visual MINTEQ calculations. However, considering the aforementioned predicted fate of pore-water Fe, formation of humic-Fe- PO_4 ternary complexes was possible (Gerke, 2010; Gerke and Hermann, 1992).

Dissolved organic carbon

Dissolved organic carbon concentrations were highest at 14 DAI and decreased over time in all substrates (Fig. 3). The FL substrate consistently had higher DOC concentrations than all other substrates after 14 DAI. In FL, DOC concentrations decreased from 123.0 ± 5.4 SE to 42.6 ± 2.05 SE $\text{mg}\cdot\text{L}^{-1}$ over the course of the study. All other substrates had 87.0 ± 4.5 SE $\text{mg}\cdot\text{L}^{-1}$ DOC at 14 DAI and between 22 and 29 $\text{mg}\cdot\text{L}^{-1}$ DOC at 91 DAI. These findings are in line with those reported by Tiemeyer et al. (2017) who observed reduced DOC leaching from peat-sand soil columns with low pH and high EC. Similarly, Shreckhise et al. (2018) observed higher DOC concentrations in leachate of pine bark columns amended with DL or DL and MF compared to those without DL. In addition to the effects of pH and EC on DOC leaching, lower DOC concentrations in substrates containing MF may be partially ascribed to FeSO_4 serving as a coagulant for DOC in pine bark. In natural waters containing high levels of DOC, Sinsabaugh et al. (1986) determined that FeSO_4 was an effective coagulant for removal of DOC.

Plant biomass and tissue nutrient levels

Relative to F, the FM and FLM substrates equally increased total dry weight (TDW) by an average of 40%, whereas FL decreased TDW by 28% (Table 3). The effect of substrates on SDW mimicked that of TDW, with highest SDW attained in FM or FLM and lowest TDW in FL (data not shown). These data indicate that $0.89 \text{ kg}\cdot\text{m}^{-3}$ MF equally improves growth of crape myrtle in limed and non-limed pine bark substrate, whereas liming with $2.97 \text{ kg}\cdot\text{m}^{-3}$ DL limits crape myrtle growth if MF is absent. Consistent with our findings, in a review of DL effects on plant growth in pine bark substrate, Altland and Jeong (2016) concluded that a supplemental MF is generally necessary in substrates containing DL to avoid pH-induced plant micronutrient deficiency. However, the effect of MF on plant growth in non-limed pine bark substrates seems

to be taxa-specific. When growing containerized crops in a pine bark-based substrate, micronutrient fertilization reduced growth of *Juniperous virginiana* (Wright and Hinesley, 1991), improved growth in nine deciduous tree species (Wright et al., 1999b) and had no effect on growth of *Rhododendron* × ‘Girards Scarlet’ (Rose and Wang, 1999) compared to plants grown in substrates not amended with a supplemental micronutrient source. In the current study, the limiting nutrients responsible for reduced growth of crape myrtle grown in FL and F could not be discerned since foliar tissue samples were not analyzed.

Total P content in plant tissue was 28% higher in plants grown in FLM compared to those grown in FL (Table 3). Since pore-water OP-P concentrations were generally higher in FL compared to other substrate treatments, observed relatively low P uptake amounts by plants in FL were not due to limiting P supply. Most likely, the absence of soluble micronutrients in FL due to relatively high pH and no supplemental MF resulted in micronutrient deficiency which subsequently limited plant growth and concomitant nutrient uptake (Ingestad and Lund, 1986). Although growth measurements over time were not recorded, weekly photographs taken of each plant indicated that at 70 DAI, plants within the FL treatment were visibly smaller and recently matured leaves displayed interveinal chlorosis and necrosis, indicating deficiency of nutrients with low (e.g., Ca, Mn) or intermediate (e.g., Fe, Zn, Cu, B, Mo) phloem-mobility (Marschner, 2012). Accordingly, higher OP-P concentrations in pour-through extracts of FL compared to FLM are partially due to relative differences in P uptake amounts by plants. Despite higher TDW of plants grown in FLM compared to F, total amounts of P in plant tissue were the same among plants in these two treatments. Hence, lower pore-water OP-P concentrations in FLM compared to F at seven of the ten sampling dates cannot be attributed to relative differences in plant uptake of P.

Since the amount of P remaining in CRF granules at experiment termination was the same across substrate treatments (11.1 ± 0.09 SE mg P remaining), and all plants were pruned to the same size at pot-up, relative PUE can be inferred by comparing total tissue P content across substrate treatments. Calculated PUE values (i.e., mg P released by CRF/mg P in plants) for F, FL, FM and FML were 0.30, 0.25, 0.29 and 0.33, respectively. Accordingly, despite $\approx 64\%$ lower pour-through OP-P concentrations (pooled over time, excluding 70, 77 and 84 DAI) in FLM compared to F, P uptake, and thus PUE, was not negatively affected. This relationship between pore-water P concentrations and P uptake suggests that the remaining quantity of OP-P in pore water (i.e., not sorbed by DL and/or MF) was high enough to maintain a sufficient level of P uptake for maximal growth. On average, OP-P concentrations in FLM were 3.3 ± 0.41 SE $\text{mg}\cdot\text{L}^{-1}$ over the course of the study. Despite the best management practice recommendation to maintain 5 to 10 $\text{mg}\cdot\text{L}^{-1}$ P in pour-through extracted substrate pore-water (Bilderback et al., 2013), studies by Havis and Baker (1985), Shreckhise et al. (2018) and Million et al. (2007) reported maximal growth of various containerized woody nursery crops when pore-water P concentrations were consistently $< 5 \text{ mg}\cdot\text{L}^{-1}$ P.

Conclusions

Amending pine bark with a combination of $0.89 \text{ kg}\cdot\text{m}^{-3}$ MF and $2.97 \text{ kg}\cdot\text{m}^{-3}$ DL reduced water-extractable OP-P and TP concentrations by, on average, 64% and 58%, respectively, when growing containerized crape myrtle for 91 days in a greenhouse environment. Depending on the substrate treatment, OP-P contributed, on average, 70 to 83 % of TP. Since the pour-through extraction method is similar to an irrigation event, we can deduce that DL and MF amendments would also reduce P leaching from containers in an outdoor nursery setting. Accordingly,

amending pine bark-based substrates with DL and MF should be considered a best management practice for reducing P runoff from nurseries. However, plant growth response to DL and MF amendments has been shown to be taxa-specific. In this research, amending the substrate with DL without MF resulted in poor health of crape myrtle. The resulting reduced growth was accompanied with less P uptake and thus higher P concentrations in substrate pore-water. Hence, adding MF and DL to pine bark will have the greatest effect on P mobility only when plant health is also optimal. Despite reduced concentrations of soluble OP-P due to sorption by DL and MF, total P uptake by plants was unaffected. However, this research could not conclude whether P sorbed by these amendments provides labile source of available P.

Acknowledgments

The authors thank Julie Brindley, Velva Groover, Anna Birnbaum and Doug Sturtz for technical assistance, as well as Carolina Bark Products and Harrell's LLC for donating pine bark and fertilizer, respectively, for this research. A special thanks is warranted to Saunders Brothers Nursery for donating plant material to Dr. Sarah White and her team for DOC sample analyses. Funding was provided by the Virginia Agricultural Experiment Station and the Hatch Program of the National Institute of Food and Agriculture (SCRI 2014-51181-22372), U.S. Department of Agriculture, the Horticultural Research Institute and the Virginia Nursery and Landscape Association.

Literature Cited

Altland, J.E. and K.Y. Jeong. 2016. Dolomitic lime amendment affects pine bark substrate pH, nutrient availability, and plant growth: A review. *HortTechnology* 26:565-573.

- Alva A.K., M.E. Sumner, and W.P. Miller. 1991. Relationship between ionic strength and electrical conductivity for soil solutions. *Soil Sci.* 152:239–242.
- Argo, W.R. and J.A. Biernbaum. 1996a. The effect of lime, irrigation-water source, and water-soluble fertilizer on root-zone pH, electrical conductivity, and macronutrient management of container root media with impatiens. *J. Amer. Soc. Hort. Sci.* 121:442–452.
- Argo, W.R. and J.A. Biernbaum. 1996b. Availability and persistence of macronutrients from lime and preplant nutrient charge fertilizers in peat-based root media. *J. Amer. Soc. Hort. Sci.* 121:453–460.
- Bilderback, T., C. Boyer, M. Chappell, G. Fain, D. Fare, C. Gilliam, B.E. Jackson, J. Lea-Cox, A.V. LeBude, A. Niemiera, J. Owen, J. Ruter, K. Tilt, S. Warren, S. White, T. Whitwell, R. Wright, and T. Yeager. 2013. *Best management practices: Guide for producing nursery crops*. 3rd ed. Southern Nursery Association, Acworth, GA.
- Boesch, D. F., R. B. Brinsfield, and R.E. Magnien. 2001. Chesapeake Bay eutrophication: scientific understanding, ecosystem restoration, and challenges for agriculture. *J. Environ. Qual.* 30:303-320.
- Broschat, T. 1995. Nitrate, phosphate, and potassium leaching from container-grown plants fertilized by several methods. *HortScience* 30:74–77.
- Broschat, T.K. and H.M. Donselman. 1985. Extractable Mg, Fe, Mn, Zn, and Cu from a peat-based container medium amended with various micronutrient fertilizers. *J. Amer. Soc. Hort. Sci.* 110:196–200.
- Browder, J.F., A.X. Niemiera, J.R. Harris, and R.D. Wright. 2005. Growth response of container-grown pin oak and Japanese maple seedlings to sulfur fertilization. *HortScience* 40:1524–1528.

- Carpernter, S.R., N.F. Caraco, D.L. Correll, R.W. Howarth, A.N. Sharpley, and V.H. Smith. 1998. Nonpoint Pollution of surface waters with phosphorus and nitrogen. *Ecological Applications* 8:559–568.
- Cavins, T.J., B.E. Whipker, and W.C. Fonteno. 2004. Establishment of calibration curves for comparing pour-through and saturated media extract nutrient values. *HortScience* 39:1635–1639.
- Correll, D.L. The role of phosphorous in eutrophication of receiving waters: A review. *J. Environ. Qual.* 27:261–266.
- Dennis, J.H., R.G. Lopez, B.K. Behe, C.R. Hall, C. Yue, B.L. Campbell. 2010. Sustainable production practices adopted by greenhouse and nursery plant growers. *HortScience* 45:1232–1237.
- Diara, C., L. Incrocci, G. Incrocci, G. Carmassi, A. Pardossi, P. Marzioletti, P. Cozzi, F. Fibbi, and R. Clemens. 2014. Reduction of nutrient run-off by the use of coated slow-release fertilizers on two container-grown nursery crops. *Acta Hort.* 1034:85–92.
- Diggle, P.J. 1988. An approach to the analysis of repeated measurements. *Biometrics* 44:959–971.
- Du, C., J. Zhou, and A. Shaviv. 2006. Release characteristics of nutrients from polymer-coated compound controlled release fertilizers. *J. Polym Environ.* 14:223–230.
- Fain, G.B., C.H. Gilliam, K.M. Tilt, J.W. Olive, and B. Wallace. 2000. Survey of best management practices in container production nurseries. *J. Environ. Hort.* 18:142–144.
- Fonteno, W.C., C.T. Hardin, and J.P. Brewster. 1995. Procedures for determining physical properties of horticultural substrates using the NCSU Porometer. Horticultural Substrates Laboratory. NC State University, Raleigh, NC.

- Gerke, J. 2010. Humic (organic matter)-Al(Fe)-phosphate complexes: An underestimated phosphate form in soils and source of plant-available phosphate. *Soil Sci.* 175:417–425.
- Gerke, J., and R. Hermann. 1992. Adsorption of orthophosphate to Humic-Fe-Complexes and to amorphous Fe-Oxide. *Z. Pflanzenernähr. Bodenk.* 155:233–236.
- Handreck, K.A. 1991a. Interactions between iron and phosphorus in the nutrition of *Banksia ericifolia* L.f. var. *ericifolia* (Proteaceae) in soil-less potting media. *Aust. J. Bot.* 39:373–84.
- Handreck, K.A. 1991b. Available phosphorus in potting media: Extractants and interpretation of extract data. *Commun. Soil Sci. Plant Anal.* 22:529–557.
- Handreck, K.A. 1996. Comparison of phosphate-phosphorous and total phosphorus in DTPA extracts for assessing plant-available phosphorus in soilless potting media. *Commun. Soil Sci. Plant Anal.* 27:2125–2135.
- Handreck, K.A. 1996. Losses of iron in leachates from organic components of potting media. *Commun. Soil Sci. Plant Anal.* 27:2065–2077.
- Handreck, K.A. 1992. Iron phosphorous interactions in in the nutrition of seedling macadamia in organic potting media. *Australian journal of experimental horticulture.* 32:773–779.
- Havis, J.R., and J.H. Baker. 1985. Influence of liming rate and phosphorus leaching from a peat-sand medium. *J. Environ. Hort.* 3:74–76.
- Huett, D.O. and S.C. Morris. 1999. Fertiliser use efficiency by containerised nursery plants 3.* Effect of heavy leaching and damaged fertilizer prills on plant growth, nutrient uptake, and nutrient loss. *Aust. J. Agric. Res.* 50:217–222.
- Johnson, N. L. 1949. Systems of frequency curves generated by methods of translation. *Biometrika* 36:149–176.

- Karaca, S., A. Gürses, M. Edjer, and M. Açıkyıldız. 2004. Kinetic modeling of liquid-phase adsorption and phosphate on dolomite. *J. Colloid Interface Sci.* 277:257–263.
- Karaca, S., A. Gürses, M. Edjer, and M. Açıkyıldız. 2006. Adsorptive removal of phosphate from aqueous solutions using raw and calcinated dolomite. *J. Hazard. Mater.* B128: 273–279.
- Khan, M.N. and F. Mohammad. 2014. Eutrophication: Challenges and Solutions. In Ed. Ansari, A.A., and S.S. Gill. *Eutrophication: Causes, Consequences and Control Volume 2*. Springer, Dordrecht Heidelberg London New York.
- Lea-Cox, J. D., A.G. Ristvey. 2003. Why are nutrient uptake efficiencies so low in ornamental plant production. *Proc. South. Nurs. Assoc. Res. Conf.* 48:116–122.
- Lindsay, W.L. 1979. *Chemical Equilibria in Soils*. John Wiley, New York.
- Lu, W., J.L. Sibley, C.H. Gilliam, J.S. Bannon, and Y. Zhang. 2006. Estimation of U.S. bark generation and implications for horticultural industries. *J. Environ. Hort.* 24:29–34.
- Mack, R., J.S. Owen, A.X. Niemiera, and J. Latimer. 2017. Virginia nursery and greenhouse grower survey of best management practices. *HortTechnology* 27:386–392.
- Mangwandi, C., A. B. Albadarin, Y. Glocheux, G. M. Walker. Removal of ortho-phosphate from aqueous solution by adsorption onto dolomite. *J. Environ. Chem. Eng.* 2:1123–1130.
- Marschner, H. 2012. *Mineral nutrition of higher plants*. 3rd ed. Academic Press, San Diego, CA.
- McGinnis, M.S., S.L. Warren, and T.E. Bilderback. 2009. Replacing conventional nursery crop nutrient inputs with vermicompost for container production of *Hibiscus moscheutos* L. ‘Luna Blush’. *HortScience* 44:1690–1703.
- Michalak, A.M., E.J. Anderson, D. Beletsky, S. Boland, N.S. Bosch, T.B. Bridgeman, J.D. Chaffin, K.Cho, R. Confesor, I. Daloglu, J.V. DePinto, M.A. Evans, G.L. Fahnenstiel, L.

- He, J.C. Ho, L. Jenkins, T.H. Johengen, K.C. Kuo, E. LaPorte, X. Liu, M.R. McWilliams, M.R. Moore, D.J. Posselt, R.P. Richards, D. Scavia, A. L. Steiner, E. Verhamme, D.M. Wright, and M.A. Zagorski. 2015. Record-setting algal bloom in Lake Erie caused by agricultural and meteorological trends consistent with expected future conditions. *Proc. Natl. Acad. Sci.* 110:6448–6452
- Million, J., T. Yeager, and J. Albano. 2007. Consequences of excessive overhead irrigation on runoff during container production of sweet viburnum. *J. Environ. Hort.* 25:117–125.
- Moore, P.A., Jr., and D.M. Miller. 1994. Decreasing phosphorus solubility in poultry litter with aluminum, calcium, and iron amendments. *J. Environ. Qual.* 23:325–330.
- Ogden, R.J., F.A. Pokorny, H.A. Mills, and M.G. Dunavent. 1987. Elemental status of pine bark-based potting media. *Hort. Rev.* 9:103–131.
- Ogutu, R. A., and K.A. Williams. 2009. Phosphate sorption of calcined materials used as components of soilless root media characterized in laboratory studies. *HortScience* 44:431–437.
- Owen, J.S., Jr., S.L. Warren, T.E. Bilderback, and J.P. Albano. 2007. Industrial mineral aggregate amendment affects physical and chemical properties of pine bark substrates. *HortScience* 42:1287–1294.
- Owen, J.S., S.L. Warren, T.E. Bilderback, and J.P. Albano. 2008. Phosphorus rate, leaching fraction, and substrate influence on influent quantity, effluent nutrient content, and response of a containerized woody ornamental crop. *HortScience* 43:906–912
- Rich, D. 2005. The removal of total phosphorus from natural waters by precipitation. Ph.D. diss., Northwestern International Univ., Evanston, IL.

- Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2004. Nutrient uptake, partitioning and leaching losses from container-nursery production systems. *Acta Hort.* 630:321–328.
- Ristvey, A.G., J.D. Lea-Cox, and D.S. Ross. 2007. Nitrogen and phosphorus uptake efficiency and partitioning of container-grown azalea during spring growth. *J. Amer. Soc. Hort. Sci.* 132:563–572.
- Rose, M.A., and H. Wang. 1999. Comparison of micronutrient sources for container rhododendron. *HortTechnology.* 9:220–224.
- Ruter, J.M. 2004. Rate of calcined clay incorporation influences phosphorus retention in a pine bark substrate. *Proc. South. Nurs. Assoc. Res. Conf.* 49:23–24.
- Schindler, D.W., R.E. Hecky, D.L. Findlay, M.P. Stainton, B.R. Parker, M.J. Paterson, K.G. Beaty, M. Lyng, and S.E.M. Kasian. 2008. Eutrophication of lakes cannot be controlled by reducing nitrogen input: Results of a 37-year whole-ecosystem experiment. *Proc. Nat. Acad. Sci. USA.* 105:11254–11258.
- Sherman, L.A., and P. Barak. 2000. Solubility and dissolution kinetics of dolomite in Ca-Mg-HCO₃/CO₃ solutions at 25° and 0.1 MPa carbon dioxide. *Soil Sci. Soc. Am. J.* 64:1959–1968.
- Shreckhise, J.H., J.S. Owen, Jr., and A.X. Niemiera. 2018. Growth response of three containerized woody plant taxa to varying low phosphorus fertilizer concentrations. *HortScience* (In Press).
- Shreckhise, J.H. 2018. Sol. Phosphorus Requirement and Chemical Fate in Containerized Nursery Crop Production. Ph.D. diss., Virginia Tech, Blacksburg, VA.

- Sinsabaugh, R.L., III, R.C. Hoehn, W.R. Knocke, and A.E. Linkins, III. 1986. Removal of dissolved organic carbon by coagulation with iron sulfate. *J. Am. Water Works Assoc.* 78(5):74–82.
- Tiemeyer, B., N. Pfaffner, S. Frank, K. Kaiser, S. Fieldler. 2017. Pore water velocity and ionic strength effects on DOC release from peat-sand mixtures: Results from laboratory and field experiments. *Geoderma* 296:86–97.
- Tyler, H.H., S.L. Warren, and T.E. Bilderback. 1996. Reduced leaching fractions improve irrigation use efficiency and nutrient efficacy. *J. Environ. Hort.* 14:199–204.
- Warren, S.L., T.E. Bilderback, and H.H. Kraus. 2001. Method of fertilizer application affects nutrient losses of controlled-release fertilizer. *Acta Hort.* 548:349–355.
- Warren, S.L., T.E. Bilderback, and H.H. Tyler. 1995. Efficacy of three nitrogen and phosphorus sources in container-grown azalea production. *J. Environ. Hort.* 13:147–151.
- Wolfinger, R. 1993. Covariance structure selection in general mixed models. *Commun. Statist.* 22:1079–1106.
- Wright, A.N., A.X. Niemiera, J.R. Harris, and R.D. Wright. 1999a. Micronutrient fertilization of woody seedlings essential regardless of pine bark pH. *J. Environ. Hort.* 17:69–72.
- Wright, A.N., A.X. Niemiera, J.R. Harris, and R.D. Wright. 1999b. Preplant lime and micronutrient amendments to pine bark affect growth of seedlings of nine container-grown tree species. *HortScience* 34:669–673.
- Wright, R.D. and L.E. Hinesley. 1991. Growth of containerized Eastern redcedar amended with dolomitic limestone and micronutrients. *HortScience* 26:143–145.
- Xu, N., H. Yin, Z. Chen, S. Liu, M. Chen, and J. Zhang. 2014. Mechanisms of phosphate retention by calcite: effects of magnesium and pH. *J. Soils Sediments.* 14:495–503.

- Yuan, X., W. Xia, J. An, W. Yang, and J. Yin. 2014. Removal of phosphate anions from aqueous solutions using dolomite as adsorbent. *Adv. Mater. Res.* 864–867:1454–1457.
- Yuan, X., X. Wentag, J. An, J. Yin, X. Zhou, and W. Yang. 2015. Kinetic and thermodynamic studies on the phosphate adsorption removal by dolomite mineral. *J. Chem.* 1–8.

Table 1. Degrees of freedom (df), F-values and P-values for analysis of variance (ANOVA) to determine significant effects of substrate treatment, time (1 to 91 DAI) and the substrate \times time interaction on total phosphorus (P), orthophosphate-P (OP-P), calcium (Ca), iron (Fe), dissolved organic carbon (DOC), pH and electrical conductivity (EC) in pour-through extracts of container-grown *Lagerstroemia* ‘Natchez’. Substrates were amended with 2.97 kg·m⁻³ of a polymer-coated 19N–2.6P–10.8K controlled-release fertilizer (CRF) and either no additional amendments (F), 2.97 kg·m⁻³ dolomite (FL), 0.89 kg·m⁻³ micronutrient fertilizer (FM) or both dolomite and micronutrient fertilizer (FLM).

ANOVA Source	df	F-value	P-value
Total P			
Substrate	3	23.2	<0.0001
Time	9	44.4	<0.0001
Substrate \times Time	27	5.0	<0.0001
OP-P			
Substrate	3	27.9	<0.0001
Time	9	49.6	<0.0001
Substrate \times Time	27	7.3	<0.0001
Ca			
Substrate	3	188.9	<0.0001
Time	9	24.9	<0.0001
Substrate \times Time	27	13.0	<0.0001
Fe			
Substrate	3	9.9	0.0001
Time	9	24.6	<0.0001
Substrate \times Time	27	3.0	<0.0001
DOC			
Substrate	3	68.4	<0.0001
Time	9	154.5	<0.0001
Substrate \times Time	27	2.5	0.0003
pH			
Substrate	3	3506.4	<0.0001
Time	9	30.6	<0.0001

Substrate × Time	27	15.6	<0.0001
EC			
Substrate	3	48.5	<0.0001
Time	9	20.3	<0.0001
Substrate × Time	27	12.8	<0.0001

Table 2. Saturation indices calculated by Visual MINTEQ for phosphorus species saturated with respect to the solid phase (positive values) in pour-through extracts collected at various intervals over the course of 91 days from containerized *Lagerstroemia* ‘Natchez’ grown in a pine bark substrate amended with 2.97 kg·m⁻³ of a polymer-coated 19N–2.6P–10.8K controlled-release fertilizer (CRF). Substrate treatments included the following: no amendments (F), 2.97 kg·m⁻³ dolomite (FL), 0.89 kg·m⁻³ micronutrient fertilizer (FM) or both dolomite and micronutrient fertilizer (FLM).

	Time (d)							
	14	35	56	63	70	77	84	91
	Saturation index							
MnHPO ₄								
F	0.9	1.0	1.3	1.2	1.0	0.8	0.6	0.3
FL	2.1	1.4	1.8	1.9	2.1	1.8	1.4	1.4
FM	1.5	1.4	1.7	1.5	1.3	1.2	0.9	0.6
FLM	3.0	2.7	2.6	2.5	2.5	2.5	2.2	1.1
Ca ₅ (PO ₄) ₃ (OH)								
F	-16.1	-14.8	-13.8	-14.4	-14.9	-15.3	-16.8	-17.6
FL	-0.4	1.3	1.3	1.3	1.6	0.6	0.1	-1.3
FM	-16.1	-16.5	-15.0	-15.7	-16.7	-16.5	-18.0	-19.5
FLM	-0.7	-0.3	-0.2	-0.4	1.7	1.7	0.9	-4.2

Table 3. Total dry weight (DW) and phosphorus (P) content (n = 5) of containerized *Lagerstroemia* ‘Natchez’ grown for 91 days in a pine bark substrate amended with 2.97 kg·m⁻³ of a polymer-coated 19N–2.6P–10.8K controlled-release fertilizer (CRF). Substrate treatments included the following: no amendments (F), 2.97 kg·m⁻³ dolomite (FL), 0.89 kg·m⁻³ micronutrient fertilizer (FM) or both dolomite and micronutrient fertilizer (FLM).

Substrate	DW (g)	Tissue-P content (mg)
F	62.9 b†	199.2 ab
FL	45.1 c	164.6 b
FM	86.8 a	194.2 ab
FLM	95.3 a	210.8 a
P-value	<0.0001	0.0188

† Within columns for each sampling date, means followed by the same letter are not significantly different according to Tukey’s HSD (0.05).

Figure captions

Fig. 1. Effect of substrate treatments on total phosphorus (TP; top) and orthophosphate-P (OP-P; bottom) concentrations over time in pour-through extracts of containerized *Lagerstroemia* 'Natchez' grown for 91 days in a pine bark substrate amended with $2.97 \text{ kg}\cdot\text{m}^{-3}$ of a polymer-coated 19N–2.6P–10.8K controlled-release fertilizer (CRF). Substrate treatments included the following: no amendments (F), $2.97 \text{ kg}\cdot\text{m}^{-3}$ dolomite (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM) or both dolomite and micronutrient fertilizer (FLM). Different vertically aligned letters next to means indicate significant difference among substrate treatments (F, FL, FM and FLM) within the corresponding sampling date via Tukey's HSD ($n = 5$; $P < 0.01$). Non-transformed values are reported. Vertical bars represent standard error of the mean.

Fig. 2. Effect of substrate treatments on electrical conductivity (EC; top) and pH (bottom) over time in pour-through extracts of containerized *Lagerstroemia* 'Natchez' grown for 91 days in a pine bark substrate amended with $2.97 \text{ kg}\cdot\text{m}^{-3}$ of a polymer-coated 19N–2.6P–10.8K controlled-release fertilizer (CRF). Substrate treatments included the following: no amendments (F), $2.97 \text{ kg}\cdot\text{m}^{-3}$ dolomite (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$ micronutrient fertilizer (FM) or both dolomite and micronutrient fertilizer (FLM). Non-transformed values are reported. Ionic strength (IS) was calculated as $(\text{EC} \times 0.012) - 0.0002$ based on Alva et al. (1991). Vertical bars represent standard error of the mean.

Fig. 3. Effect of substrate treatments on calcium (Ca; top), iron (Fe; middle) and dissolved organic carbon (DOC; bottom) concentrations over time in pour-through extracts of containerized *Lagerstroemia* 'Natchez' grown for 91 days in a pine bark substrate amended with $2.97 \text{ kg}\cdot\text{m}^{-3}$ of a polymer-coated 19N–2.6P–10.8K controlled-release fertilizer (CRF). Substrate treatments included the following: no amendments (F), $2.97 \text{ kg}\cdot\text{m}^{-3}$ dolomite (FL), $0.89 \text{ kg}\cdot\text{m}^{-3}$

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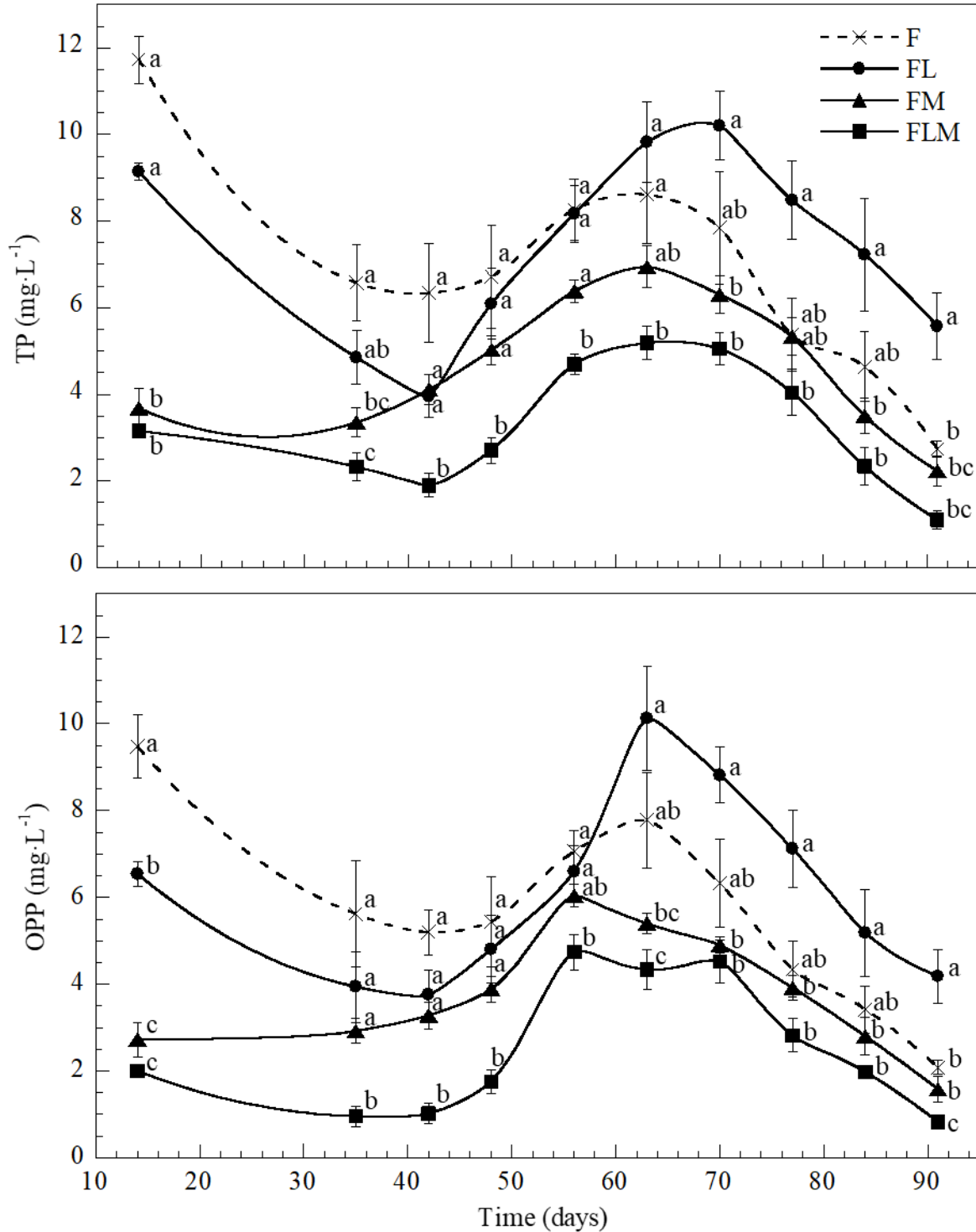


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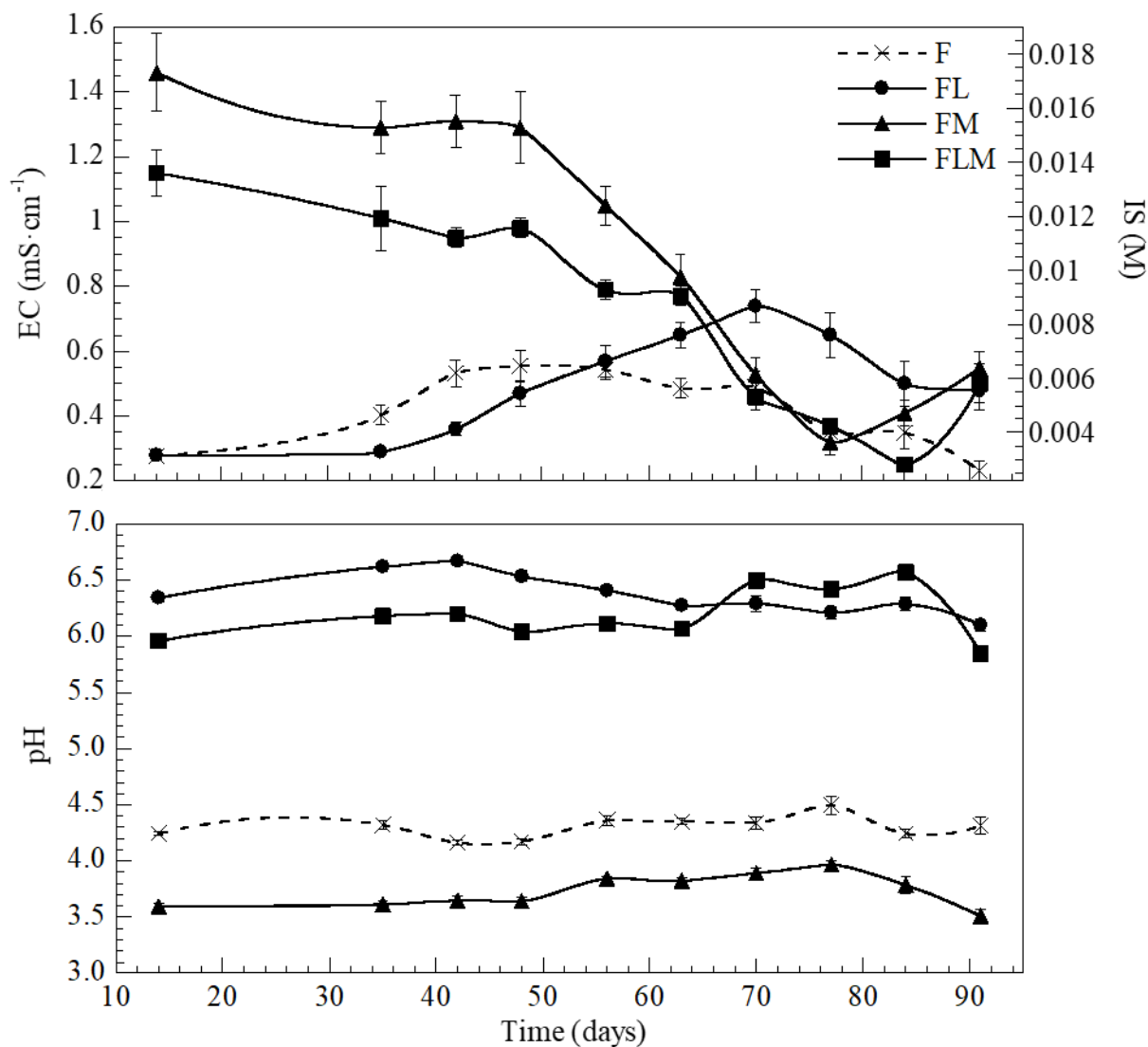


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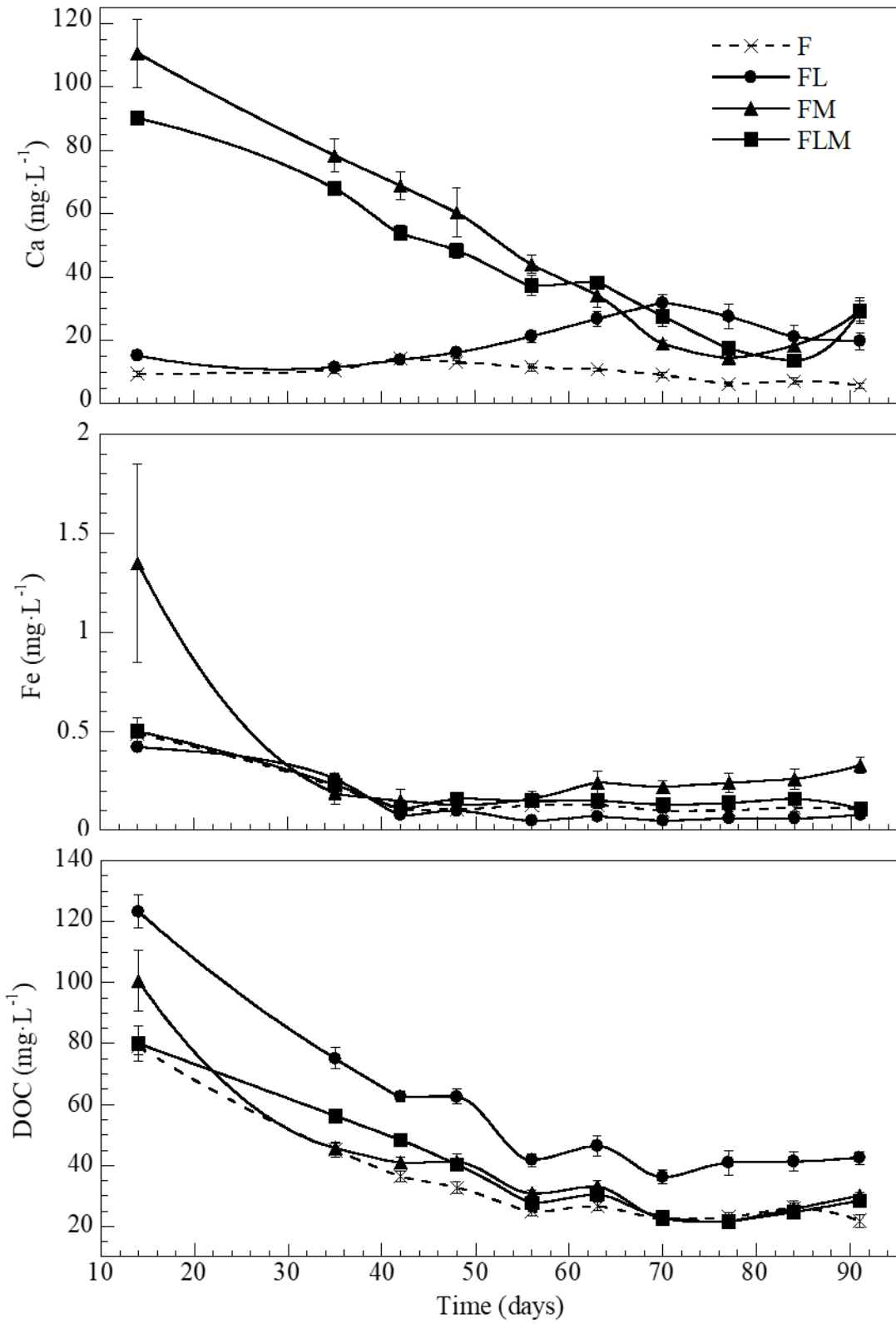


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Conclusions

Among the two low-P fertilization studies, all four taxa studied achieved maximal growth when pore-water P concentrations were consistently lower than $5 \text{ mg}\cdot\text{L}^{-1}$, the minimum concentration recommended by BMPs. As was discussed in Chapter 3, *H. macrophylla* achieved maximal growth when fertilized with 18N–1.3P–10K CRF at a rate of 0.3 g P per 3.8 L container incorporated at the substrate surface. In comparison, the standard CRF formulation used for containerized nursery crop production (e.g., 18N–2.6P–10K) typically contains approximately twice this amount of P; hence, in general, P fertilization of *H. macrophylla* may be reduced by as much as 50% compared to the industry standard. According to the recent census of horticulture specialties, on 1 January 2015, U.S. nursery operations had over 10.8 million *Hydrangea* in production, representing 13% of all deciduous shrubs produced in the U.S. (second only to shrub roses). A majority of *Hydrangea* produced in U.S. nurseries are from only four species, one of which being *H. macrophylla*. Hence, industry-wide adoption of a low-P CRF for *H. macrophylla* production would likely have major implications for improving PUE and reducing P runoff from containerized nursery sites. However, additional research is needed to determine if P requirement of *Hydrangea* is cultivar-specific.

Results presented in Chapter 4 indicate that DL incorporated into pine bark at a rate of $4.15 \text{ kg}\cdot\text{m}^{-3}$ is capable of reducing OP-P concentrations in leachate by 50%, even after forty-eight 2.6 cm irrigation events. From 15 to 48 DAI, incorporating both DL and MF into pine bark had the same effect on OP-P leaching as DL alone. The effects of MF on OP-P leaching were apparent during the first five irrigation event, but not thereafter. Comparing these finding to those presented in Chapter 5 highlights the impact of root absorption on OP-P leaching. In Chapter 5, OP-P leaching from pine bark amended with DL-only was generally the same as that

from the non-amended substrate. This was partially due to poor growth and concomitant P uptake of plants grown in the DL-only substrate. Plants grown in substrates containing MF or MF plus DL had equivalent biomass and tissue P content; however, only the substrate containing both DL and MF had lower pore-water OP-P concentrations than those in the non-amended substrate. Accordingly, amending pine bark with DL should be considered a BMP only if growth is either unaffected or improved by DL incorporations. Since DL is readily available and relatively inexpensive compared to other P-sorbing products (e.g., calcined clay), DL may be a superior sorbent. Further investigation is needed to determine the influence of DL rate and particle size on short- and long-term P sorption in pine bark. Additional research is also needed to determine the lability of DL-sorbed P and its potential as a slow-release P supply.