

ABSTRACT

HENRY, JOSHUA BRADY. Beneficial and Adverse Effects of Low Phosphorus Fertilization of Floriculture Species. (Under the direction of Brian Earl Whipker).

Low phosphorus (P, referring to phosphorus supplied by phosphate) fertilization provides numerous benefits to floriculture growers. It is established that low P results in compact growth and can lead to darker green or red coloration in many species. Previous work investigated the benefits of low P on floriculture species, but did not provide recommendations applicable to commercial growing practices. These studies sought to provide P fertilization recommendations for several floriculture species, using methods similar to commercial practices. Low P concentrations can be detrimental to growth when deficiency symptoms develop. Recent reports of an atypical reproductive stage P deficiency symptom which occurs on the upper foliage were investigated and characterized.

Six ornamental species were grown with custom fertilizers that provided uniform concentrations of all essential nutrients, but varied P concentrations. Alternanthera [*Alternanthera brasiliana* (L.) Kuntze] and petunia [*Petunia atkinsiana* (Sweet) D. Don ex W. H. Baxter] were grown with 0 – 80 mg·L⁻¹ P, which provided a growth response curve of low to high P supply. Angelonia (*Angelonia angustifolia* Benth.), vinca [*Catharanthus roseus* (L.) G. Don], and New Guinea impatiens (*Impatiens hawkeri* W. Bull) were grown with 0 – 20 mg·L⁻¹ P to observe growth response over time, with biweekly measurements of height and diameter. Angelonia and New Guinea Impatiens were also grown to compare growth control from low P fertilization to chemical plant growth retardants (PGRs). Ornamental peppers (*Capsicum annuum* L.) were grown with initial concentrations of 0 – 20 mg·L⁻¹ P with half later restricted to 0 mg·L⁻¹ P. This was done to investigate the effects of restricting P later in the production cycle on ultimate plant growth and health. Quadratic growth plateaus were developed for each species and

optimal P concentrations for growth control are presented. These experiments all indicated that current P fertilization regimens are likely greater than necessary, and most greenhouse bedding plants required 5 – 13 mg·L⁻¹ P to maximize growth.

Ornamental peppers and chrysanthemums (*Chrysanthemum morifolium* Ramat.) were used to investigate symptoms of reproductive stage P deficiency on the upper foliage. To induce these symptoms, ornamental peppers were initially grown with 0 – 20 mg·L⁻¹ P and later restricted to 0 mg·L⁻¹ P. Plants grown with less than 10 mg·L⁻¹ P concentrations developed typical lower leaf symptomology while plants grown with 10 mg·L⁻¹ P concentrations developed symptoms of chlorosis, necrotic spotting, and olive green leaf spotting on the upper foliage. Methods were refined for chrysanthemums, and symptoms were successfully induced on three cultivars. Symptoms varied by cultivar, and a complete description accompanied by tissue P concentrations is provided.

Alternanthera and geranium (*Pelargonium x hortorum* L. H. Bailey) were grown with low P concentrations to enhance red foliar coloration. Restricting P later in the production cycle resulted in plants with significantly redder leaf coloration, without development of adverse P deficiency symptoms. Using low and restricted P fertilization also provided growth control, resulting in plants with several enhanced attributes that are considered valuable in ornamental species. A concluding chapter is provided to illustrate how low P fertilization may be best implemented for the production of floriculture species, and what may be done to limit the occurrence of detrimental deficiency symptoms.

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Beneficial and Adverse Effects of Low Phosphorus Fertilization of Floriculture Species

by
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A thesis submitted to the Graduate Faculty of
North Carolina State University
in partial fulfillment of the
requirements for the degree of
Master of Science

Horticultural Science

Raleigh, North Carolina

2017

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DEDICATION

To my brilliant wife Amy, and her constant love and support.

BIOGRAPHY

Joshua Henry was born in Cleveland, Ohio on January 22, 1993 to Chris and Chris Crites. Henry graduated summa cum laude from The Ohio State Agricultural Technical Institute in May 2013 as recipient of the Director's Award, and later graduated summa cum laude with his B.S. from The Ohio State University in December 2014. Henry interned at Smith Gardens in Aurora, Oregon during the spring of 2015 prior to beginning his graduate work at North Carolina State University (NCSU) in August 2015. In May 2017, he will begin his doctoral work at NCSU.

ACKNOWLEDGMENTS

I would like to thank Dr. Brian Whipker for all of the support and opportunities. You have exposed me to so many different aspects of floriculture and have done so much to help me succeed. I could not have accomplished nearly as much without your guidance. Thank you to Dr. Paul Nelson and Dr. Brian Jackson for serving on my committee, and providing useful insight into my research. Dr. Penelope Perkins, thank you so much for letting me use your lab, and helping me learn color determination. This work enabled me to learn a new set of skills and knowledge I did not expect to obtain. To Ingram McCall, thank you for the many hours of harvesting, data collection, and taking care of our many projects. I do not know how I could have completed my research without you. Thanks to Paul Cockson for your help with my research. Your eagerness to learn will get you far.

Thanks to my wife Amy, for all of the love and support over the past two years. I appreciate all of the early mornings and late nights taking data and harvesting studies with me. Thank you for maintaining my sanity when the workload was too great, and always being my ice cream buddy. To my parents, thank you so much for your willingness to support my horticulture habit from the time I was young. You always encouraged me to explore this field that you previously knew so little about. Thank you for gardening with me, finding volunteer opportunities for me, and always pushing me to get out of my comfort zone.

To everyone who has encouraged me to pursue my passion for plants, thank you. Without my fourth grade gardening class taught by the Ohio State Extension Master Gardeners, I certainly would not be where I am today. Thank you to Dr. Bob McMahon, Dr. Peg McMahon, Dr. Laura Deeter, and countless others for providing me with knowledge and mentorship over the course of my education.

Lastly, I would like to thank all those who contributed to this research. Thanks to the Fred C. Gloeckner Foundation, American Floral Endowment Altman Family Scholarship, The Garden Club of America, and numerous others who contributed to my education and research. Without your support, this research would not have been possible. Additional thanks goes to Dümmer Orange for providing the plant materials used in numerous studies. I would also like to express our gratitude to Sun Gro Horticulture for providing the peat moss used in these studies.

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Chapter 1

Introduction

Introduction

Producers of floriculture crops strive to cultivate compact and healthy plants which are considered high quality and attractive for consumers. There are a number of ways in which growers control the growth of their crops. Chemical growth regulators such as plant growth retardants (PGRs) have long been the most commonly used forms of growth control in ornamental production (Whipker, 2017). A concern associated with PGRs is that they may not be labelled for certain crops. This is especially true for vegetable plant growers who are often very limited on what chemicals can be used (Nelson et al., 2012). A number of other commercial methods for controlling plant growth are commonly used, often in association with one another. These methods include water stress, nutrient restriction, light and temperature manipulation, and use of smaller growing containers (Dole and Wilkins, 2005). All of these methods can potentially help to produce more compact plants. The primary aspects of this research concern nutrient restriction, specifically of phosphorus (P, referring to phosphorus supplied by phosphate).

Restriction of P can be very effective and easy to implement in short-term crops, such as plug production (Nelson et al., 2012), but long-term crops may be more difficult to successfully manipulate. This is especially true in greenhouse bedding crops that are typically produced in soilless substrate, which tends to have a very limited P holding capacity (Whipker, 2014). Without adequate application of P, long-term crops have the potential to run out of the initial charge of P in the substrate and may have to begin reallocating P from older plant tissues, leading to the development of deficiency symptoms (Mills and Jones, 1996). Nevertheless, P also has the widest window between beneficial control of plant growth due to mild nutrient stress, and detrimental deficiency symptoms when compared with limitation of other essential nutrients.

This makes P a safer and potentially easier input to be manipulated for regulation of plant growth.

Phosphorus is one of three primary macrolelements along with nitrogen (N) and potassium (K) essential for plant growth, and it is needed in the lowest amount. It is most highly available for plant uptake at a low pH in the soilless substrate most commonly used in bedding plant production (Bailey, 1996). In fertilizer formulas, P is expressed in the form of phosphorus oxide (P_2O_5), and is represented by the second number of a fertilizer formula such as 20N–10 P_2O_5 –20 K_2O (20N–4.4P–16.6K). Phosphate is actively taken up into the plant via either a co-transport or antiport system, leading to 100 – 1,000 times greater P content in the root tissue and xylem than in the growing substrate (Mills and Jones, 1996). For leaf tissue levels, a range of approximately 0.2 – 0.5% P of total dry weight is considered sufficient for most plants. Deficiency typically occurs below this range and can result in darker green or purple foliage (Figure 1.1) (Mills and Jones, 1996). This can in part be attributed to higher anthocyanin production in P deficient leaves and other tissues (Henry et al., 2012).



Figure 1.1. Phosphorus deficiency symptoms of lower leaf purpling on *Chrysanthemum x morifolium*. Photo credit: Josh Henry.

Phosphorus is extremely important for plant growth, serving as a major component of plant cell membranes, nucleic acids, and adenosine triphosphate (ATP). It also plays a major role in photosynthesis and photorespiration among other important plant processes (Hernández and Munné-Bosch, 2015). Without adequate P, numerous deficiency symptoms occur in different plant tissues. The root to shoot ratio becomes greater under sub-optimal P conditions, with increased secondary root growth (Khandan-Mirkohi and Schenk, 2009; Mengel et al., 2001). In foliar tissues, chlorosis may occur due to a decrease in chlorophyll. Red or purple coloration may also develop on the older leaves, or intensify due to increased anthocyanins (Hernández and Munné-Bosch, 2015).

Most of the common forms of P deficiency are well documented, though Whipker (2014) reported symptoms occurring that do not appear to have been previously described. Necrosis

may begin to form on the upper leaves under P deficient conditions, when terminally flowering plants such as garden mums are in the reproductive stage. This necrosis appears to occur when there are two sinks competing for limited supplies of P: the flowers and developing fruit, and the actively growing upper foliage. When these sinks compete for P, it appears that the reproductive tissues take precedence, and reallocated P supplies bypass the upper foliage in favor of the reproductive tissues. Although this symptomology appears to be less common than the typical symptoms, it may become more of an issue as growers continue to adopt a low P fertilization regiment, to achieve plant compactness.

Excessive applications of agricultural nutrients including P have caused many water quality issues in recent years. The algae blooms occurring in Lake Erie due to excessive P is just one example of these problems (Wines, 2014). Many municipalities and agencies are also becoming more aware of issues associated with excessive nutrient application and runoff into water sources. Greenhouse and nursery growers in the Chesapeake Bay watershed have been encouraged to use improved management practices to reduce unnecessary runoff and improve the bay water quality (Majsztrik and Lea-Cox, 2013). In the Florida Everglades, the concentration of P can be up to 60 times the maximum level determined to be safe for the unique organisms that live there (Liu et al., 2015). With all of the various environmental issues associated with excessive P application, growers will need to lower their fertilization regiments in order to reduce runoff.

Lowering P fertilization strategies for greenhouse growers also comes with the added benefit of reduced costs. Fertilizer prices in general have risen in the U.S. at a very rapid pace since 2000, and P specifically has followed a very similar trend. Between 2000 and 2012, agricultural fertilizer prices have risen 189%, while P prices have risen 178% (USDA, 2012).

Additionally, there are concerns of the mineral P supply running low, making agricultural P use efficiency a more important issue than ever before (Liu et al., 2015). Developing low P fertilization strategies for floriculture crops could help control some of the environmental issues associated with excess fertilizers and potentially lead to production of more compact and commercially acceptable plants.

Phosphorus and Plant Height

Although the nitrate (NO_3^-) form of N has often been used to keep plants compact, recent findings indicate that it is the low P level in high nitrate fertilizers that accounts for compactness (Nelson et al., 2012). Most NO_3^- based fertilizers that are recommended for compact plant growth are also low in P. Commercial fertilizer formulations including 13N–0.9P–10.8K, 14N–0P–11.6K and 15N–0P–12.5K are all high in NO_3^- sources of N and contain little to no P. N is mostly supplied as calcium, magnesium or potassium nitrate (Nelson et al., 2002). Erroneously, it was thought that fertilizer formulations high in ammoniacal nitrogen (NH_4^+) result in greater plant growth. Experiments conducted by Nelson et al. (2002) using nutrient formulations containing 60% $\text{NO}_3\text{-N}$ and 40% $\text{NH}_4\text{-N}$ with varying levels of P demonstrated that plant size increased with higher P concentrations. Previously, it had been thought that higher P concentrations would only increase plant growth up to a dry matter P concentration of 0.25% (Nelson et al., 2002). These experiments indicate that P content up to 1% of seedling dry matter can significantly increase the height of several plant species.

Huang and Nelson (1994) reported that growers can potentially utilize marginally low P concentrations during bedding plant seedling production to develop more compact plants with a greater root to shoot ratio than those grown with sufficient nutrient solution P concentrations of

0.5 mM PO₄ (15.5 mg·L⁻¹ P). When seedlings were grown initially without P, they exhibited more compact growth with greater root to shoot ratio. These plants returned to the typical robust growth habit when P fertilization was initiated. Seedlings given a continuous low P concentration of 0.15 mM PO₄ (4.7 mg·L⁻¹ P) exhibited a more compact growth habit than those grown with P concentrations of 0.5 mM PO₄ (15.5 mg·L⁻¹ P) (Huang and Nelson, 1994).

Nelson et al. (2002) also conducted experiments with varying ratios of NO₃-N and NH₄-N, but with a constant concentration of P to determine if plant height was affected by this ratio, as had been thought for many years. Results indicate that there was no significant difference in height regardless of the type of N fertilizer used. In fact, NO₃-N may actually increase plant size when NO₃⁻ concentrations are increased and P remains constant (Nelson et al., 2012). This confirms that P is the primary nutrient that can be used in controlling plant size and specifically height, whereas NH₄-N is primarily attributed to leaf expansion.

In a study by Baas et al. (1995) involving low P fertilization of poinsettias, it was found that P concentrations lower than those typically applied did not cause deficiency or control growth. Applying P at a concentration of 0.24 mmol/L (7.4 mg·L⁻¹ P) in an ebb and flow system did not control growth, leading to the conclusion that lower P concentrations could still be applied to achieve growth control without exhibiting deficiency symptoms. *Impatiens walleriana* provides another good example of this, as they were more compact without exhibiting any negative factors when grown with reduced P. *Pelargonium x hortorum* and *Salvia* also developed more compact growth habits from lower P fertilization, however flowering was delayed (Baas et al., 1995).

Akhtar et al. (2007) conducted experiments using various Brassica species to observe the effects of limited P fertilization. Using ten different cultivars grown hydroponically, it was found

that both the shoot and root dry matter accumulation was significantly less when lower P concentrations were used, as was the overall leaf area. This study also indicated that there was a wide variability in P use efficiency among not only different species but also among cultivars of the same species. Plants vary in their ability to tolerate low P stress, so optimal concentrations for producing compact growth of ornamentals will likely vary across taxa.

Low P fertilization practices are becoming more widely used in commercial greenhouse production (McMahon, 2011). Utilizing a marginal P deficiency can result in desirable growth control and provide overall compactness without development of advanced symptoms such as reddening, chlorosis, necrosis, and leaf abscission. This marginal P deficiency may be accomplished for short term crops by lowering or eliminating P; however, there is a risk for long term crops developing a severe P deficiency without sufficient P applications.

Reproductive Stage Phosphorus Deficiency

Deficiency symptoms associated with P are generally observed when plant tissue comprises less than 0.2% or 2,000 mg·kg⁻¹ P (Mills and Jones, 1996). Typical symptoms are often described as a reddening or purpling (Figure 1.2) of the lower foliage, an overall darker green coloration, stunted growth, delayed flowering, and greater root lengths (Epstein and Bloom, 2005; Marschner, 1995; Mengel et al., 2001). Symptoms may sometimes manifest as olive green spots (Figure 1.3) of the lower leaves (Whipker, 2014). The occurrence of red coloration is most common and tends to occur most often in cool, wet conditions that are conducive to P deficiency. Olive green leaf spots have more of a tendency to occur during warm temperatures (Whipker, 2014). In either case, deficiency symptoms initially begin to form on the lower leaves due to the fact that P is mobile within the plant. As P becomes deficient, the plant

translocates P from lower plant tissue to the actively growing areas, leading to symptom development on lower leaves. Leaves developing early symptoms may exhibit a dull green coloration which later develops into chlorosis and eventual necrosis.



Figure 1.2. *Impatiens hawkeri* displaying P deficiency symptoms of stunting and reddening of the lower foliage may be seen on the plant on the left, compared to the plant on the right which was provided with a higher concentration of phosphorus. Photo credit: Josh Henry.



Figure 1.3. A leaf from *Capsicum annuum* displaying P deficiency symptoms of necrotic and olive green spots. Photo credit: Josh Henry.

Many factors contribute to P deficiency including available P in the substrate, pH, moisture level, substrate temperature and root health (Mills and Jones, 1996). When there is insufficient P available in the substrate or nutrient solution, this can prevent plants from accumulating sufficient levels of P. Other potential factors relate to inadequate root uptake regardless of the P concentration available in the soil. In the case of excessively high or low moisture in the soil, the roots ability to take up P is reduced, as is the case with soil temperature (Yan et al., 2012). Similar issues will occur when the roots are unhealthy even though environmental conditions and P levels are sufficient in the substrate (Marschner, 1995; Yan et al., 2012).

In the case of a reproductive stage P deficiency, plants that exhibit terminal flowering still translocate P from lower leaves; however, there are two competing sinks for nutrients. We hypothesize that when the plant cannot acquire P from external sources such as the substrate, stores in the lower foliage will translocate toward the developing upper leaves, flowers, and fruits. As reproduction may be considered the ultimate role of the plant, the limited P resources appear to bypass the upper foliage in favor of the flowers and fruits. In this case, the plant is putting more of its resources into reproduction than sustaining its own growth.

In earlier cases of this type of deficiency, it is possible to see the lower and upper leaves begin to turn dull green in coloration, with the upper leaves then becoming chlorotic and later necrotic (Figure 1.4). This symptomology on the lower foliage supports our hypothesis that P is still being translocated out of the lower foliage and into the flowers. Since the upper foliage develops symptoms but the flowers initially remain healthy, it would appear that a bypass of P is indeed occurring. As the severity of the disorder progresses, necrosis may begin to develop in the flower itself, as P eventually becomes deficient throughout all plant tissues (Whipker, 2014).



Figure 1.4. A *Capsicum annuum* plant displaying P deficiency symptoms on the upper foliage. Leaves just below the fruit become chlorotic and necrotic, quickly abscising from the plant. Photo credit: Josh Henry.

Phosphorus Impact on Anthocyanin Production

Anthocyanins are water-soluble pigments that can be found in the vacuoles of all plant parts, both above and below ground (Gould et al., 2009). For ornamental plants, anthocyanins found in the leaves and flowers are most important and contribute a wide range of colors that make them desirable for consumers. Some plants have naturally high levels of anthocyanins that contribute to their red coloration regardless of health status. Many other plant species are known to increase production of anthocyanins in response to increased stress. According to Gould et al. (2009), these conditions can include “strong light, UV-B radiation, temperature extremes,

drought, ozone, N and P deficiencies, bacterial and fungal infections, wounding, herbivory, herbicides, and various pollutants”.

Increased foliar anthocyanin production is one of the primary symptoms commonly associated with P deficiency in plants (Sarker and Karmoker, 2011). This leads to a reddening or purpling that typically occurs on older foliage, and can also account for the “darker green” coloration that sometimes accompanies P deficiency. The vacuoles of the leaf epidermal cells house accumulated anthocyanins which are thought to provide a defense role against excessive sunlight (Hernández and Munné-Bosch, 2015). This photoprotective role would depend on the position of the leaf in the canopy, the thickness of that leaf, and the location of anthocyanin concentration within the leaf tissue (Henry et al., 2012). Anthocyanins would likely play a photoprotective role when located in the leaf epidermis and not in the mesophyll (Henry et al., 2012). Although an exhaustive list of the functions of anthocyanins are currently unknown, it is widely accepted that they play a variety of protective roles.

Henry et al. (2012) conducted experiments to observe the effects on green or red coleus plants given a low ($1 \mu\text{M}$ or $\sim 0.1 \text{ mg}\cdot\text{L}^{-1}$ of P) or high ($50 \mu\text{M}$ or $\sim 5 \text{ mg}\cdot\text{L}^{-1}$ of P) concentration of P. They hypothesized that the naturally red coleus plants containing high levels of anthocyanins might fare better in terms of overall growth under low P conditions than would the green genotypes. Using various light intensities to coincide with the low and high P concentrations, it was actually discovered that the red coleus plants did not have any advantage over the green coleus, and the accumulated biomass of the two differently colored coleus were similar (Henry et al., 2012). This demonstrates that plants grown with low P concentrations are generally more compact unless they are native to areas of low P, such as Australia. This can

account for the fact that many Australian floriculture species are very P efficient and require only low levels of P in order to optimize growth (Gikaara et al., 2004; Nelson et al., 2012).

Limited supply of both P and N can lead to such conditions that decrease growth and lead to higher anthocyanin production (Gould et al., 2009). It is believed that because P deficiency stress limits plant growth, the number of cells that produce anthocyanins and the amount those cells produce both increase (Gould et al., 2009). As growth becomes limited, primary metabolism decreases, and secondary metabolism, such as anthocyanin production increases. There is evidence that indicates certain molecules and precursors that normally go toward primary metabolic processes, such as phenylalanine, go toward secondary metabolic processes when growth is less (Gould et al., 2009). Anthocyanins are actually formed by the metabolism of phenylpropanoid, which is formed from phenylalanine (Glover and Martin, 2012).

Aside from this additional availability of molecular precursors, there are a number of active regulatory processes that may also be controlled by growth and nutrient stress conditions. Genes associated with transcription factors and structural genes that regulate anthocyanin biosynthesis may be expressed more under P deficient conditions (Hosokawa, 2013). An increase in sugar accumulation due to P deficiency may be another factor that leads to higher production of anthocyanins (Hammond et al., 2011; Sarker and Karmoker, 2011).

Popular bedding plants such as zonal geraniums (*Pelargonium x hortorum*) can benefit from lower P application due to the darkening of the foliar bands. Phosphorus stress darkens the bands, which can increase its value as an ornamental bedding plant (Baas et al., 1995). This darkening, often accompanied by an increase in red or purple pigmentation is attributed to increased anthocyanin production onset by P stress. Conversely, P stress may also lead to negative factors such as increased time before flower initiation. This was also found to occur

with zonal geraniums in the study by Baas et al. (1995). For this reason, lower P regiments may sometimes be more beneficial for plants produced for their foliage rather than their flower, though effectiveness certainly varies by species. This issue may be overcome by providing adequate P during the initial period of growth, and then cutting P later in the production cycle.

Research done by Ulrychová and Sosnová (1970) reported that tomato (*Solanum lycopersicum*) plants that were given no P in a nutrient solution developed anthocyanin levels up to five times greater than the levels found in control plants. They also noted that both light quantity and temperature seem to have additive effects on anthocyanin production regardless of cultivar and P concentration in the nutrient solution. Additional research conducted by Rajendran et al. (1992) found that anthocyanin levels in carrot (*Daucus carota*) callus tissue grown in cell culture were significantly higher under both P and N stress when compared to the control. When limiting available P, anthocyanin content as a percent of total dry weight was about 2.25 times greater than the control.

Other plants such as Chinese kale (*Brassica alboglabra*) are available as either green or mauve cultivars, which exhibit different concentrations of anthocyanins within the epidermal cells of their flower stalks. When P is limited, kale plants that are normally green may become mauve in color (Chen et al., 2013). This is another case where lowering the concentration of P may affect the quality of a plant and its value in a potentially positive manner. In experiments conducted by Chen et al. (2013), P concentrations of 30 (normal), 7.5 (low) and 0 mg·L⁻¹ (deficient) were used to observe the effects on plant growth and pigmentation using a green and a mauve Chinese kale cultivar. Results indicated that growth was only significantly less between 7.5 and 0 mg·L⁻¹, though yield was significantly lower with every reduction of P concentration. Additionally, the anthocyanin content was not significantly different between the normal and low

P concentrations, but was significantly greater when no P was supplied. This may indicate that additional P concentrations must be used between low and sufficient levels to better determine if there is a possibility of increasing anthocyanin content without decreasing mass accumulation too drastically.

The link between P deficiency and anthocyanin production has long been established (Mengel et al., 2001; Ulrychová and Sosnová, 1970), though there has been little research explaining the use of reduced P fertilization concentrations in order to enhance pigmentation due to increased anthocyanins. Plants that are naturally high in anthocyanins including coleus and red cabbage may not change significantly in anthocyanin concentration regardless of P fertilization concentration or tissue P concentration (Boldt, 2013). Additionally, decreased shoot P concentrations have been observed alongside increased anthocyanin concentrations in sunflower (Boldt, 2013).

Phosphorus deficient plants with a naturally red foliage display a more intense red coloration when compared to P sufficient plants. Although most studies indicate an increase in anthocyanin production and therefore overall coloration changes (Chen et al., 2013), they also indicate a significant control of plant growth. A number of plants including *Brassica alboglabra* and *Solanum lycopersicum* have already been reported (Chen et al., 2013; Ulrychová and Sosnová, 1970) to respond to low P levels by increasing anthocyanin production. These plants may be good candidates for finding optimal concentrations for improved coloration fertilization regimens.

Other methods of increasing anthocyanin content in plants may be emerging with the advancement of light-emitting diodes (LEDs) (Owen and Lopez, 2015). Environmental factors such as increased light intensity have been found to increase anthocyanin production and

accumulation in lettuce and enhance the red coloration in the foliage of multiple lettuce cultivars (Owen and Lopez, 2015). Additional environmental parameters such as temperature are also found to affect anthocyanin production, and may act as additional variables for determining ways to improve red coloration in some plants (Bolt, 2013). By combining multiple growing conditions including optimal P concentrations for enhanced coloration, various light sources, intensities and temperatures, it may be possible to significantly alter the red coloration of certain species without compromising the overall health and quality of the plants.

Potential Challenges

While it is already well established that limited P fertilization can greatly impact plant growth and specifically plant size, specific concentrations for height control are relatively unavailable. Work done by Nelson et al. (2002, 2012) indicates that there is a small range of concentrations toward the lower end of P fertilization which has the greatest effect on plant growth. Preliminary experiments on various ornamental bedding plants also seem to indicate that a fertilizer P concentration between 5 – 10 mg·L⁻¹ applied as a constant liquid feed may be sufficient for many plant species when grown in peat based substrate. This range of P concentrations is in agreement with those suggested for growth control by McMahon (2011).

It appears that the range of 0 – 10 mg·L⁻¹ is of the greatest importance to the various aspects of this research. Although Nelson's research was comprehensive for bedding plant plug production, more work is required to determine optimal P concentrations for bedding plants throughout the entire production period. Other experiments are in agreement with the fact that P concentrations above the 5 – 10 mg·L⁻¹ range do little to alter overall growth or pigmentation due to anthocyanin accumulation (Akhtar et al., 2007). Determining P concentrations and regiments

to optimize compact growth and enhanced coloration of certain ornamental crops is a primary portion of this intended research.

A major issue that may occur while trialing such low P fertilization concentrations is occurrence of P deficiency symptoms. While there are already several well documented symptoms known to occur on a wide variety of plants, the symptomology described by Whipker (2014) does not appear to be described in any other literature. While conducting experiments with low P fertilizer concentrations, it would be beneficial to attempt to replicate these types of symptoms. Using low, but adequate concentrations of P during the majority of the production period, and later eliminating P from the fertilization regiment may be a good strategy to use to induce these reproductive stage P deficiency symptoms.

Research Objectives

The three primary objectives of this research are to establish optimal fertilizer P concentrations for controlling growth of greenhouse grown ornamentals, determine if moderate P deficiency may be used to enhance coloration of red-leaved plants, and to induce and describe the symptoms of reproductive stage P deficiency described by Whipker (2014). Recent research has established that there is indeed potential to successfully control growth using low P fertilization during plug production (Nelson et al., 2012). Additional studies are required to develop optimal P concentrations for the wide range of different floricultural crops that are produced.

Boldt (2013) noted that further research was needed to determine factors associated with increasing anthocyanin content in ornamentals. As P stress contributes significantly to anthocyanin accumulation, P fertilization regiments appear crucial for improving foliar

coloration of ornamental plants. Inducing mild P deficiency has the potential to increase red coloration, but care must be taken to avoid the negative aspects of P deficiency, which can damage plants beyond marketability.

Utilizing low P fertilization brings with it the risk of developing P deficiency symptoms. This research aims to better understand the symptoms of reproductive stage P deficiency, and provide recommendations for growers to avoid this issue. Determining P concentration recommendations for bedding plant growers could help to optimize crop production, leading to plants with more valuable characteristics while utilizing less fertilizer.

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Chapter 2

Growth Response of Herbaceous Ornamentals to Low Phosphorus Fertilization

Abstract

A series of experiments were conducted to investigate the effects of increasing phosphorus (P, referring to phosphorus supplied by phosphate) concentrations on the overall growth and development of six horticultural species. In experiment 1, alternanthera [*Alternanthera brasiliana* (L.) Kuntze] and petunia [*Petunia atkinsiana* (Sweet) D. Don ex W. H. Baxter] were grown using fertilizer P concentrations of 0, 1.25, 2.5, 5, 10, 20, 40, and 80 mg·L⁻¹ at every irrigation to determine upper and lower bounds of plant growth response to P. Experiment 2 was conducted to observe growth response to various constant P concentrations over time. Two cultivars each of New Guinea impatiens (*Impatiens hawkeri* W. Bull), vinca [*Catharanthus roseus* (L.) G. Don], and angelonia (*Angelonia angustifolia* Benth.) were grown using concentrations of 0, 2.5, 5, 10, and 20 mg·L⁻¹ P applied at every irrigation. Biweekly measurements were collected for plant height and diameter. In experiment 3, *Capsicum annuum* ‘Tango Red’ plants were initially grown using concentrations of 0, 2.5, 5, 10, and 20 mg·L⁻¹ P at every irrigation. After six weeks, half of the plants grown with each P concentration were then switched to 0 mg·L⁻¹ P to observe whether plants could be supplied with sufficient levels of P for the first half of production, and then finished without P to order to keep them compact. All experiments were analyzed using linear or quadratic regression, and quadratic nonlinear regression with plateaus to determine a best fit for the data. In most cases, growth plateaus were selected and used to determine which P concentrations would result in maximum growth for several parameters. These experiments all indicated that current P fertilization regimens exceed the P requirements of these plants, and that most greenhouse bedding plants only require a continual supply of 5 – 13 mg·L⁻¹ P to maximize growth.

Introduction

Producers of floriculture crops strive to cultivate compact and healthy plants which are considered high quality and attractive for consumers. Growers use a variety of methods to control growth in their crops (Dole and Wilkins, 2005), however chemical growth regulators such as plant growth retardants (PGRs) are the most commonly used method in ornamental production (Whipker, 2017). A concern associated with PGRs is that they are not labelled for all crops. This is especially true for vegetable growers who are limited on chemical options (Nelson et al., 2012). Numerous cultural methods for controlling plant growth can be used, often in association with one another. These methods include drought stress, nutrient restriction, light and temperature manipulation, and use of smaller growing containers (Dole and Wilkins, 2005; Gibson et al., 2007). These methods produce more compact plants, although this study only focused on nutrient restriction, specifically of phosphorus (P).

Although the nitrate (NO_3^-) form of N has often been used to keep plants compact, recent findings indicate that it is the low P level in high nitrate fertilizers that accounts for compactness (Nelson et al., 2012). Most NO_3^- based fertilizers that are recommended for compact plant growth are also low in P. Commercial fertilizer formulations including 13N–0.9P–10.8K, 14N–0P–11.6K and 15N–0P–12.5K are all high in NO_3^- sources of N and contain little to no P. N is mostly supplied as calcium, magnesium or potassium nitrate (Nelson et al., 2002). Erroneously, it was thought that fertilizer formulations high in ammoniacal nitrogen (NH_4^+) result in greater plant growth. Experiments conducted by Nelson et al. (2002) used constant ratios of N source, but varied concentrations of P, and found that plant size increased with increasing P concentrations. Previously, it had been thought that higher P concentrations would only increase plant growth until plant P concentration reached 0.25% of total dry matter (Nelson et al., 2002).

Phosphorus restriction can be effective and easy to implement for short-term crops, such as with young plant (plug) production (Nelson et al., 2012), but long-term crops may be more difficult to successfully manipulate. This is especially true in greenhouse bedding crops that are typically produced in soilless substrates, which have limited P holding capacity (Marconi and Nelson, 1984; Whipker, 2014). Without adequate P, long-term crops have the potential to deplete the initial charge of P in the substrate and may have to begin recycling and reallocating P from older plant tissues, leading to the appearance of deficiency symptoms on the lower leaves (Mengel et al., 2001). Nevertheless, P also has the widest window between beneficial control of plant growth due to mild nutrient stress, and detrimental deficiency symptoms when compared with limitation of other essential nutrients. After the critical value of sufficient P has been reached, most plants exhibit a broad growth plateau where luxury P consumption does not change growth (Nelson et al., 2012). This makes P a safer and potentially easier input to be manipulated for regulation of plant growth.

Deficiency symptoms associated with P are commonly observed when dry plant tissue comprises less than 0.2% or 2,000 mg·kg⁻¹ P (Mills and Jones, 1996). Typical symptoms are often described as a reddening or purpling of the lower foliage, an overall darker green coloration, stunted growth, delayed flowering, and greater root lengths (Epstein and Bloom, 2005; Marschner, 1995; Mengel et al., 2001). For leaf tissue concentrations, a range of approximately 0.2–0.5% of total plant dry weight is generally considered sufficient P for most plants (Mills and Jones, 1996).

Excessive applications of agricultural nutrients including P have caused many water quality issues in recent years. The algae blooms occurring in Lake Erie due to excessive P is just one example of these problems (Wines, 2014). Many municipalities and agencies are also

becoming more aware of the issues associated with excessive nutrient application and runoff into water sources. Greenhouse and nursery growers in the Chesapeake Bay watershed have been encouraged to use improved management practices to reduce unnecessary nutrient runoff and improve the bay water quality (Majsztrik and Lea-Cox, 2013). In the Florida Everglades, P concentrations can be 60 times the maximum level determined safe for the unique organisms that live there (Liu et al., 2015). With the various environmental issues associated with excessive P application, growers will need to reduce their P fertilization strategies in order to limit runoff.

Many commercial fertilizers used in greenhouse production and mixed at recommended concentrations can supply greater P concentrations than required by plants, as is the case with 20N–8.7P– 16.6K. This fertilizer mixed at a concentration of 200 mg·L⁻¹ N would provide 87 mg·L⁻¹ P. One recommendation for growth control in greenhouse crops by McMahon (2011) suggests using concentrations of only 5 – 10 mg·L⁻¹ P. This study aims to determine the quantity of P greenhouse crops require for optimal growth, in order to provide improved recommendations for growers, with additional environmental benefits of reduced fertilizer waste and runoff.

Materials and Methods

All plants were propagated and grown in a glass greenhouse at 35°N latitude in Raleigh, NC. Greenhouse day/night temperature set points were 23.9/18.3°C. Plants were grown under natural photoperiod. The substrate used for the entire experiment was an 80:20 (v:v) mix of Canadian sphagnum peat moss (Conrad Fafard, Agawam, MA) and horticultural coarse perlite (Perlite Vermiculite Packaging Industries, Inc., North Bloomfield, OH), with added dolomitic lime at 8.875 kg/m³ (Rockydale Agricultural, Roanoke, VA) and AquaGro 2000·G Wetting

Agent (Aquatrols, Cherry Hill, NJ) at 600.3 g/m^3 . This custom substrate was used to ensure that there was no initial charge of P.

Fertilization began on the day of transplant, and fertilizers were custom blends of the following individual technical grade salts: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, KNO_3 , KH_2PO_4 , K_2SO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Mg}(\text{NO}_3)_2$, FeDTPA, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{ZnCl}_2 \cdot 7\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, H_3BO_3 , and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (Appendix A). Phosphorus (referring to phosphate-phosphorus) concentrations were varied among treatments while other essential nutrients were adjusted to remain as constant as possible. Nitrogen (N) and potassium (K) were held at $150 \text{ mg} \cdot \text{L}^{-1}$, with all other essential microelements remaining constant. All N was supplied from NO_3^- sources. Fertilizer solution was mixed in 100 L barrels, and applied via sump pumps (Model 1A, Little Giant Pump Co., Oklahoma City, OK) connected to 1.9 cm black irrigation tubing fitted with either drip rings (Dramm USA, Manitowoc, WI) or dribble tubes (Dramm USA, Manitowoc, WI).

Several growth parameters were recorded for each species. Plant height was measured from the rim of the pot to the highest point on the plant, and diameter was recorded by averaging the widest point and the axis perpendicular to that. Substrate pH and electrical conductivity (EC) were obtained using the Pour-Thru method (Cavins et al., 2005), and recorded using a HI 9813-6 portable meter (Hanna Instruments, Woonsocket, RI).

Experiment 1

Concentrations of 0, 1.25, 2.5, 5, 10, 20, 40, and $80 \text{ mg} \cdot \text{L}^{-1}$ P were used to determine the upper and lower bounds of growth response to P. One cultivar of alternanthera and two cultivars of petunia were grown in this experiment. All plants were rooted from cuttings in 128 cell plug trays with cell dimensions of $2.7 \times 2.7 \times 3.8 \text{ cm}$ (length x width x depth). Once cuttings had

rooted out to the edge of the cell, they were transplanted into 5-inch diameter pots (Dillen, Middlefield, OH) with dimensions of 12.7 x 9.2 cm (diameter x depth) and a volume of 0.8 L. During the course of the experiment, the maximum temperature was 27.1°C, the minimum was 15.1°C, and the mean was 19.7°C.

Alternanthera (*Alternanthera brasiliana*) ‘Brazilian Red’ cuttings were stuck on 4 Aug. 2015 and rooted under mist. Cuttings were not supplied with any fertilizer until treatments began. After the cuttings had rooted out, they were transplanted on 27 Aug. 2015. Seven single plant replicates were grown for each of the eight P fertilizer treatments. Plants were measured and destructively harvested six weeks after transplant, on 8 Oct. 2015.

Petunia (*Petunia atkinsiana*) ‘Surprise Sky Blue’ and ‘Potunia Neon’ cuttings (Dümmen Orange, Columbus, OH) were stuck on 28 Sept. 2015 and rooted under mist. ‘Surprise Sky Blue’ was chosen to represent a petunia with a trailing growth habit, and ‘Potunia Neon’ was grown to represent a petunia with a compact growth habit. Cuttings were not supplied with any fertilizer until cuttings were transplanted on 23 Oct. 2015. The experiment was completely randomized with eight single plant replicates of each of the eight treatments. After eight weeks of growth, a destructive harvest was completed on both cultivars on 17 Dec. 2015. Substrate pH and EC were recorded, and leachate was submitted for analysis at the North Carolina Department of Agriculture & Consumer Services (NCDA&CS, Raleigh, NC).

Upon termination of each species, measurements were taken, and tissue samples were collected from the most recently matured leaves. These tissue samples were washed in a solution of 0.5 M hydrochloric acid (HCl), followed by a rinse of deionized water. The remaining vegetative tissues were cut off at the substrate and dried. Tissue samples were also allowed to dry for at least 72 hours at 70°C, and total plant dry mass was recorded. After drying, tissue

samples were ground using a Thomas Wiley® Mini-Mill (Thomas Scientific, Swedesboro, NJ), and analyzed for nutrient concentrations by AgSource Laboratories (Lincoln, NE). Total N was processed by Kjeldahl digestion, and determined via flow injection analysis (FIA). Extractable K was processed by 2% acetic acid digestion, and determined via inductively coupled plasma mass spectrometry (ICP-MS). Total P and all other plant minerals were processed by nitric acid/hydrogen peroxide digestion, and determined via ICP-MS.

Experiment 2

Two cultivars each of angelonia (*Angelonia angustifolia*), vinca (*Catharanthus roseus*), and New Guinea impatiens (*Impatiens hawkeri*) were grown in this study. All cuttings were stuck or seeds sown into 128-cell plug trays. During the course of the experiment, the maximum temperature was 35.4°C, the minimum was 15.8°C, and the mean was 23.7°C. Experiments were conducted under natural photoperiod, with 50% shade (Ludwig Svensson Inc., Charlotte, NC), drawn between 11:00 and 15:00. Phosphorus fertilizer treatments of 0, 2.5, 5, 10, or 20 mg·L⁻¹ were used, with N and K remaining constant at 150 mg·L⁻¹ each. Tubing was fitted with either drip rings (Dramm USA, Manitowoc, WI) for angelonia and New Guinea impatiens or Dribble Tubes (Dramm USA, Manitowoc, WI) for vinca. Biweekly measurements were collected for plant height and diameter.

Angelonia

A. angustifolia ‘Sungelonia Blue’ and ‘Sungelonia White’ cuttings (Dümmen Orange, Columbus, OH) were stuck on 3 May 2016. Rooted cuttings were transplanted on 27 May 2016 into 5-inch pots. A final destructive harvest was conducted eight weeks after transplant.

Catharanthus

C. roseus ‘Cora Burgundy’ and ‘Pacifica Blush XP’ (Fred C. Gloeckner & Co., Inc., Harrison, NY) were planted on 23 Apr. 2016. Once roots reached the edges of the cell walls, they were transplanted into 4.5-inch standard pots (Dillen, Middlefield, OH) with dimensions of 11.4 x 9.7 cm (diameter x depth) and a volume of 0.6 L on 2 June 2016. A final destructive harvest was conducted seven weeks after transplant.

Impatiens

I. hawkeri ‘Tamarinda Dark Red’ and ‘Pure Beauty Red on Pink’ cuttings (Dümmen Orange, Columbus, OH) were stuck on 3 May 2016. ‘Tamarinda Dark Red’ was chosen to represent a green leaf cultivar, and ‘Pure Beauty Red on Pink’ was chosen to represent a dark leaf cultivar. Once the cuttings were well rooted, plugs were transplanted on 24 May into 5-inch pots. Ten weeks after transplant, the experiment was terminated and plants were destructively harvested.

Experiment 3

Ornamental peppers (*Capsicum annuum*) ‘Tango Red’ seeds (Fred C. Gloeckner & Co., Inc., Harrison, NY) were sown on 6 Apr. 2016 into 1204 flat inserts with cell dimensions of 5.7 x 3.8 x 5.4 cm (length x width x depth). Peppers were transplanted on 13 May into 5.5-inch diameter pots (Dillen, Middlefield, OH) with dimensions of 13.7 x 11.6 cm (diameter x depth) and a volume of 1.28 L. Plants were initially grown using concentrations of 0, 2.5, 5, 10, and 20 mg·L⁻¹ P. After six weeks, 12 plants from each non-zero P concentration were switched to 0 mg·L⁻¹ P, while twelve plants remained on their original P concentration. Upon switching, all

pots received a drench of unfertilized water with a 50% leaching fraction, in order to leach P out of the substrate.

There were three destructive harvests, which occurred at six, nine, and eleven weeks after transplant. At each harvest, growth parameters were measured for six individual plants replicates. Plant height and diameter were recorded using the previously described methods. Substrate pH and EC were recorded, and leachate from four single plants replicates submitted to NCDA&CS for nutrient analysis. Tissue samples of the most recently matured leaves were also collected at each harvest from four single plant replicates, and handled utilizing procedures previously described for nutrient analysis.

Data Analysis

Height, diameter, and dry mass were analyzed and used to calculate a growth index (GI) (Equation 1) which was based off the equation for GI presented by Krug et al. (2010). For petunia, a modified GI was calculated which excluded height, due to the fact height was not measured for this species.

Equation 1

$$GI = \frac{\text{height} + \frac{\text{diameter 1} + \text{diameter 2}}{2} + \text{dry mass}}{3}$$

Data obtained from all experiments were analyzed using SAS (version 9.4; SAS Institute, Cary, NC). Height, diameter, dry mass, substrate pH, and EC data were subjected to PROC GLM and the means were separated by Tukey' honestly significant differences at $P \leq 0.05$. Data was combined between cultivars whenever there were no interactions between P concentration and

cultivar. PROC REG was used to regress the data and determine the best fit linear or quadratic model for the different P concentrations. PROC NLIN was also used to determine best fit quadratic plateau models (Equation 2) for each growth parameter.

Equation 2

$$Y_i = \begin{cases} \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \varepsilon_i & \text{if } X < X_0 \\ Y_0 + \varepsilon_i & \text{if } X \geq X_0 \end{cases}$$

Where:

$$X_0 = -\frac{\hat{\beta}_1}{2\hat{\beta}_2}, \text{ and}$$

$$Y_0 = \hat{\beta}_0 - \frac{\hat{\beta}_1^2}{4\hat{\beta}_2}$$

Quadratic models obtained from PROC REG and quadratic plateau models obtained from PROC NLIN were then compared and selected based on highest r^2 values. X_0 values provided for growth plateaus indicate the P concentration at which each growth parameter reached its respective maximum, past which point no increased growth was observed.

Results

Experiment 1

Alternanthera ‘Brazilian Red’ plants (Figure 2.1) exhibited a growth response best suited to quadratic regression with a plateau. From visual observations, it was noted that plant size reached its maximum at $\sim 5 \text{ mg}\cdot\text{L}^{-1}$ P. This visual observation is supported by the data for height, diameter, and dry mass. The model for height (Figure 2.2 A) plateaued at $4.37 \text{ mg}\cdot\text{L}^{-1}$ P,

specified by the X_0 value, indicating that further increasing P concentration would not result in increased plant height past this quantity. Plant diameter (Figure 2.2 B) displayed a similar growth plateau in which diameter did not change significantly with applications greater than $5.64 \text{ mg}\cdot\text{L}^{-1}$ P. The model for dry mass (Figure 2.3 A) also plateaued with a low P concentration of $7.04 \text{ mg}\cdot\text{L}^{-1}$ P. These models together indicate that increased growth may not be achieved with P concentrations greater than $4 - 7 \text{ mg}\cdot\text{L}^{-1}$. The model for GI determined the optimal P concentration across the major growth parameters of height, diameter, and dry mass (Figure 2.3 B). This model plateaus at $5.5 \text{ mg}\cdot\text{L}^{-1}$ P, indicating that maximum growth of alternanthera was achieved with this P concentration.

Tissue P concentrations ranged from 0.10% of dry matter in plants grown without P to 0.50% in plants grown with $20 \text{ mg}\cdot\text{L}^{-1}$ P (Table 2.1). Foliar P concentrations were significantly lower for plants grown without P than plants grown with $2.5 \text{ mg}\cdot\text{L}^{-1}$ P or more. No significant increases in foliar P concentrations were observed with fertilizer P concentrations greater than $20 \text{ mg}\cdot\text{L}^{-1}$ P. Foliar N concentrations were lowest for plants grown with $0 - 1.25 \text{ mg}\cdot\text{L}^{-1}$ P, and were highest in plants grown with $10 - 80 \text{ mg}\cdot\text{L}^{-1}$ P. In contrast, foliar K concentrations were highest in plants grown with $1.25 - 2.5 \text{ mg}\cdot\text{L}^{-1}$ P, and lowest in plants grown with $80 \text{ mg}\cdot\text{L}^{-1}$ P. Calcium (Ca) and magnesium (Mg) concentrations followed similar increasing trends to each other. Plants grown without P had lowest foliar Ca and Mg concentrations, while plants grown with $5 - 80 \text{ mg}\cdot\text{L}^{-1}$ P had the highest. Foliar zinc (Zn) concentrations were highest in plants grown without P and lowest in plants grown with $5 - 10 \text{ mg}\cdot\text{L}^{-1}$ P. High P concentrations are known to induce Zn deficiency (Epstein and Bloom, 2005; Marschner, 1995). Although higher P concentrations resulted in plants with significantly lower foliar Zn concentrations, these Zn concentrations were above the sufficient concentration of 20 parts per million (ppm) (Epstein and Bloom, 2005).

Foliar manganese (Mn) concentrations were highest in plants grown with 0 – 1.25 mg·L⁻¹ P, and were lowest in plants grown with 5 – 40 mg·L⁻¹ P.

Visual differences in plant growth may be observed for *P. atkinsiana* ‘Surprise Sky Blue’ and ‘Potunia Neon’ (Figure 2.4). Plants grown with less than 5 mg·L⁻¹ P exhibited P deficiency symptoms, ranging from mild to severe stunting, with lower leaf chlorosis, necrosis, and in many cases, complete floral inhibition. Quadratic regression models with plateaus provided a best fit for diameter (Figure 2.5 A) and dry mass (Figure 2.5 B) for these plants. Plant diameter plateaued at 8.31 and 8.81 mg·L⁻¹ P, respectively for ‘Surprise Sky Blue’ and ‘Potunia Neon’. The amount of P required to reach these growth plateaus was similar for both cultivars, despite the fact that maximum diameter of ‘Surprise Sky Blue’ was nearly double that of ‘Potunia Neon’.

Maximum dry mass accumulation for ‘Potunia Neon’ was roughly half that of ‘Surprise Sky Blue’ (Figure 2.5 B). Maximum dry mass for ‘Surprise Sky Blue’ was reached at 11.49 mg·L⁻¹ P, while ‘Potunia Neon’ plateaued at 14.11 mg·L⁻¹ P. This indicates that although there were no significant increases in plant diameter above 8.31 – 8.81 mg·L⁻¹ P, dry mass continued to accumulate with greater P concentrations. GI provides a more accurate account of where maximum growth was achieved for each cultivar (Figure 2.6). Maximum growth was achieved for ‘Surprise Sky Blue’ and ‘Potunia Neon’ at 8.72 and 9.08 mg·L⁻¹ P, respectively. This indicates that optimal P concentrations of ~9 mg·L⁻¹ can be used to provide maximum growth for these two petunia cultivars.

Models for substrate pH (Figure 2.7 A) and EC (Figure 2.7 B) for petunia also exhibited trends that demonstrate plant growth response to increasing concentrations of P. ‘Surprise Sky Blue’ substrate pH values followed a similar growth plateau to other measured parameters,

where pH plateaued above P concentrations of $6.41 \text{ mg}\cdot\text{L}^{-1}$. ‘Potunia Neon’ did not exhibit a plateau within the range of $0 - 20 \text{ mg}\cdot\text{L}^{-1}$, and instead followed a quadratic regression trend. In this model, pH gradually increased, and then began to drop with increasing P concentrations above P concentrations of $50 \text{ mg}\cdot\text{L}^{-1}$. The increase in pH between P concentrations of 0 and $6.41 \text{ mg}\cdot\text{L}^{-1}$ P for ‘Surprise Sky Blue’ can be explained by the fact that high NO_3^- concentrations resulted in the fertilizer being basic. With higher P concentrations, EC decreased, indicating greater fertilizer uptake. As NO_3^- absorption increased by the plant, hydroxide (OH^-) and bicarbonate (HCO_3^-) secretion significantly increased substrate pH (Marschner, 1995).

This trend in pH may be further supported by observing the trend in EC. Substrate conductivity decreased from a maximum of 2.4 mS/cm , before plateauing at 1.9 mS/cm at P concentrations greater than $8.69 \text{ mg}\cdot\text{L}^{-1}$. Substrate EC decreases as plants uptake fertilizer, which occurs with greater concentrations of P. This trend can be explained by the limited plant growth which occurred with $0 - 8.69 \text{ mg}\cdot\text{L}^{-1}$ P, which resulted in a lower demand for nutrients. As plants take up more P and more fertilizer, pH increases, to the point at which fertilizer uptake reaches equilibrium and plateaus. These overall trends in substrate pH and EC are not specifically due to P uptake alone, but due to the fertilizer salts being basic in nature, with all N provided by NO_3^- sources.

Foliar P concentrations ranged from 0.05% in plants grown without P, up to 1.21% in plants grown with $80 \text{ mg}\cdot\text{L}^{-1}$ P (Table 2.2). Plants grown with $80 \text{ mg}\cdot\text{L}^{-1}$ P had significantly higher foliar P concentrations than plants grown with any other P concentration. This eludes to the fact that plants exhibit luxury P consumption past the point plants have reached maximum size in response to P (Nelson et al., 2012). Foliar N concentrations were lowest in plants grown with $5 - 10 \text{ mg}\cdot\text{L}^{-1}$ P. Foliar K concentrations were lowest in plants grown without P, and highest

in plants grown with 5 or 20 – 80 mg·L⁻¹ P. These trends in foliar nutrient concentrations illustrate the influence of P fertilization on the absorption and accumulation of other essential elements.

Experiment 2

Over the course of ten weeks, height and diameter of *Impatiens hawkeri* plants followed similar overall growth trends. Means of plant height were plotted for ‘Tamarinda Dark Red’ (Figure 2.8 A) to compare P concentrations over time. Plants grown with 5 – 10 mg·L⁻¹ P increased with a similar trend. Plants grown with 2.5 mg·L⁻¹ P had similar height to plants grown with greater concentrations for the initial four weeks, before trends diverged, and increases in plant height were less over time. Weekly means for height of ‘Pure Beauty Red on Pink’ (Figure 2.8 B) were similar between plants grown with 10 and 20 mg·L⁻¹ P, with lower concentrations following similar increasing trends over time.

Diameter of ‘Tamarinda Dark Red’ (Figure 2.8 C) increased over time for plants grown at all P concentrations, except those grown without P. Plants grown without P exhibited decreasing overall diameter, which was due to changes in overall leaf architecture. Over time, leaves of ‘Tamarinda Dark Red’ plants grown without P became upward oriented, resulting in plants having a narrower appearance (Figure 2.9). Diameter of ‘Pure Beauty Red on Pink’ (Figure 2.8 D) exhibited a similar trend as ‘Tamarinda Dark Red’, with the exception that plants grown without P also increased in diameter over time. Although ‘Pure Beauty Red on Pink’ plants were still severely stunted, they were not as severely effected as ‘Tamarinda Dark Red’ (Figure 2.9).

Quadratic growth plateau models demonstrate optimal concentrations of P for height and diameter of each New Guinea impatiens cultivar. The model for height (Figure 2.10 A) indicated that maximum height was achieved using P concentrations of 10.07 mg·L⁻¹ for ‘Pure Beauty Red on Pink’ and 8.06 mg·L⁻¹ for ‘Tamarinda Dark Red’. After this point, there was no significant increase in plant height with increasing P concentrations. The model for diameter (Figure 2.10 B) indicated that maximum diameter was achieved using P concentrations of 14.1 mg·L⁻¹ for ‘Pure Beauty Red on Pink’ and 10.08 mg·L⁻¹ for ‘Tamarinda Dark Red. This demonstrates that although maximum height was reached with P concentrations of ~8 – 10 mg·L⁻¹, diameter may still increase with higher P concentrations. Plateaus for GI illustrate which P concentrations can be used for maximum overall growth (Figure 2.11). ‘Tamarinda Dark Red’ reached maximum growth with 9.64 mg·L⁻¹ P, and ‘Pure Beauty Red on Pink’ reached maximum growth with 12.42 mg·L⁻¹ P.

C. roseus ‘Cora Burgundy’ plants exhibited similar increasing trends for plant height over the course of six weeks (Figure 2.12 A) for all P concentrations, except 0 mg·L⁻¹. Plants grown without P were stunted for the entire experiment in terms of overall height, and were roughly the same size as they had been as plugs, prior to transplant (Figure 2.13). Height of ‘Pacifica XP Blush’ plants (Figure 2.12 B) exhibited a nearly identical growth trend to ‘Cora Burgundy’, with the exception that these plants were generally smaller at every measurement date. ‘Cora Burgundy’ and ‘Pacifica XP Bush’ plants grown without P did not increase in height over time, and appeared similar to the plugs that had been transplanted at the beginning of the experiment, six weeks earlier (Figure 2.13).

Trends in diameter of ‘Cora Burgundy’ (Figure 2.12 C), and ‘Pacifica XP Blush’ (Figure 2.12 D) both indicate that maximum diameter was achieved for each P concentration after four

weeks of growth. Both cultivars exhibited high levels of growth between two and four weeks after transplant for all P concentrations except 0 mg·L⁻¹. Without P, these plants did not increase in height or diameter over time. In contrast, plants grown with 2.5 mg·L⁻¹ P grew over time and matured to the point of producing flowers, but were stunted and exhibited mild symptoms of chlorosis on the lower leaves. Plants grown without P were flowerless, and did not change in size over the course of six weeks. This indicates that vinca does not require high amounts of P, but was severely affected without any additional supply of P. This severe stunting may also have occurred due to the fact that these plants were grown from seed, whereas New Guinea impatiens and angelonia were grown from cuttings. Cuttings likely have higher stores of P than the small seeds of vinca, indicating that plants grown from cuttings could still achieve some level of growth without an additional supply of P.

Growth plateaus for vinca indicated that maximum height were achieved for ‘Cora Burgundy’ and ‘Pacifica XP Blush’ using P concentrations of 8.05 mg·L⁻¹ and 8.0 mg·L⁻¹, respectively (Figure 2.14 A). This illustrates that although these two cultivars exhibited different maximum height, they achieved their individual maxima with similar P concentrations. Growth plateaus for diameter (Figure 2.14 B) indicate that maximum diameters for ‘Cora Burgundy’ and ‘Pacifica XP Blush’ occurred with 3.8 and 4.56 mg·L⁻¹, respectively. These concentrations are very low, indicating that vinca requires low P concentrations to achieve maximum diameter. Overall GI values demonstrate that ‘Cora Burgundy’ required 6.74 mg·L⁻¹ P to achieve maximum growth, while ‘Pacifica XP Blush’ required 6.43 mg·L⁻¹ P (Figure 2.15). Little difference was observed between optimal P concentrations for these two cultivars.

A. angustifolia ‘Sungelonia White’ height increased over the course of eight weeks, for all P concentrations (Figure 2.16 A). Plants grown without P increased in height, though were

significantly stunted as compared to plants grown with 2.5 mg·L⁻¹ P. ‘Sungelonia Blue’ plants had similar increasing trends in height for all P concentrations (Figure 2.16 B). Diameter of ‘Sungelonia White’ (Figure 2.16 C) and ‘Sungelonia Blue’ (Figure 2.16 D) increased over time for all P concentrations, except those grown with 0 mg·L⁻¹. Plants grown without P decreased in diameter over time. This was due to changes in leaf architecture, as the leaves of plants grown without P were oriented upward, while all other plants had leaves held perpendicular to the stem.

Growth plateaus for ‘Sungelonia White’ and ‘Sungelonia Blue’ illustrate that maximum height was achieved using P concentrations of 4.21 mg·L⁻¹ and 4.51 mg·L⁻¹, respectively (Figure 2.17 A). Maximum diameter was achieved using P concentrations of 8.2 mg·L⁻¹ for ‘Sungelonia White’ and 9.76 mg·L⁻¹ for ‘Sungelonia Blue’ (Figure 2.17 B). When combining height, diameter, and dry mass into a single GI, ‘Sungelonia White’ reached maximum growth with 7.27 mg·L⁻¹ P, and ‘Sungelonia Blue’ reached maximum growth with 8.78 mg·L⁻¹ P (Figure 2.18).

Experiment 3

Six weeks after transplant, significant differences in several growth parameters of ornamental peppers were observed among the five different P concentrations (Table 2.3). Height was greatest for plants grown with 20 mg·L⁻¹ P, and was lowest for plants grown without any P. Diameter, dry mass, and GI also followed this same trend. Substrate pH was significantly lower for plants grown with 0 and 2.5 mg·L⁻¹ P, and higher for plants grown with 10 and 20 mg·L⁻¹ P. Although there were differences in height, diameter, dry mass, GI, and substrate pH, there were no significant differences in substrate EC.

After nine weeks, measurements were also taken for plants grown with the initial five P concentrations for six weeks then restricted to 0 mg·L⁻¹ P (Table 2.4). Plants that were restricted

will be referred to as having an initial concentration of P, to differentiate from plants that maintained a continuous P concentration throughout the experiment. Plant height was least for plants grown without P. Plants grown with 10 and 20 mg·L⁻¹ P were tallest. Plants grown with initial concentrations of 20 mg·L⁻¹ P were significantly shorter than those grown with continuous concentrations of 20 mg·L⁻¹ P; however, there were not significant differences among other treatments of the same initial or continuous P concentration. Diameter was also significantly less for plants grown without P, and was greatest for plants grown with continuous concentrations of 10 or 20 mg·L⁻¹ P, or an initial concentration of 20 mg·L⁻¹ P. Dry mass was least for plants grown with 0 mg·L⁻¹ P, or 2.5 mg·L⁻¹ P initially or continuously. Plants grown with 20 mg·L⁻¹ P had the greatest dry mass. When combining all of these parameters into the GI, plants grown without P had the least growth, while plants grown with 20 mg·L⁻¹ P had the most.

At week eleven, growth parameters followed similar trends to what was observed at week nine (Table 2.5). Height was greatest for plants grown with continuous concentrations of 10 and 20 mg·L⁻¹ P, or an initial concentration of 20 mg·L⁻¹ P, and plants grown without P were the shortest. Diameters for plants grown with 10 and 20 mg·L⁻¹ P were the greatest, while plants grown without P were the smallest. Dry mass was greatest for plants grown with 20 mg·L⁻¹ P, and was least for plants grown with 0 mg·L⁻¹ P, or initial concentrations of 2.5 mg·L⁻¹ P. GI was least for plants grown without P and greatest for plants grown with 10 or 20 mg·L⁻¹ P. This demonstrates that growth of *C. annuum* ‘Tango Red’ can be controlled by using continuous concentrations less than 10 mg·L⁻¹ P, or any restricted P concentration.

Although low or restricted P concentrations could successfully control growth, a number of plants developed detrimental symptoms of P deficiency. Upon termination of the experiment, P deficiency symptoms of chlorosis, necrosis, and leaf abscission occurred on plants grown

without P or with a continuous concentration of $2.5 \text{ mg}\cdot\text{L}^{-1}$ P. Plants grown without P also did not set fruit and had similar height as when they were initially transplanted. Additionally, all plants that had been switched to a $0 \text{ mg}\cdot\text{L}^{-1}$ P fertilization regiment developed at least some level of P deficiency symptoms. Symptoms were less severe for plants grown with the highest initial concentration of $20 \text{ mg}\cdot\text{L}^{-1}$ P, however symptoms still developed. The fact that symptoms developed on plants grown with the highest initial P concentration illustrates how P may be restricted to control growth; however, P was still required to maintain healthy growth. Without at least a low P concentration being supplied via fertilization, P in the substrate and in the plant was depleted and led to deficiency symptoms developing.

Analysis of the most recently matured foliage demonstrates how tissue P concentrations change with fertilizer P concentrations over time (Table 2.6). Tissue P concentrations were highest for plants grown with $20 \text{ mg}\cdot\text{L}^{-1}$ P at weeks six and nine. At week eleven, however, P tissue values decreased significantly compared to what they were two weeks earlier. In fact, tissue P concentrations for plants grown with $20 \text{ mg}\cdot\text{L}^{-1}$ P for eleven weeks were nearly half what they had been at week nine. Tissue P concentrations also decreased in plants grown with $10 \text{ mg}\cdot\text{L}^{-1}$ P, from their highest point at week six. Some of this decrease in tissue P concentrations may be attributed to the fact that plants were developing fruit by week eight, and the fruit were acting as a sink for P in the vegetative tissues (Marschner, 1995). There were no other significant decreases in plant tissue P concentrations over time for plants grown with continuous P concentrations less than $10 \text{ mg}\cdot\text{L}^{-1}$ P, or for any restricted P treatment.

Leachate collected from Pour-Thru analysis demonstrated several trends in substrate nutrient concentrations over the course of the experiment. For leachate collected six weeks after transplant (Table 2.7), P concentrations in solution were the highest for plants grown with 20

mg·L⁻¹ P. Conversely, N and K concentrations in the solution increased with decreasing fertilizer P concentrations. This demonstrates that because plant growth was limited due to low P, absorption of N and K was also limited. Nine weeks after transplants (Table 2.8), P concentrations in the substrate of 20 mg·L⁻¹ P were significantly greater than in every other fertilizer treatment. Plants grown with continuous concentrations of 10 mg·L⁻¹ P or less, or any restricted concentration absorbed most of the P available in the substrate.

Nitrogen and K concentrations in the leachate followed similar trends after nine weeks (Table 2.8) as they had after six. Both N and K concentrations were significantly higher in the substrate of plants grown without P. Additionally, N and K concentrations were the lowest in plants grown with continuous concentrations of 10 or 20 mg·L⁻¹ P, or initial concentrations of 20 mg·L⁻¹ P. After three weeks of P being restricted to 0 mg·L⁻¹, only plants grown with a continuous or initial concentration of 10 mg·L⁻¹ P differed significantly from each other in leachate N and K concentrations. All other P treatments were similar among plants grown with the same initial P concentration.

Eleven weeks after transplant (Table 2.9), P concentrations in the leachate followed an identical trend to what was observed at week nine. Once again, the substrate of plants grown with 20 mg·L⁻¹ P had significantly higher P concentrations in the leachate than all other treatments. This also is in agreement with the plateau for GI (Figure 2.19), indicating further growth is not achieved with P concentrations greater than 13.1 mg·L⁻¹ P. Leachate N concentrations were highest for plants grown without P; however, no significant differences were observed among N concentrations from any other fertilization treatment. K concentrations in the leachate were also highest for plants grown without P, and lowest for plants grown with continuous P concentrations of 10 or 20 mg·L⁻¹ P, and initial concentrations of 5 or 20 mg·L⁻¹ P.

These trends all illustrate how plants limited by P have low levels of P in the substrate, as they absorbed the majority of what was available. This rapid response makes it feasible to implement a low P ($2.5 - 5 \text{ mg}\cdot\text{L}^{-1}$) fertilization shift during production once the plant has achieved marketable size in order to manage growth. Plants grown with $0 \text{ mg}\cdot\text{L}^{-1}$ P had high levels of N and K, as they were unable to utilize these nutrients due to limited growth. Conversely, P levels were highest in the substrate of plants grown with $20 \text{ mg}\cdot\text{L}^{-1}$ P, as these plants reached their maximum growth potential due to P. N and K in the substrate were lowest for these plants, as they were able to achieve maximum growth, and thus, use more of the N and K available from the fertilizer.

Discussion

Low P concentrations were successful at controlling plant growth for several plant species. For all species grown in this study, growth plateaus for GIs were used to determine optimal P concentrations for maximum growth. This was done because GI aggregated several measurements of plant size, providing a more robust value to determine maximum growth. Table 2.10 lists optimal P concentrations in terms of GI for each cultivar grown in this study. Overall, P concentrations ranging from 5.50 to $13.1 \text{ mg}\cdot\text{L}^{-1}$ P resulted in maximum growth. Using P concentrations lower than the maximum concentration in Table 2.10 can be used to control growth in the corresponding species. Concentrations of $2.5 \text{ mg}\cdot\text{L}^{-1}$ P typically led to the development of some level of P deficiency symptoms, depending on the species.

These results deviate from the findings of Hansen and Nielsen (2000, 2001), as significant growth control was not achieved in four different species when lowering P concentrations from 31 to $4.7 \text{ mg}\cdot\text{L}^{-1}$. Hansen and Nielsen (2000, 2001) found that P

concentrations of $1.5 \text{ mg}\cdot\text{L}^{-1}$ provided significant height control when compared with their highest concentration of $31 \text{ mg}\cdot\text{L}^{-1}$ P. Although these findings are in agreement with this study, P concentrations of $2.5 \text{ mg}\cdot\text{L}^{-1}$ typically led to plants that were extremely small and even developed mild symptoms of P deficiency. This was especially true for ornamental peppers and petunia which had higher maximum P concentrations than the other species, and had the most severe P deficiency symptoms with P concentrations of $2.5 \text{ mg}\cdot\text{L}^{-1}$ P or less. Results from Hansen and Nielsen (2000, 2001) indicated that growing plants with just $1.5 \text{ mg}\cdot\text{L}^{-1}$ provided significant growth control with no detrimental effects on appearance or flowering.

The discrepancy between the findings of this study and the findings of Hansen and Nielsen (2000, 2001) may be explained by the differences in substrate and fertility. Hansen and Nielsen used kiln dried clay as the substrate, which was treated with a P buffer. This buffer supplied P at a constant concentration that releases steadily upon each irrigation, rather than being supplied through a liquid fertilizer. This is because certain clays such as allophane have a very high ability to adsorb and deliver P over time (Oh et al., 2016). This P buffer provided a more constant P supply (Borch et al., 1998; Oh et al., 2016), thus preventing deficiency symptoms from developing even when P concentrations are very low. In fact, other studies have found that plants grown in P buffered substrates can achieve maximum growth with just $0.093 \text{ mg}\cdot\text{L}^{-1}$ P (Lynch et al., 1991). This low concentration would result in detrimental P deficiency symptoms in the peat based substrates typically used in bedding plant production. Peat based substrates have a very low ability to adsorb P, and thus, P leaches out of the substrate quite readily (Marconi and Nelson, 1984). In this study, a peat and perlite based substrate was used in conjunction with a constant liquid feed fertilization program, which is more typical of practices used in greenhouse production of ornamental bedding plants. As the methods in this study

replicate typical production practices, results may be used directly to provide fertilizer recommendations to growers.

Much of the low P work that has been done with bedding plants has utilized a P buffer, and have suggested using P concentrations ranging from 0.093 – 1.5 mg·L⁻¹ for compact and healthy growth (Borch et al., 1998; Hansen and Nielsen, 2000, 2001). Borch et al. (1998) used low P to investigate the effects of growth control on *Impatiens walleriana* Hook. f. and *Tagetes patula* L.; however, P buffered substrate was compared with liquid feed fertilization. Plants were grown using P buffer concentrations of 0.093 and 0.28 mg·L⁻¹, and compared with plants grown using liquid feed fertilization with a P concentration of 46.5 mg·L⁻¹. Even with these differences in P concentration, no significant height control was observed in either species (Borch et al., 1998). Conversely, significant growth control was achieved for each species grown in this study by utilizing P concentrations below 5 – 10 mg·L⁻¹.

Conclusions

In most cases, plants required very low concentrations of P to achieve maximum height and diameter. Species grown in this study required only ~5 – 13 mg·L⁻¹ P to achieve maximum growth, indicating that many common commercial fertilizers supply far more P than greenhouse bedding plants require. For instance, 20N–8.7P–16.6K mixed at a low rate of 100 mg·L⁻¹ N would supply crops with approximately 43 mg·L⁻¹ P. This amount of P can be nearly nine times greater than what is required for species such as *Alternanthera*, which reached maximum growth with 5.50 mg·L⁻¹ P. Results from this experiment illustrate how little P is required for most greenhouse species, and may be directly applied to growing techniques to improve fertilization strategies.

Although P concentrations may be significantly lowered relative to current practices and recommendations, it is still important to note that different species have different P requirements for optimal growth (Table 2.10). For instance, alternanthera required only $5.50 \text{ mg}\cdot\text{L}^{-1}$ P to achieve maximum growth, while ornamental peppers required $13.1 \text{ mg}\cdot\text{L}^{-1}$ P. This illustrates how even though optimal P concentrations are low for both of these species, alternanthera requires less than half the P required by ornamental peppers to achieve maximum overall growth. Higher P concentrations may be required by ornamental peppers to support fruit development. This is further evidenced by the fact that leaf tissue P concentrations decreased in P sufficient plants as peppers were developing and maturing. It has been found that remobilized N and P can account for 90% of these elements in developing flowers and fruit, indicating high P requirements to support these reproductive structures (Epstein and Bloom, 2005; Marschner, 1995).

A number of commercial fertilizers can be used by growers to supply optimal P concentrations in the range of $5 - 15 \text{ mg}\cdot\text{L}^{-1}$. For instance, 13N–0.9P–10.8K Cal Mag mixed at $75 - 225 \text{ mg}\cdot\text{L}^{-1}$ N, or 15N–2.2P–12.5K Cal Mag mixed at $50 - 100 \text{ mg}\cdot\text{L}^{-1}$ N could be used to supply $5 - 15 \text{ mg}\cdot\text{L}^{-1}$ P. Additionally, growers may alternate between two commercial fertilizers to apply an average within this optimal range of P. For instance, a high P fertilizer formulation such as 20N–4.4P–16.6K mixed at $100 \text{ mg}\cdot\text{L}^{-1}$ N could be used in conjunction with a low P fertilizer such as 13N–0.9P–10.8K Cal Mag. There are many options available to commercial growers that may aid them in producing healthy and compact plants by utilizing a low P fertilization regiment.

Acknowledgements

We are grateful for the funding support provided by Fred C. Gloeckner Foundation, American Floral Endowment Altman Family Scholarship, and The Garden Club of America. We would also like to express our gratitude to Dümme Orange for providing cuttings, and for peat moss provided by Sun Gro Horticulture.

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Table 2.1. Means and minimum significant differences for most recently matured leaf tissue nutrient concentrations of *Alternanthera brasiliana* ‘Brazilian Red’.

Phosphorus Concentration (mg·L ⁻¹)	Element						
	Nitrogen	Phosphorus	Potassium	Magnesium	Calcium	Zinc	Manganese
0	4.0 d ¹	0.10 e	6.2 bc	1.0 d	1.0 d	81.0 a	558.5 a
1.25	4.2 d	0.15 de	7.1 a	1.4 c	1.2 c	59.8 b	472.2 ab
2.5	4.6 c	0.20 d	6.9 ab	1.5 bc	1.5 bc	54.4 b	440.2 bc
5	5.0 b	0.29 c	6.0 cd	1.6 abc	1.7 ab	42.4 d	327.5 d
10	5.2 ab	0.36 b	5.6 cd	1.5 abc	1.7 ab	42.9 cd	274.2 d
20	5.4 a	0.50 a	5.9 cd	1.7 ab	1.8 a	53.6 bc	321.5 d
40	5.2 ab	0.49 a	5.6 cd	1.8 a	1.8 a	51.3 bcd	353.7 cd
80	5.4 a	0.48 a	5.2 d	1.6 ab	1.6 ab	51.1 bcd	425.6 bc
MSD ²	0.30	0.057	0.98	0.26	0.26	10.92	89.86

¹ Lower case letters signify minimum significant differences among all phosphorus concentrations for each individual element. Means with different letters are significantly different at $P \leq 0.05$.

² Minimum significant differences (MSD) are listed for each element.

Table 2.2. Means and minimum significant differences for most recently matured leaf tissue nutrient concentrations of *Petunia atkinsiana* ‘Surprise Sky Blue’.

Phosphorus Concentration (mg·L ⁻¹)	Element		
	Nitrogen	Phosphorus	Potassium
0	4.1 d ¹	0.05 e	3.9 e
1.25	4.5 c	0.10 de	5.2 d
2.5	5.7 b	0.13 de	5.7 cd
5	6.1 a	0.25 cd	6.6 abc
10	6.1 a	0.32 c	6.0 bcd
20	5.6 b	0.68 b	7.0 ab
40	5.7 b	0.79 b	7.4 a
80	5.6 b	1.21 a	7.0 ab
MSD ²	0.36	0.146	1.17

¹ Lower case letters signify minimum significant differences among all phosphorus concentrations for each individual element. Means with different letters are significantly different at $P \leq 0.05$.

² Minimum significant differences (MSD) are listed for each element.

Table 2.3. Means and minimum significant differences for growth parameters, and substrate pH and EC of *Capsicum annuum* ‘Tango Red’, six weeks after transplant.

Phosphorus Concentration (mg·L ⁻¹)	Growth Parameter					
	Height (cm)	Diameter (cm)	Dry Mass (g)	Growth Index	pH	EC ¹
0	2.8 d ²	5.5 d	0.1 d	2.8 d	6.4 b	3.2 a
2.5	9.4 c	20.0 c	1.0 c	10.2 c	6.4 b	3.1 a
5	11.6 bc	27.0 b	2.4 b	13.6 b	6.6 ab	2.6 a
10	12.2 b	28.6 b	2.8 b	14.5 b	6.8 a	2.9 a
20	15.0 a	33.3 a	4.6 a	17.6 a	6.8 a	2.5 a
MSD ³	2.41	3.74	0.88	2.17	0.22	0.96

¹ EC = electrical conductivity

² Lower case letters signify minimum significant differences among all phosphorus concentrations for each growth parameter. Means with different letters are significantly different at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for each growth parameter.

Table 2.4. Means and minimum significant differences for growth parameters, and substrate pH and EC of *Capsicum annuum* ‘Tango Red’, nine weeks after transplant.

Fertilization Regiment ¹	Phosphorus Concentration (mg·L ⁻¹)	Growth Parameter					
		Height (cm)	Diameter (cm)	Dry Mass (g)	Growth Index	pH	EC ²
Continuous	0	5.6 e ³	7.6 f	0.1 f	4.4 g	6.5 d	3.9 a
	2.5	12.8 d	17.8 e	0.6 f	10.4 f	6.9 bc	2.6 b
	5	17.2 c	26.4 c	2.4 d	15.3 d	7.1 ab	2.6 b
	10	22.5 ab	37.4 a	5.2 b	21.7 b	7.3 a	2.7 ab
	20	25.0 a	38.6 a	6.4 a	23.3 a	7.2 a	2.8 ab
Restricted	2.5	12.6 d	15.8 e	0.4 f	9.6 f	6.8 c	2.8 ab
	5	16.1 c	22.9 d	1.4 e	13.4 e	6.8 c	3.3 ab
	10	20.7 b	31.8 b	3.3 c	18.6 c	7.1 a	2.7 ab
	20	20.1 b	36.6 a	5.1 b	20.6 b	7.1 a	3.0 ab
	MSD ⁴	2.89	3.29	0.57	1.56	0.22	1.22

¹ Continuous phosphorus (P) plants were grown with 0, 2.5, 5, 10, or 20 mg·L⁻¹ P. Restricted P plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for six weeks, that was then restricted to 0 mg·L⁻¹ P for three weeks.

² EC = electrical conductivity

² Lower case letters signify minimum significant differences among all P concentrations for each growth parameter. Means with different letters are significantly different at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for each growth parameter.

Table 2.5. Means and minimum significant differences for growth parameters, and substrate pH and EC of *Capsicum annuum* ‘Tango Red’, eleven weeks after transplant.

Fertilization Regime ¹	Phosphorus Concentration (mg·L ⁻¹)	Growth Parameter					
		Height (cm)	Diameter (cm)	Dry Mass (g)	Growth Index	pH	EC ²
Continuous	0	6.2 e ³	6.2 f	0.1 g	4.1 f	6.4 d	3.8 a
	2.5	13.7 cd	16.6 de	0.7 ef	10.3 e	6.9 bc	1.4 c
	5	19.6 b	27.6 c	2.9 d	16.7 c	7.1 bc	1.8 bc
	10	23.8 a	39.3 a	7.7 b	23.6 a	7.4 a	2.4 b
	20	22.2 ab	41.1 a	9.3 a	24.2 a	7.4 a	2.7 b
Restricted	2.5	12.9 d	13.6 e	0.4 fg	9.0 e	7.0 bc	1.2 c
	5	16.1 c	18.7 d	1.3 e	12.0 d	6.9 c	1.3 c
	10	20.0 b	26.4 c	2.6 d	16.3 c	7.0 bc	1.8 bc
	20	22.4 ab	34.1 b	6.9 c	21.1 b	7.2 b	2.4 b
	MSD ⁴	2.87	3.73	0.64	1.71	0.23	0.97

¹ Continuous phosphorus (P) plants were grown with 0, 2.5, 5, 10, or 20 mg·L⁻¹ P. Restricted P plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for six weeks, that was then restricted to 0 mg·L⁻¹ P for five weeks.

² EC = electrical conductivity

² Lower case letters signify minimum significant differences among all P concentrations for each growth parameter. Means with different letters are significantly different at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for each growth parameter.

Table 2.6. Means and minimum significant differences for most recently matured leaf tissue phosphorus concentrations in *Capsicum annuum* ‘Tango Red’, at six, nine, and eleven weeks after transplant.

Week	Fertilizer Phosphorus Concentration (mg·L ⁻¹) ¹								
	Continuous					Restricted			
	0	2.5	5	10	20	2.5	5	10	20
6	0.11 ²	0.16 de ³	0.19 cde	0.39 b	0.55 a	-	-	-	-
9	0.10	0.14 de	0.13 de	0.21 cd	0.51 a	0.11 de	0.11 de	0.11 de	0.14 de
11	0.12	0.13 de	0.16 de	0.19 cde	0.27 c	0.10 e	0.14 de	0.15 de	0.10 e

¹ Continuous phosphorus (P) plants were grown with 0, 2.5, 5, 10, or 20 mg·L⁻¹ P. Restricted P plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for six weeks, that was then restricted to 0 mg·L⁻¹ P for five weeks.

² Values for the plants grown with 0 mg·L⁻¹ P were from a single tissue sample from several plants, and thus were not included in the statistical analysis. They are reported only for reference.

³ Lower case letters signify significant differences among all P concentrations and weeks. Means with different letters are significantly different at $P \leq 0.05$. The minimum significant difference was 0.107.

Table 2.7. Means and minimum significant differences for leachate primary macronutrient concentrations of *Capsicum annuum* ‘Tango Red’ substrate, six weeks after transplant.

Phosphorus Concentration (mg·L ⁻¹)	Element		
	Nitrogen	Phosphorus	Potassium
0	295 a ¹	0.4 c	222 a
2.5	298 a	1.2 c	160 b
5	218 ab	1.9 bc	106 c
10	188 ab	4.5 b	105 c
20	110 b	10.5 a	70 c
MSD ²	139.8	2.64	39.8

¹ Lower case letters signify minimum significant differences among all phosphorus concentrations for each individual macronutrient. Means with different letters are significantly different at $P \leq 0.05$.

² Minimum significant differences (MSD) are listed for each macronutrient.

Table 2.8. Means and minimum significant differences for leachate primary macronutrient concentrations of *Capsicum annuum* ‘Tango Red’ substrate, nine weeks after transplant.

Fertilization Regime ¹	Phosphorus Concentration (mg·L ⁻¹)	Element		
		Nitrogen	Phosphorus	Potassium
Continuous	0	418 a ²	0.4 b	330 a
	2.5	222 b	0.4 b	208 b
	5	185 bc	0.7 b	102 c
	10	69 cd	1.3 b	12 e
	20	42 d	8.1 a	8 e
Restricted	2.5	278 b	0.8 b	222 b
	5	261 b	0.4 b	115 c
	10	211 b	0.3 b	86 cd
	20	77 cd	1.0 b	28 de
	MSD ³	133.1	2.02	65.8

¹ Continuous phosphorus (P) plants were grown with 0, 2.5, 5, 10, or 20 mg·L⁻¹ P. Restricted P plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for six weeks, that was then restricted to 0 mg·L⁻¹ P for three weeks.

² Lower case letters signify minimum significant differences among all P concentrations for each individual macronutrient. Means with different letters are significantly different at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for each macronutrient.

Table 2.9. Means and minimum significant differences for leachate primary macronutrient concentrations of *Capsicum annuum* ‘Tango Red’ substrate, eleven weeks after transplant.

Fertilization Regime ¹	Phosphorus Concentration (mg·L ⁻¹)	Element		
		Nitrogen	Phosphorus	Potassium
Continuous	0	304 a ²	0.3 b	293 a
	2.5	62 b	0.7 b	89 bc
	5	117 b	0.9 b	89 bc
	10	53 b	1.4 b	23 e
	20	46 b	4.8 a	27 de
Restricted	2.5	52 b	0.3 b	86 bcd
	5	64 b	0.4 b	68 cde
	10	133 b	0.3 b	130 b
	20	105 b	0.3 b	60 cde
	MSD ³	89.8	1.84	59.5

¹ Continuous phosphorus (P) plants were grown with 0, 2.5, 5, 10, or 20 mg·L⁻¹ P. Restricted P plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for six weeks, that was then restricted to 0 mg·L⁻¹ P for three weeks.

² Lower case letters signify minimum significant differences among all P concentrations for each individual macronutrient. Means with different letters are significantly different at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for each macronutrient.

Table 2.10. Summary of optimal phosphorus concentrations to achieve maximum growth for each cultivar, based on GI plateaus.

Plant Species 'Cultivar'	Phosphorus Concentration (mg·L ⁻¹)
<i>Alternanthera brasiliana</i>	
'Brazilian Red'.....	5.50
<i>Angelonia angustifolia</i>	
'Sungelonia Blue'.....	8.78
'Sungelonia White'.....	7.27
<i>Capsicum annuum</i>	
'Tango Red'.....	13.1
<i>Catharanthus roseus</i>	
'Cora Burgundy'.....	6.74
'Pacifica XP Blush'.....	6.43
<i>Impatiens hawkeri</i>	
'Pure Beauty Red on Pink'.....	12.4
'Tamarinda Dark Red'.....	9.64
<i>Petunia atkinsiana</i>	
'Potunia Neon'.....	9.08
'Surprise Sky Blue'.....	8.72

Alternanthera brasiliana

'Brazilian Red'

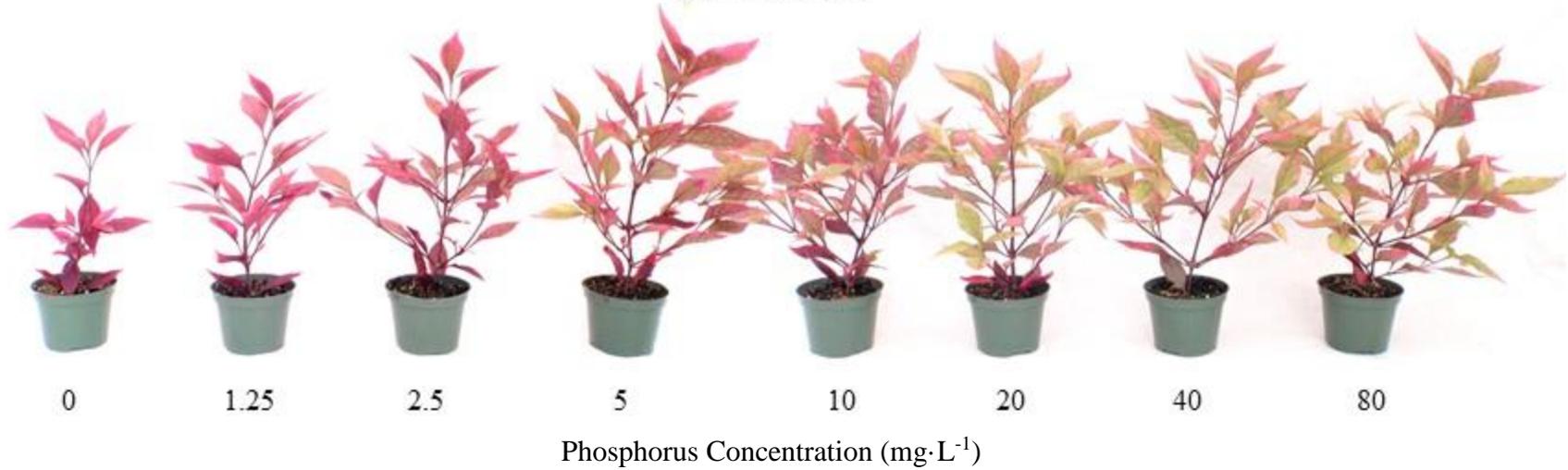


Figure 2.1. *Alternanthera brasiliana* 'Brazilian Red' plants grown with 0, 1.25, 2.5, 5, 10, 20, 40, and 80 mg·L⁻¹ P. Visual differences in height, diameter, and overall appearance may be observed here. Plant growth plateaued at ~5 mg·L⁻¹ P, and no further increases occurred at higher P concentrations.

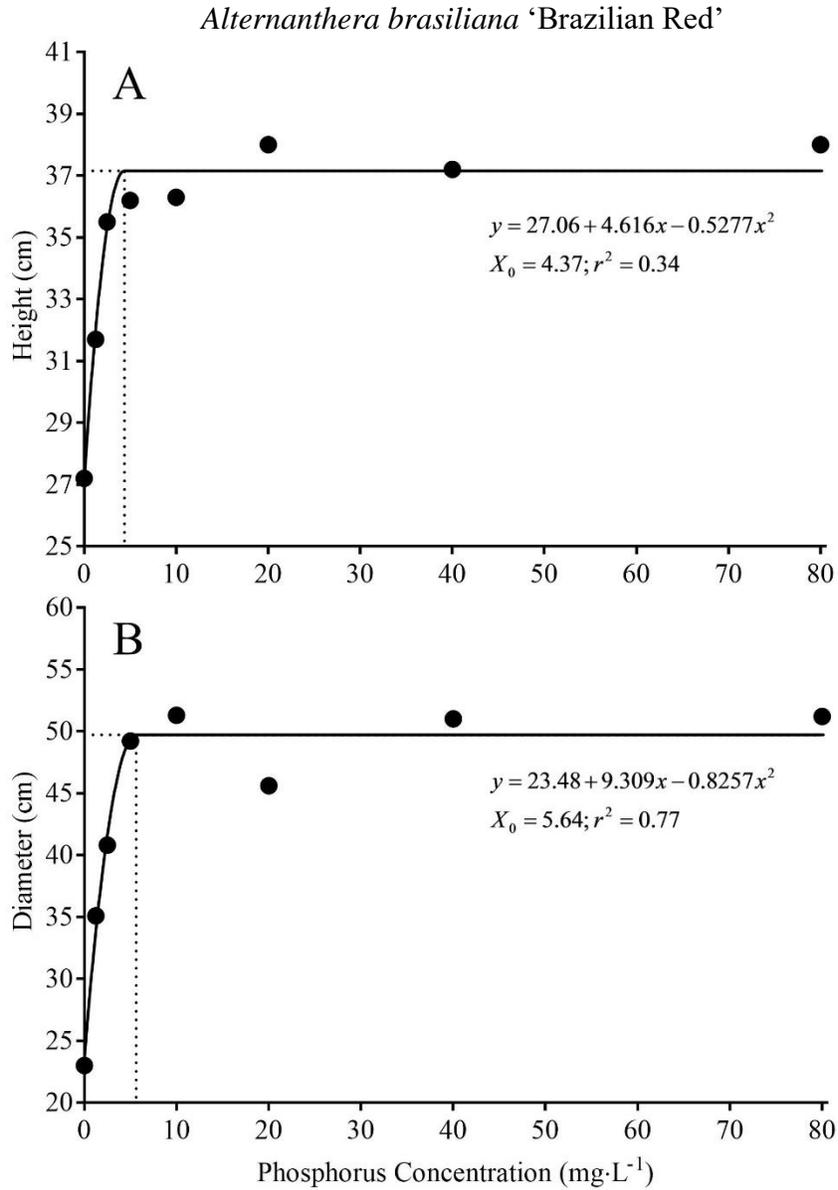


Figure 2.2. Nonlinear regression models with plateau for height (A) and diameter (B) of *Alternanthera brasiliana* 'Brazilian Red'. Regression lines were generated from means of each treatment ($n = 7$). Equations, r^2 , and X_0 values are located below the corresponding line. Dotted lines represent X_0 values, where the model plateaus, along with corresponding height or diameter. Models significant at $P \leq 0.0001$.

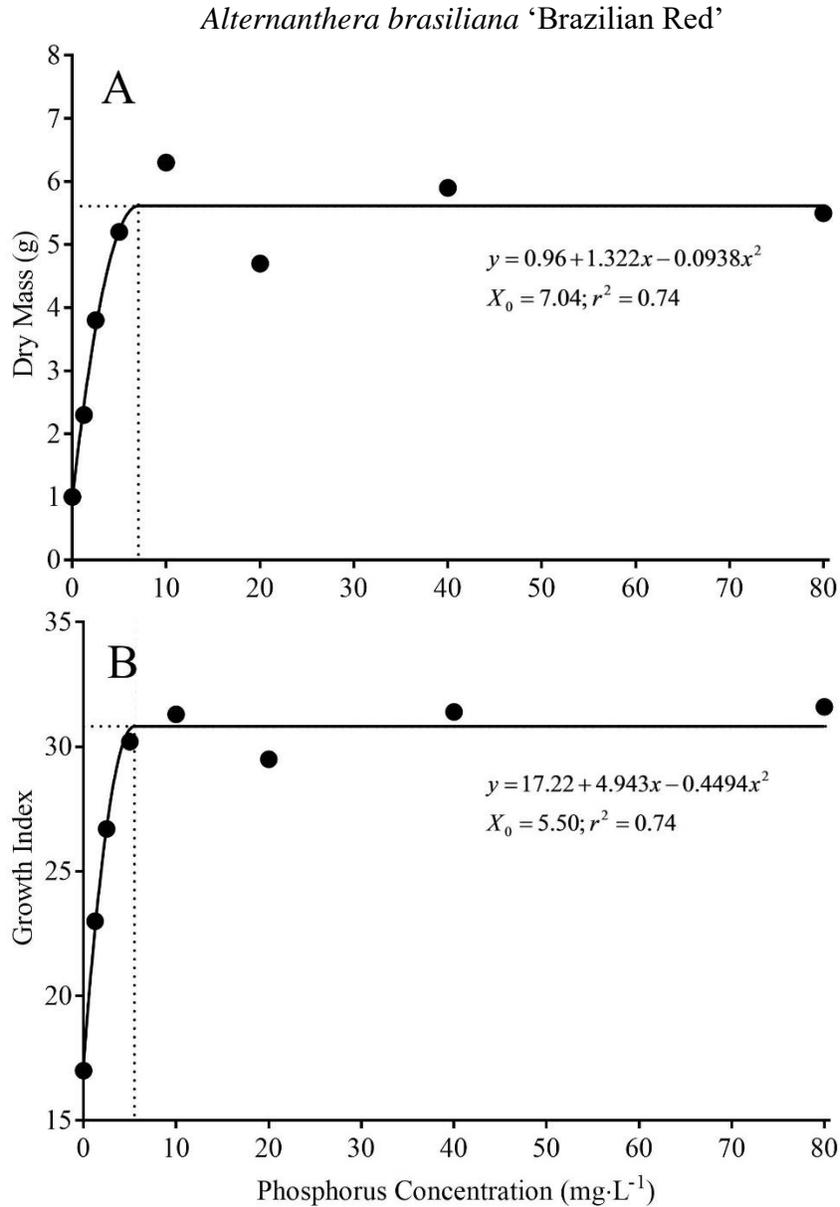


Figure 2.3. Nonlinear regression model with plateau for dry mass (A) and growth index (B) of *Alternanthera brasiliana* 'Brazilian Red'. Regression lines were generated from means of each treatment ($n = 7$). Equation, r^2 , and X_0 values are located below the corresponding lines. Dotted lines represent the X_0 value, where the model plateaus, along with corresponding dry mass or growth index. Models significant at $P \leq 0.0001$.

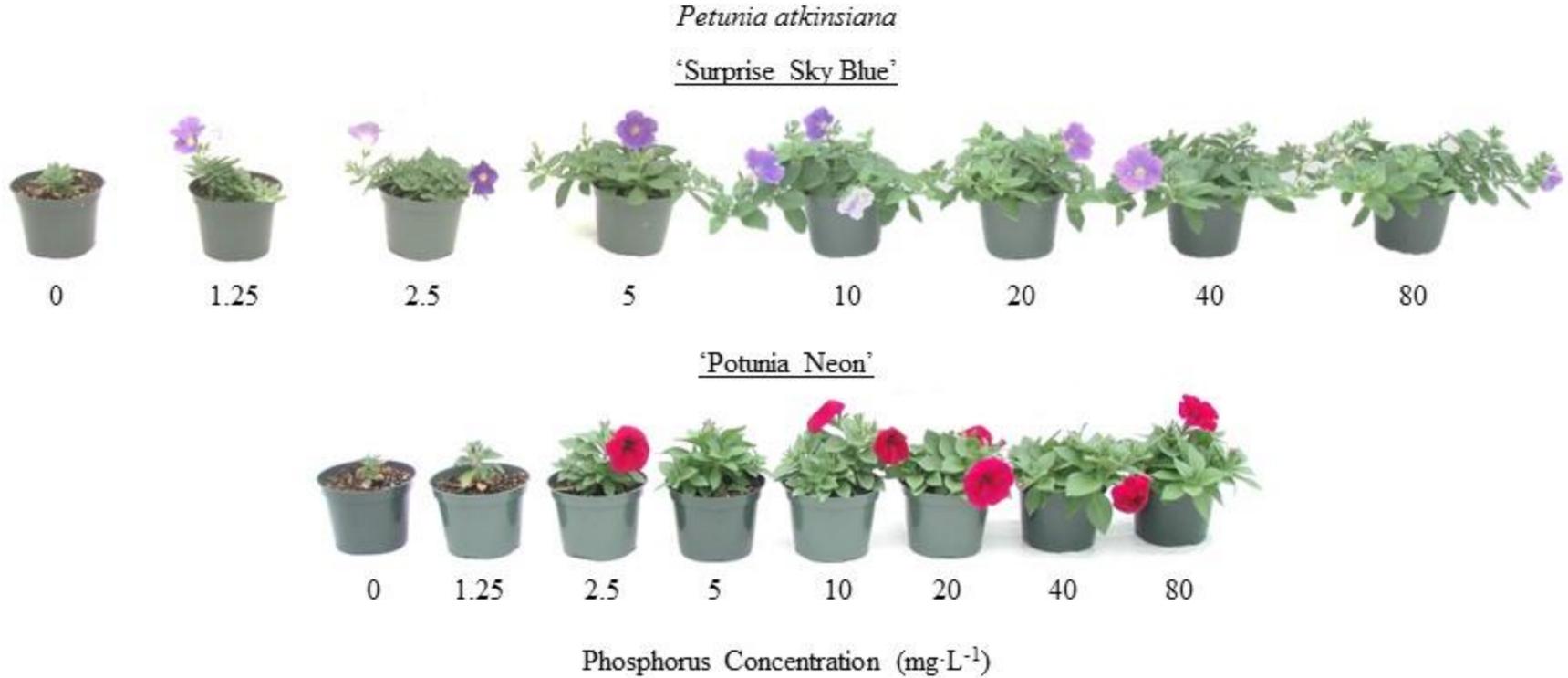


Figure 2.4. *Petunia atkinsiana* 'Surprise Sky Blue' and 'Potunia Neon' plants grown with 0, 1.25, 2.5, 5, 10, 20, 40, and 80 $\text{mg}\cdot\text{L}^{-1}$ P. Visual differences in height, diameter, and overall appearance may be observed here. Plant growth plateaued at $\sim 10 \text{ mg}\cdot\text{L}^{-1}$ P, and no further increases occurred at higher P concentrations.

Petunia atkinsiana ‘Surprise Sky Blue’ and ‘Potunia Neon’

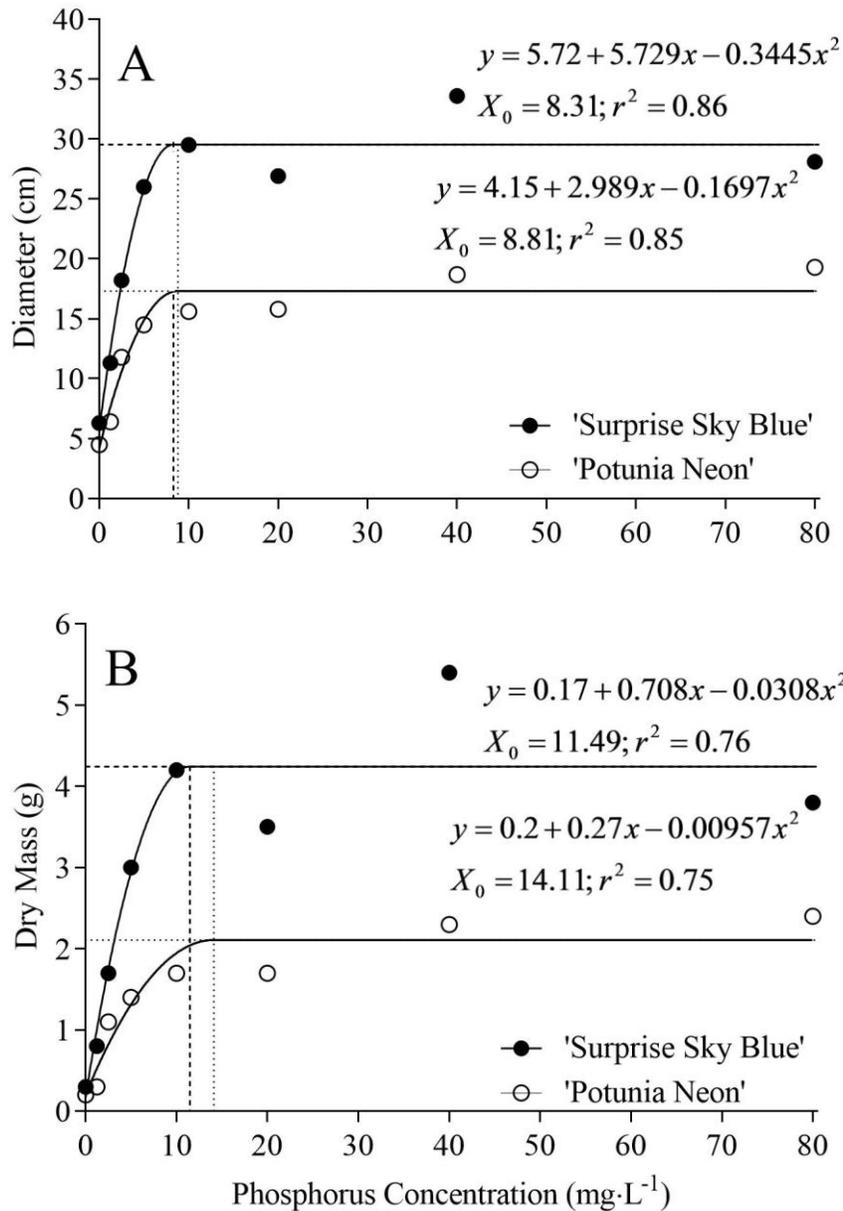


Figure 2.5. Nonlinear regression model with plateau for plant diameter (A) and dry mass (B) of *Petunia atkinsiana* ‘Surprise Sky Blue’ and ‘Potunia Neon’ upon termination of the experiment. Regression lines were generated from means of each treatment ($n = 8$). Equations, r^2 , and X_0 values for plateau models are located above the corresponding lines. Dotted lines represent the X_0 values, where the model plateaus, along with corresponding diameter or dry mass. Models significant at $P \leq 0.0001$.

Petunia atkinsiana 'Surprise Sky Blue' and 'Potunia Neon'

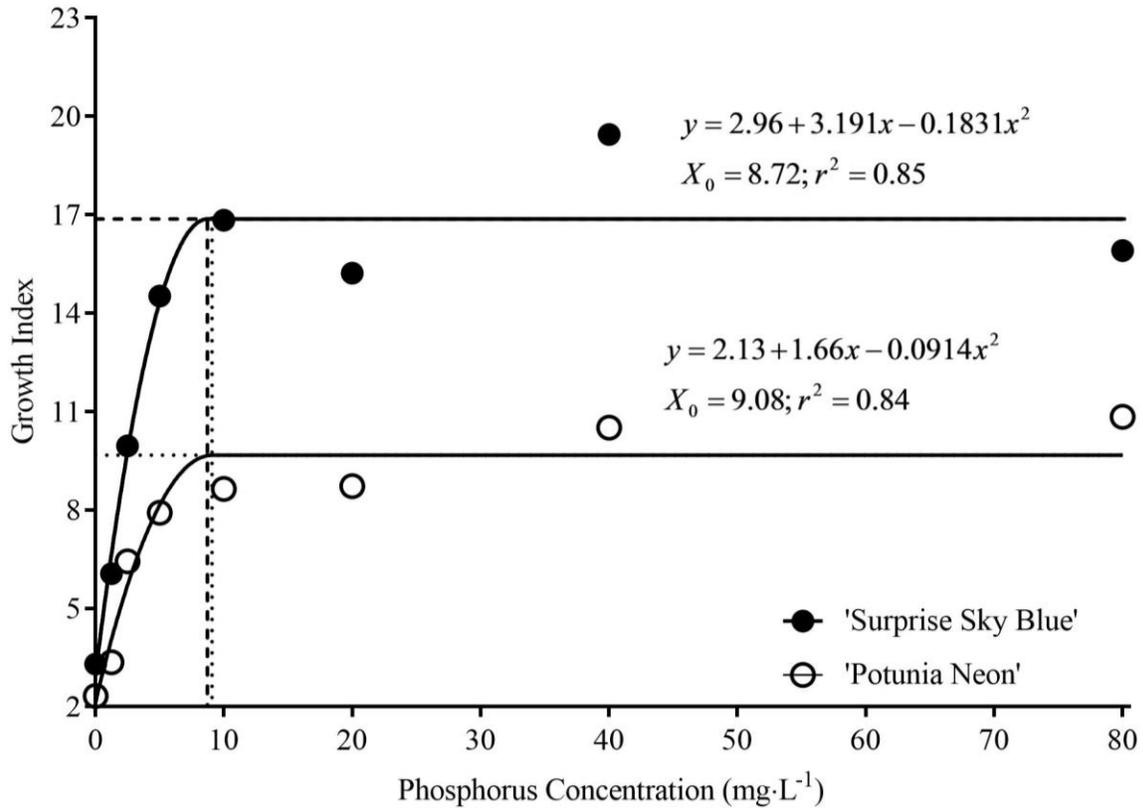


Figure 2.6. Nonlinear regression plateaus and quadratic regression model for growth index (GI) of *Petunia atkinsiana* 'Surprise Sky Blue' and 'Potunia Neon' upon termination of the experiment. Regression lines were generated from means of each treatment (n = 8). Equations, r^2 , and X_0 values for plateau models are located above the corresponding lines. Dotted lines represent the X_0 values, where the model plateaus, along with corresponding GI. Models significant at $P \leq 0.0001$.

Petunia atkinsiana ‘Surprise Sky Blue’ and ‘Potunia Neon’

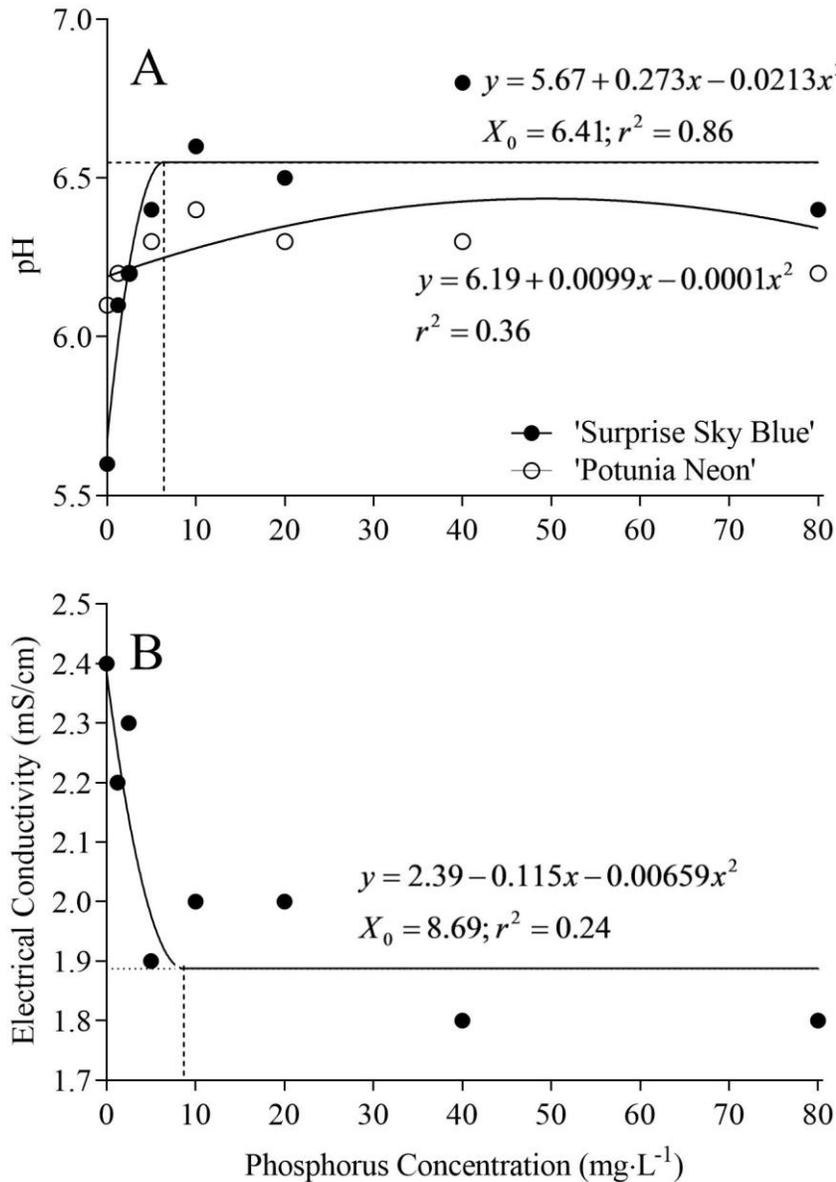


Figure 2.7. Nonlinear regression plateaus and quadratic regression model for substrate pH (A) and electrical conductivity (B) of *Petunia atkinsiana* ‘Surprise Sky Blue’ and ‘Potunia Neon’ upon termination of the experiment. Electrical conductivity (EC) was reported with data from both cultivars combined. Regression lines were generated from means of each treatment (n = 8). Equations, r^2 , and X_0 values for plateau models are located above the corresponding lines. Equation and r^2 for the quadratic regression model are located below the corresponding line. Dotted lines represent the X_0 values, where the model plateaus, along with corresponding pH or EC. Models significant at $P \leq 0.0001$.

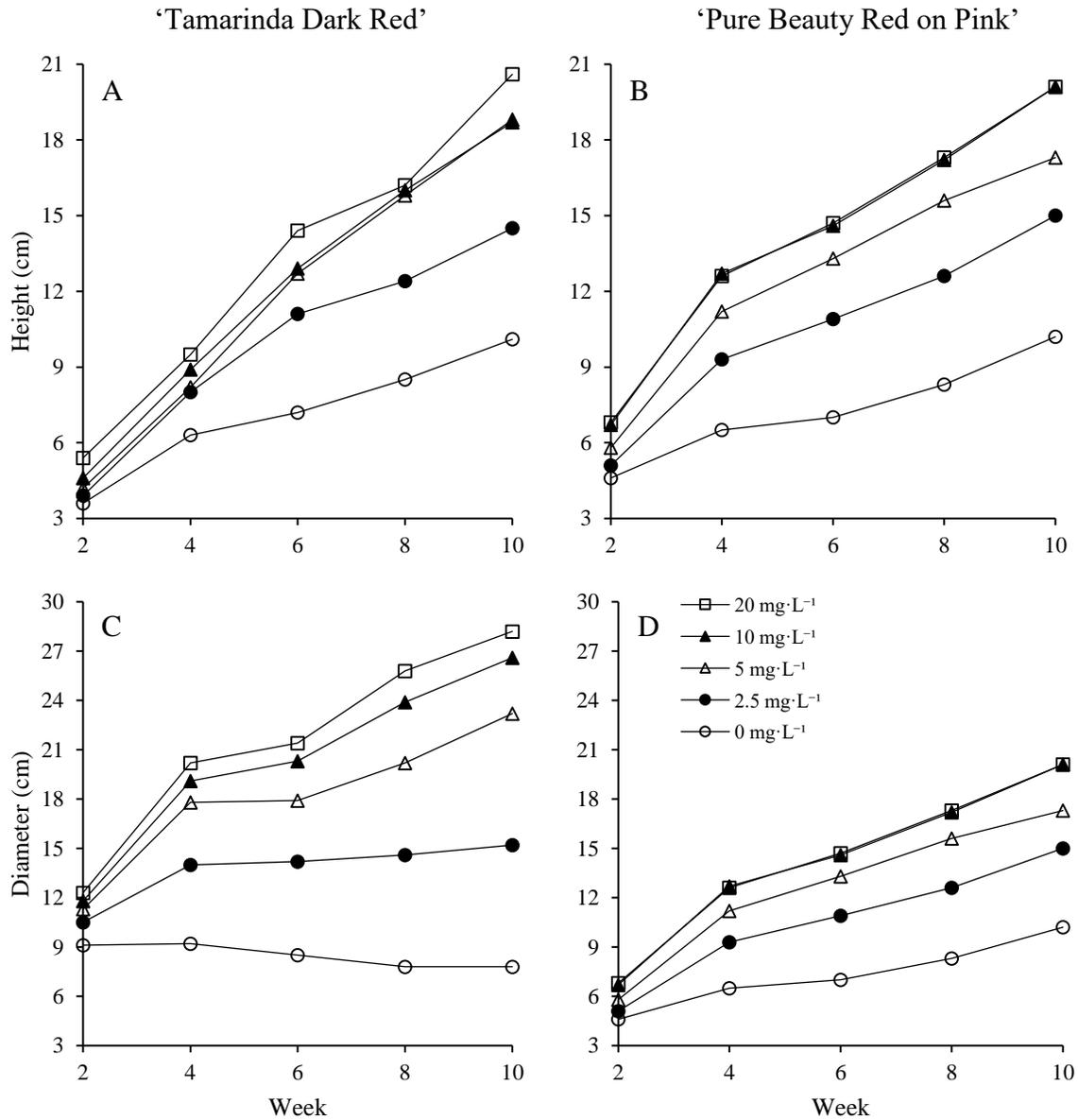


Figure 2.8. Effect of five different fertilizer P concentrations on growth means of *Impatiens hawkeri* 'Tamarinda Dark Red' and 'Pure Beauty Red on Pink' over a ten-week period. Growth in terms of height (A) and diameter (B) of 'Tamarinda Dark Red', compared with height (C) and diameter (D) of 'Pure Beauty Red on Pink'.

Impatiens hawkeri

'Tamarinda Dark Red'



'Pure Beauty Red on Pink'



0

2.5

5

10

20

Phosphorus Concentration ($\text{mg}\cdot\text{L}^{-1}$)

Figure 2.9. *Impatiens hawkeri* plants grown using P concentrations of 0, 2.5, 5, 10, and 20 $\text{mg}\cdot\text{L}^{-1}$ over the course of ten weeks. 'Tamarinda Dark Red' may be seen in the top row, and 'Pure Beauty Red on Pink' in the bottom row. Visual differences in plant size, flowering, and overall leaf architecture may be observed.

Impatiens hawkeri 'Pure Beauty Red on Pink' and 'Tamarinda Dark Red'

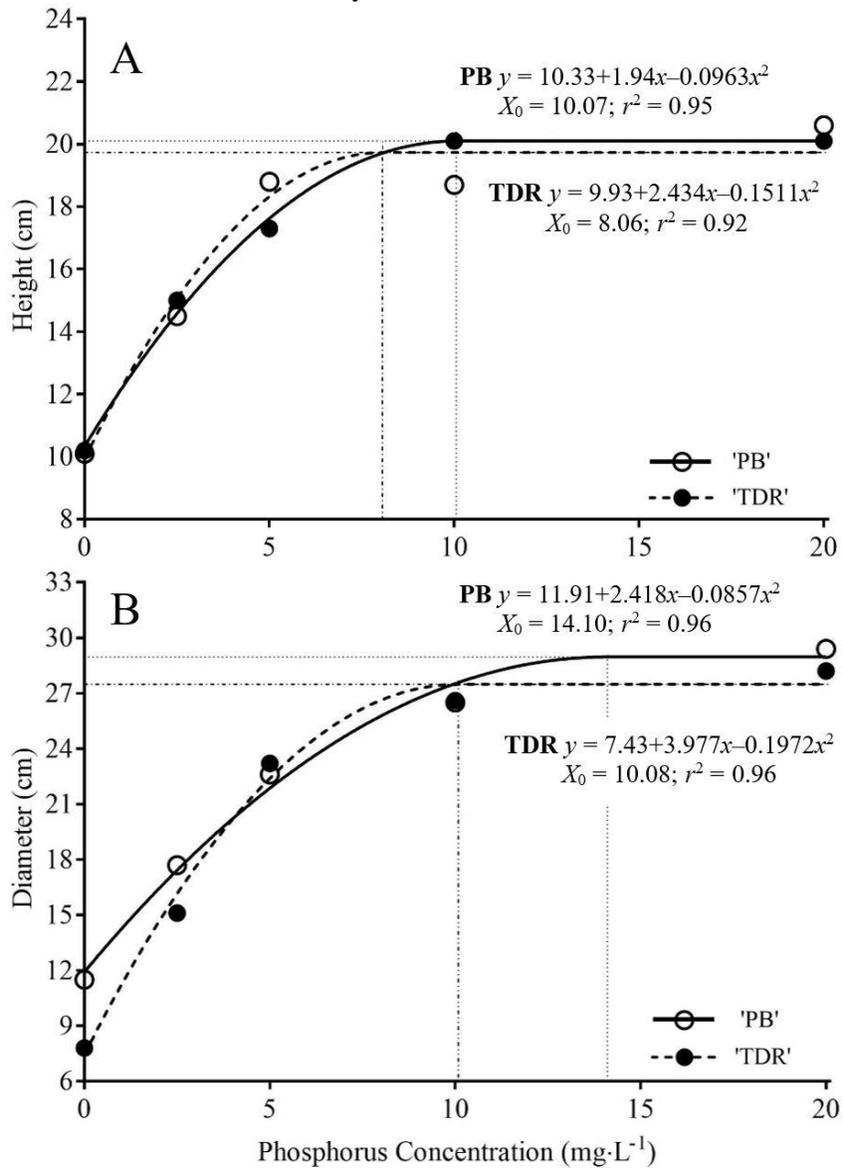


Figure 2.10. Nonlinear regression models with plateau for final height (A) and diameter (B) of *Impatiens hawkeri* 'Pure Beauty Red on Pink', denoted PB, and 'Tamarinda Dark Red', denoted TDR. Regression lines were generated from means of each treatment (n = 8). Equations, r^2 , and X_0 values are located above or below the corresponding line. Vertical and horizontal dotted lines represent X_0 values, where the model plateaus, along with corresponding height or diameter. Models significant at $P \leq 0.0001$.

Impatiens hawkeri 'Pure Beauty Red on Pink' and 'Tamarinda Dark Red'

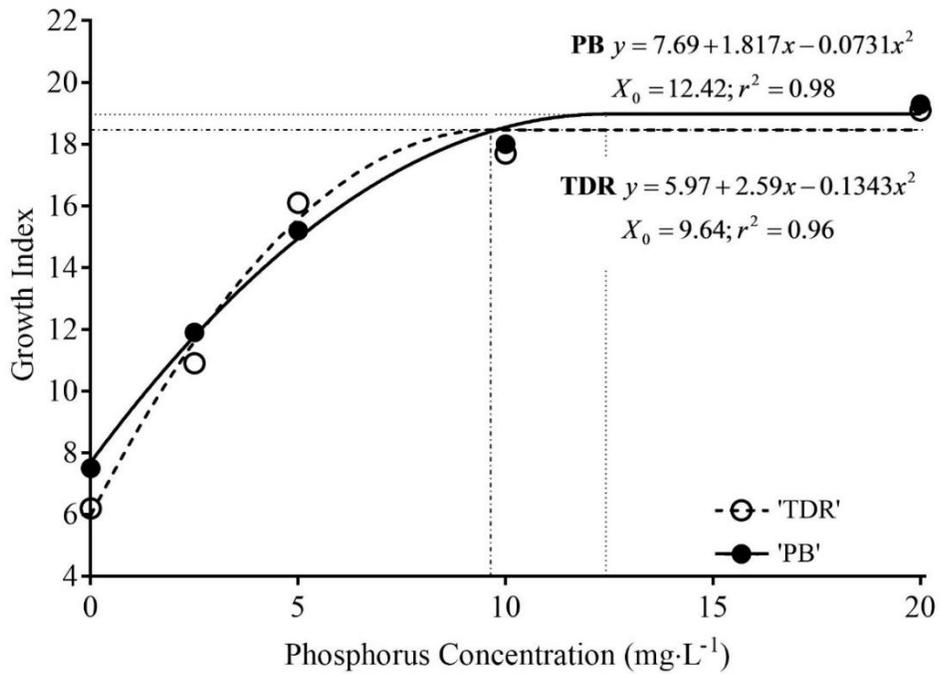


Figure 2.11. Nonlinear regression models with plateau for growth index (GI) of *Impatiens hawkeri* 'Tamarinda Dark Red', denoted TDR, and 'Pure Beauty Red on Pink', denoted PB. Regression lines were generated from means of each treatment ($n = 8$). Equations, r^2 , and X_0 values are located above or below the corresponding line. Vertical and horizontal dotted lines represent X_0 values, where the model plateaus, along with corresponding GI. Models significant at $P \leq 0.0001$.

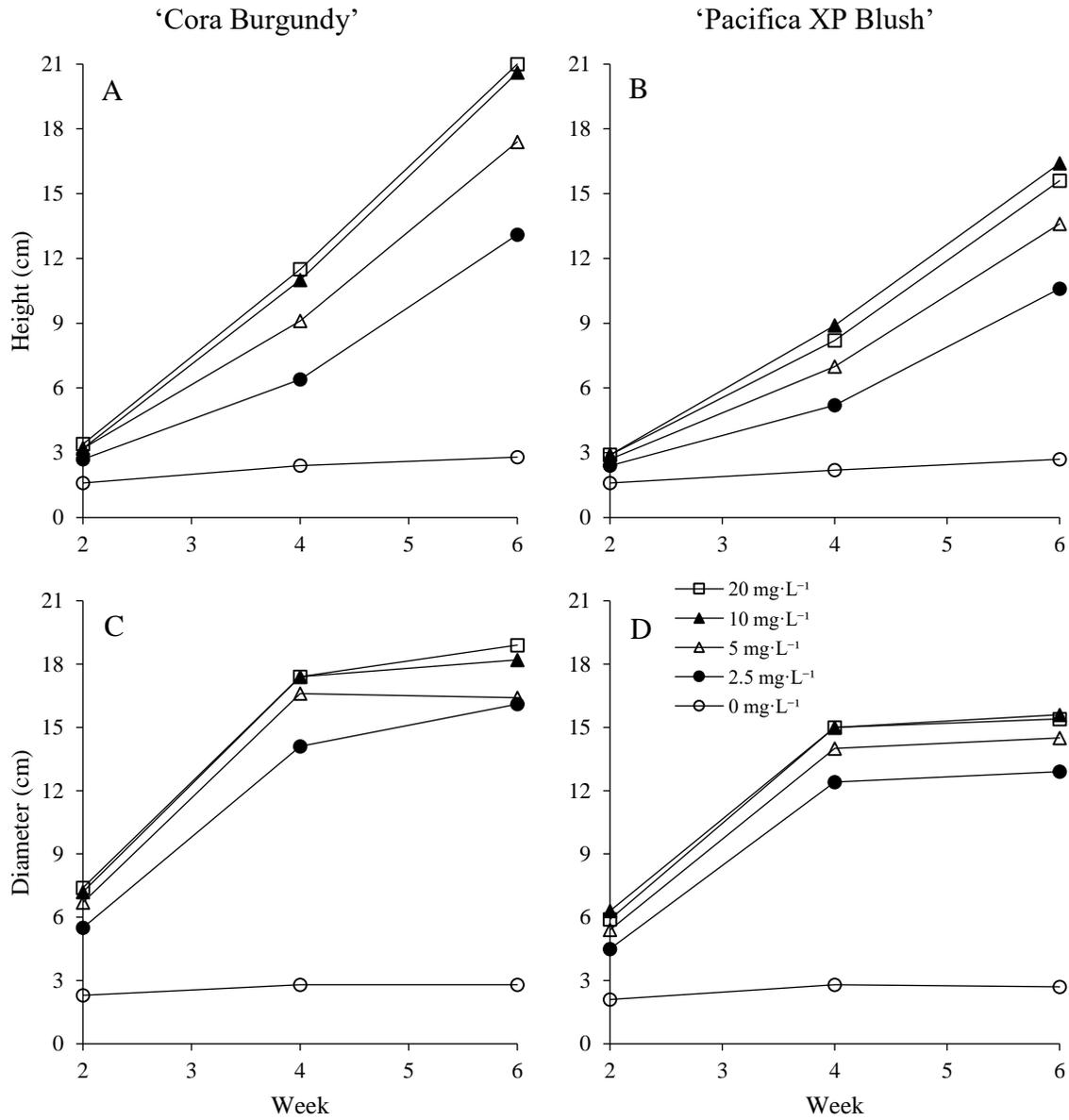


Figure 2.12. Effect of five different fertilizer P concentrations on growth means of *Catharanthus roseus* 'Cora Burgundy' and 'Pacifica XP Blush' over a six-week period. Growth in terms of height (A) and diameter (B) of 'Cora Burgundy', compared with height (C) and diameter (D) of 'Pacifica XP Blush'.

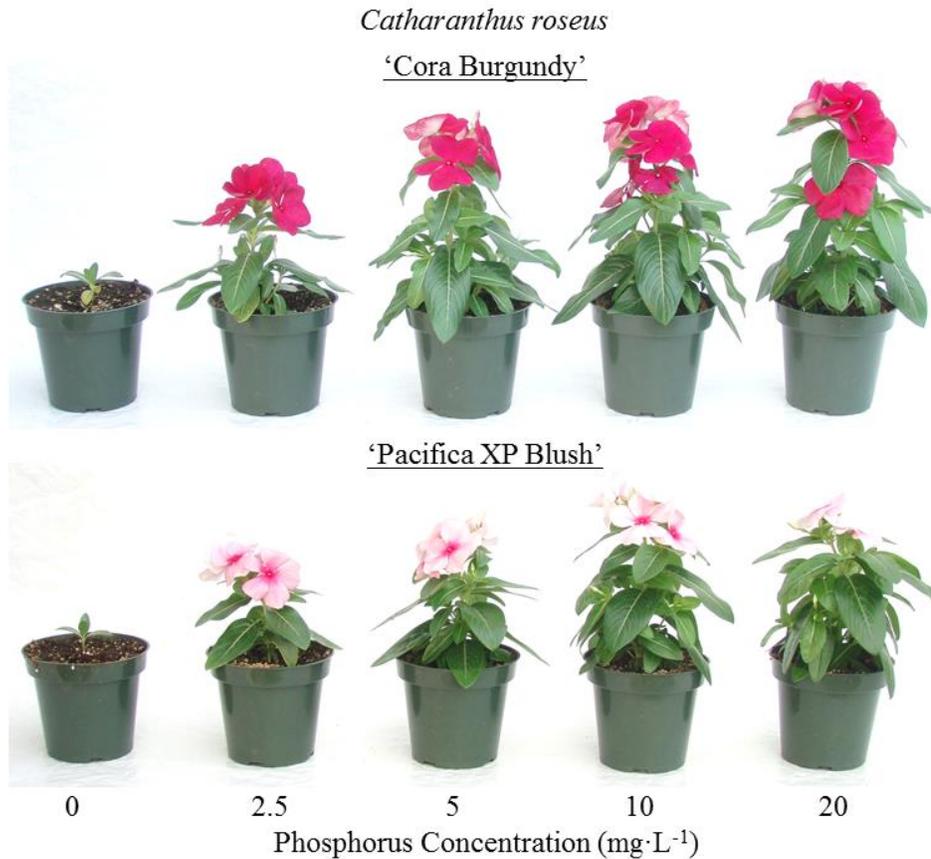


Figure 2.13. *Catharanthus roseus* plants grown using P concentrations of 0, 2.5, 5, 10, and 20 mg·L⁻¹ over a six-week period. 'Cora Burgundy' may be seen in the top row, and 'Pacifica XP Blush' in the bottom row. Visual differences in plant size and flowering may be observed. Plants grown without P did not appear different than plants at the plug stage. This is reflected in the weekly growth charts in Fig. 2.12.

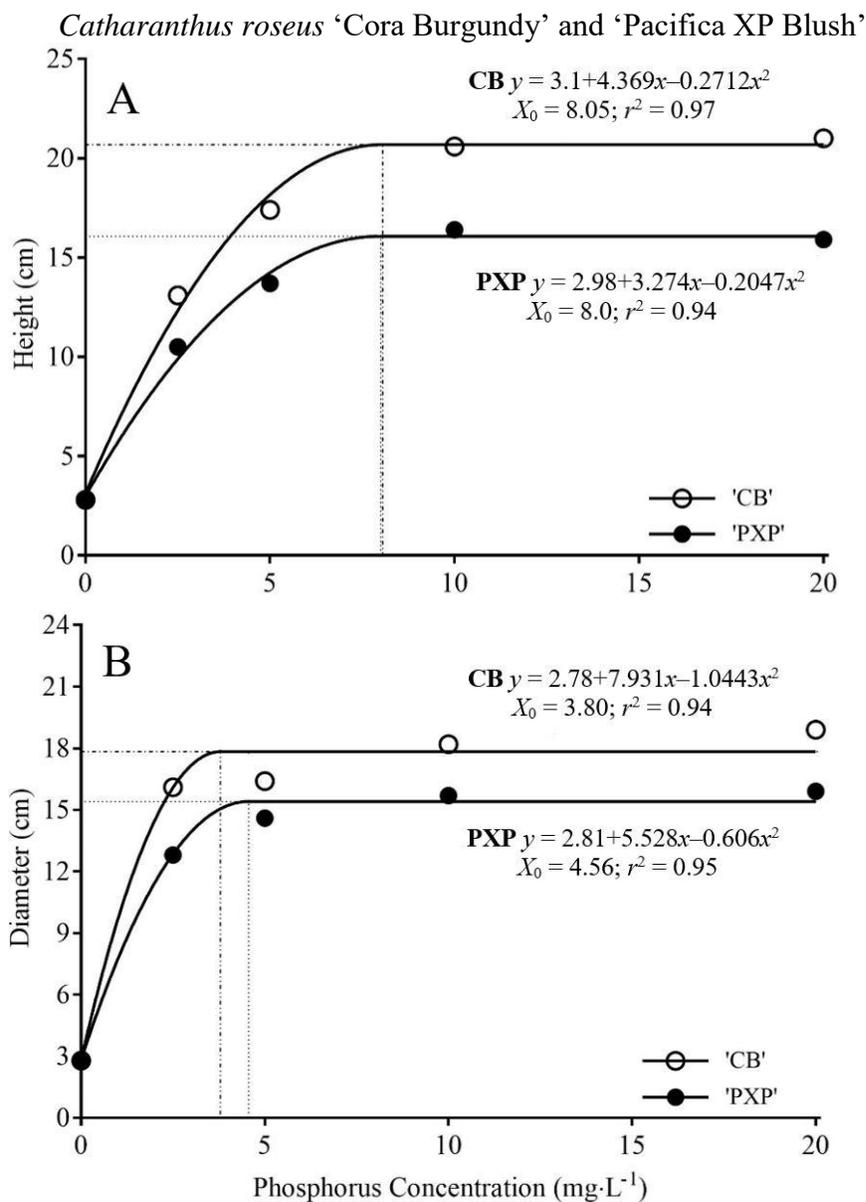


Figure 2.14. Nonlinear regression models with plateau for final height (A) and diameter (B) of *Catharanthus roseus* 'Cora Burgundy', denoted CB, and 'Pacifica XP Blush', denoted PXP. Regression lines were generated from means of each treatment (CB, n = 7; PXP, n = 8). Equations, r^2 , and X_0 values are located above or below the corresponding line. Vertical and horizontal dotted lines represent X_0 values, where the model plateaus, along with corresponding height or diameter. Models significant at $P \leq 0.0001$.

Catharanthus roseus 'Cora Burgundy' and 'Pacifica XP Blush'

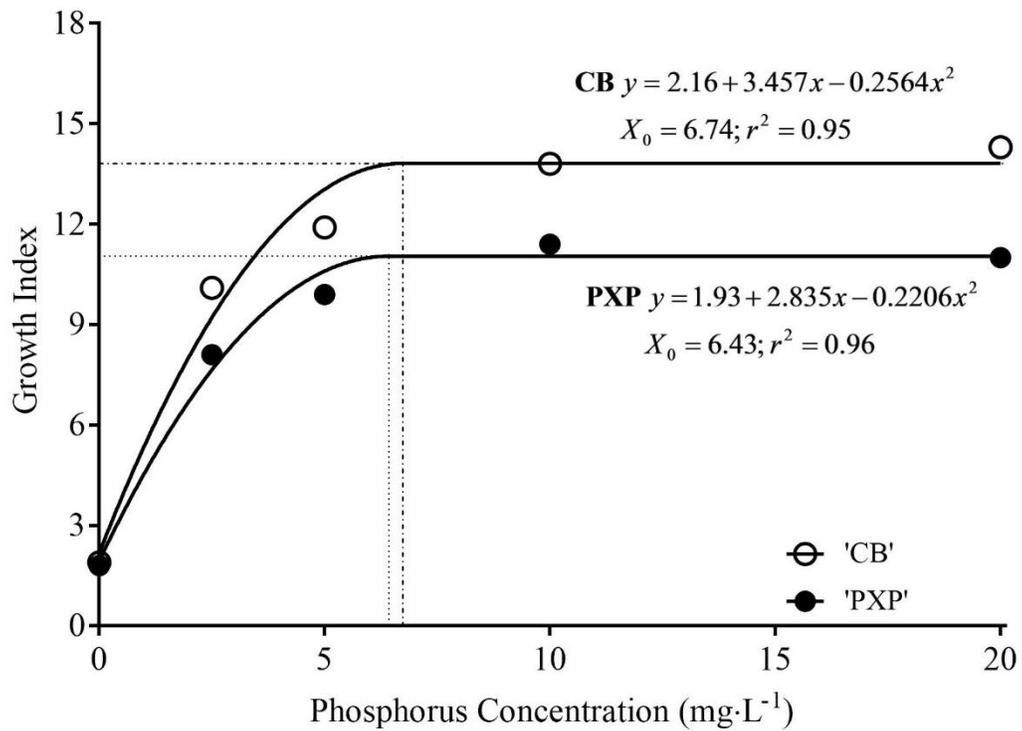


Figure 2.15. Nonlinear regression models with plateau for growth index (GI) of *Catharanthus roseus* 'Cora Burgundy', denoted CB, and 'Pacifica XP Blush', denoted PXP. Regression lines were generated from means of each treatment (CB, n = 7; PXP, n = 8). Equations, r^2 , and X_0 values are located above or below the corresponding line. Vertical and horizontal dotted lines represent X_0 values, where the model plateaus, along with corresponding GI. Models significant at $P \leq 0.0001$.

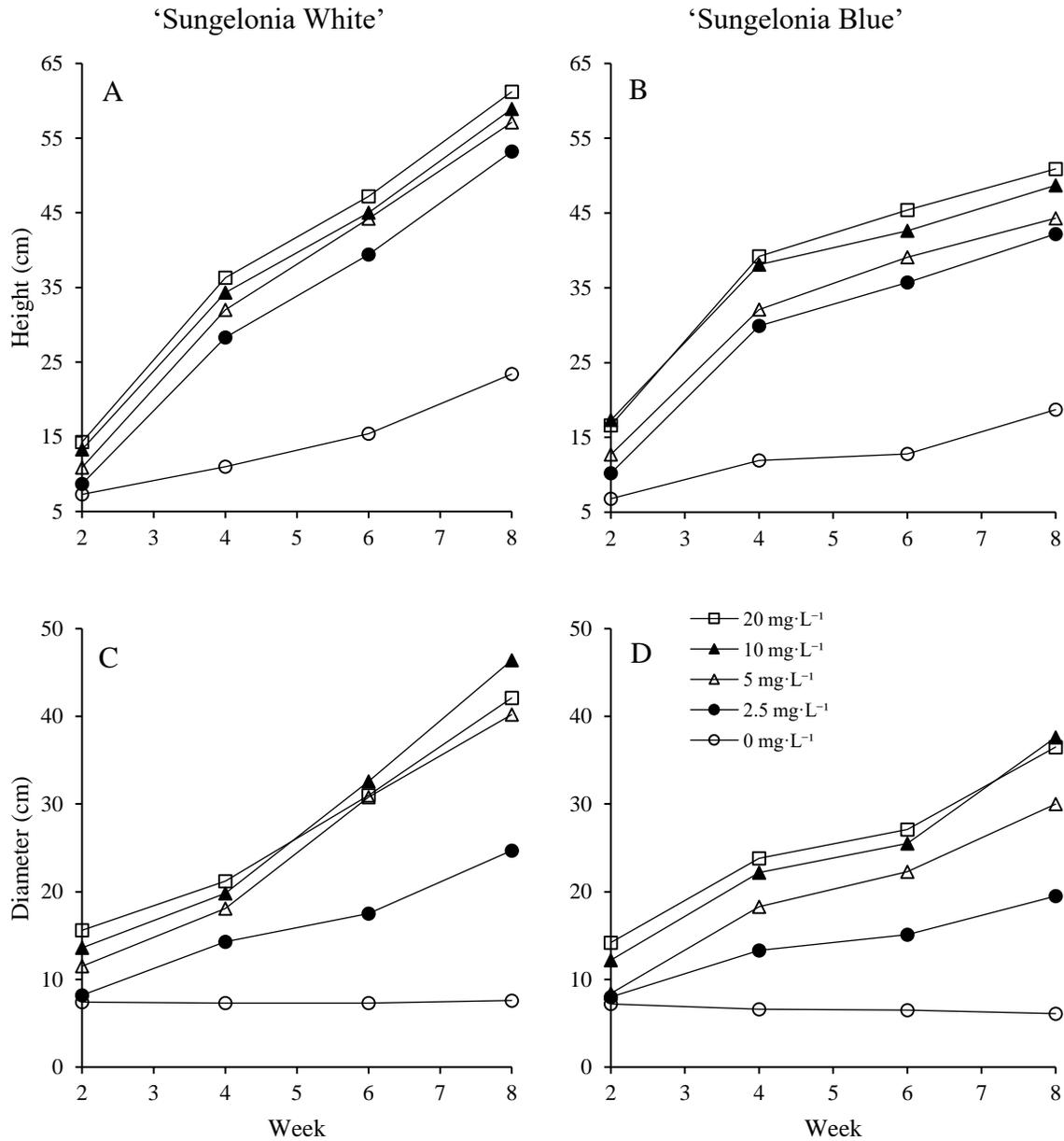


Figure 2.16. Effect of five different fertilizer P concentrations on growth means of *Angelonia angustifolia* 'Sungelonia White' and 'Sungelonia Blue' over an eight-week period. Growth in terms of height (A) and diameter (B) of 'Sungelonia White', compared with height (C) and diameter (D) of 'Sungelonia Blue'.

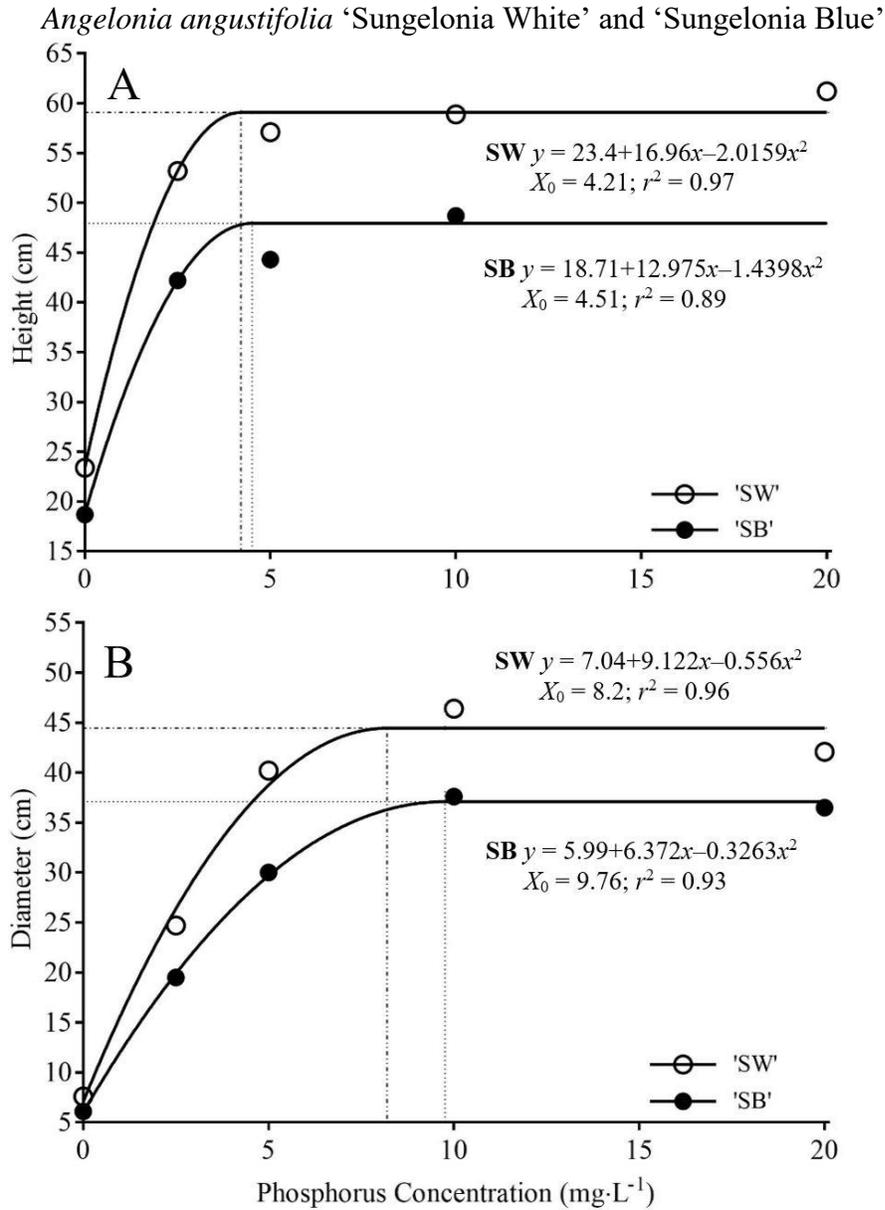


Figure 2.17. Nonlinear regression models with plateau for final height (A) and diameter (B) of *Angelonia angustifolia* ‘Sungelonia White’, denoted SW, and ‘Sungelonia Blue’, denoted SB. Regression lines were generated from means of each treatment ($n = 8$). Equations, r^2 , and X_0 values are located above or below the corresponding line. Vertical and horizontal dotted lines represent X_0 values, where the model plateaus, along with corresponding height or diameter. Models significant at $P \leq 0.0001$.

Angelonia angustifolia ‘Sungelonia White’ and ‘Sungelonia Blue’

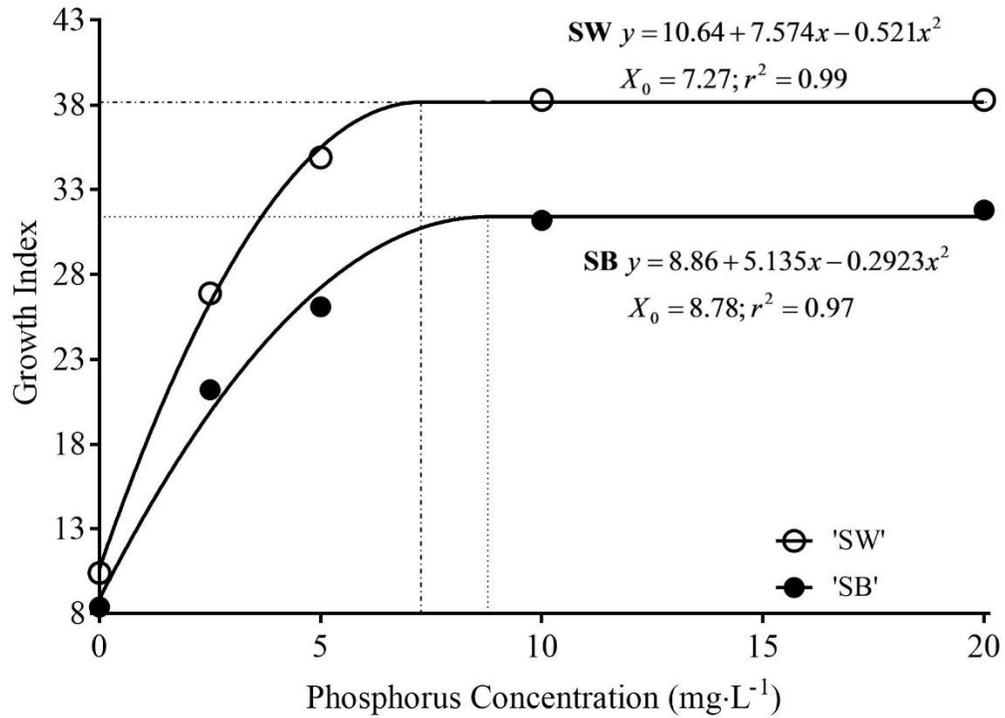


Figure 2.18. Nonlinear regression models with plateau for growth index (GI) of *Angelonia angustifolia* ‘Sungelonia White’, denoted SW, and ‘Sungelonia Blue’, denoted SB. Regression lines were generated from means of each treatment (n = 8). Equations, r^2 , and X_0 values are located above or below the corresponding line. Vertical and horizontal dotted lines represent X_0 values, where the model plateaus, along with corresponding GI. Models significant at $P \leq 0.0001$.

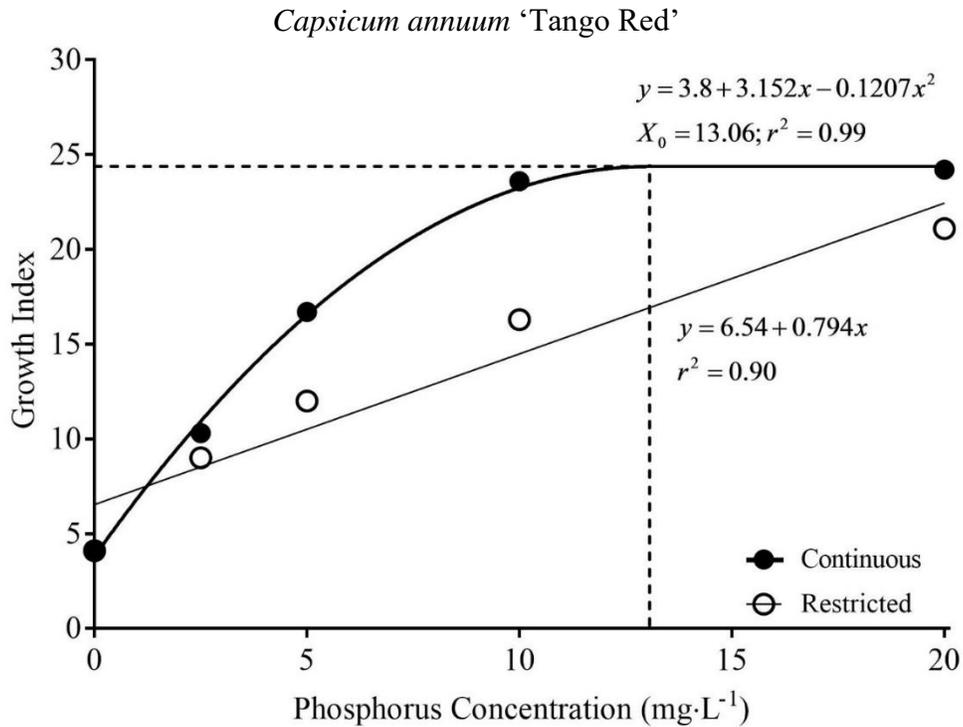


Figure 2.19. Nonlinear regression model with plateau, and linear regression model for final growth index (GI) of *Capsicum annuum* 'Tango Red' grown with continuous or restricted P concentrations. Regression lines were generated from means of each treatment ($n = 6$). Equation, r^2 , and X_0 values are located above the growth plateau model. Equations and value are located below the linear regression model. Vertical and horizontal dotted lines represent X_0 values, where the model plateaus, along with corresponding GI value. Models significant at $P \leq 0.0001$.

Chapter 3

Phosphorus Restriction as an Alternative to Chemical Plant Growth Retardants (PGRs) in Bedding Plants

Abstract

Chemical plant growth retardants (PGRs) are commonly used to produce compact and attractive bedding plants. Fewer PGRs are labeled for sensitive species due to the concern of overregulation or phytotoxicity. Growers are therefore presented with a dilemma: produce untreated plants that may be too tall, or risk applying a PGR that can potentially lead to irreversible aesthetic damage to the plant. Nutrient restriction, specifically of phosphorus (P, referring to phosphorus supplied by phosphate) may be used to limit plant height. This study was done to determine if restricting P fertilization can result in growth control comparable to plants produced with PGRs. Two cultivars each of New Guinea impatiens (*Impatiens hawkeri* W. Bull) and angelonia (*Angelonia angustifolia* Benth.) were grown using five fertilizers that varied by P concentration (0, 2.5, 5, 10, and 20 mg·L⁻¹). Nitrogen (N) and potassium (K) remained constant at 150 mg·L⁻¹ each with fertilizer applied at every irrigation. Half of the plants from each P fertilizer concentration were treated with paclobutrazol approximately half way through the production period. A P concentration of 20 mg·L⁻¹ with a PGR application was used to represent typical production practices. Upon termination of the experiment, data was collected for height, diameter, and dry mass, which were used to determine an overall growth index. Plants grown with 20 mg·L⁻¹ P and a paclobutrazol application had comparable height to plants grown with 5 mg·L⁻¹ P and no PGR in all but one cultivar. *Impatiens hawkeri* ‘Tamarinda Dark Red’ plants grown with 20 mg·L⁻¹ P and a paclobutrazol application had greater height control compared to plants grown with 5 mg·L⁻¹ P and no PGR; however, 5 mg·L⁻¹ P and no PGR still significantly controlled height compared to 20 mg·L⁻¹ P and no PGR. Overall, lower P fertilization provided significant growth control when compared to using PGRs with a higher, more commonly used concentration of P fertilization.

Introduction

Growers of floriculture crops strive to produce compact and healthy plants, which are considered high quality and attractive for consumers (Borch et al., 1998). Excessively large bedding plants are less desirable as they are more difficult to handle and are prone to breakage (van Iersel and Nemali, 2004). Damaged plants are often unmarketable, so growers utilize management practices which limit plant height and promote overall compactness. There are a number of ways in which growers manage excessive growth. Chemical growth regulators such as plant growth retardants (PGRs) have long been the most commonly used forms of growth control in ornamental production (Whipker, 2017). A concern associated with chemicals is that they are not labeled for all crops. This is especially true for vegetable plant growers who are limited on chemical options (Nelson et al., 2012).

Additionally, PGRs have the potential to cause phytotoxicity in sensitive species, leading to unmarketable plants. Although PGRs may still adequately control height in these sensitive plants, damage may occur to the flowers or foliage. This is the case for vinca [*Catharanthus roseus* (L.) G. Don], which develops symptoms of black spotting in response to paclobutrazol (Barrett and Nell, 1987), a PGR commonly used in floriculture production. New Guinea impatiens (*Impatiens hawkeri*) will develop chlorosis due to applications of chlormequat chloride, and may exhibit excessive stunting from an application of paclobutrazol, flurprimidol, or uniconazole (Currey et al., 2016). A number of other crops have also been reported to develop phytotoxicity due to applications of common PGRs, such as the application of ethephon on *Monarda* (Latimer, 2004). Damage severity often varies by cultivar and quantity of active ingredient (a.i.) that is applied to the plant.

Nutrient restriction is an alternative way to limit growth and control height in greenhouse crops (Gibson et al., 2007). Restriction of phosphorus (P) has a direct effect on limiting internode elongation, resulting in more compact plants (Nelson et al., 2012). Phosphorus also has the widest window between beneficial control of plant growth due to mild nutrient stress, and detrimental deficiency symptoms when compared with limitation of other essential nutrients. After the critical value of sufficient P has been reached, most plants exhibit a broad plateau where luxury P consumption does not further encourage excessive growth (Nelson et al., 2012). This makes P a safer and potentially easier input to manipulate for regulation of plant growth.

Research has determined that growers can utilize lower P concentrations during bedding plant seedling production to develop compact plants with a greater root to shoot ratio than those grown with sufficient P concentrations (Huang and Nelson, 1994). This growth management strategy has been suggested for seedling production (Nelson et al., 2012), but requires further investigation and refinement to determine if it may be adapted to control growth over the course of an entire production cycle. P restriction may be successfully implemented for production of seedlings, which takes only a few weeks, whereas an entire production cycle may take several months (McMahon, 2011). Over an extended period of time, P concentrations become depleted, leading to deficiency symptom development. This is especially true for bedding plants produced in typical soilless substrates which have a limited P holding capacity (Marconi and Nelson, 1984; Whipker, 2014). Concentrations of 5 – 10 mg·L⁻¹ P have been recommended for growth control (McMahon, 2011), although little research has been published regarding low P as an alternative to conventional PGRs.

A study by Hansen and Nielsen (2001) investigated low P fertility as an alternative to chemical PGRs. *Pentas lanceolata* (Forssk.) Deflers were grown with 1.5, 4.7, and 31 mg·L⁻¹ P,

and *Rosa-hybrid* were grown using P concentrations of 1.5 and 15.5 mg·L⁻¹. In this study, significant height control for *Pentas* was only observed between P concentrations of 1.5 and 31 mg·L⁻¹, and not with P concentrations of 4.7 mg·L⁻¹ (Hansen and Nielsen, 2001). Additional work by Hansen and Nielsen (2000) found similar results using P concentrations of 0.31, 1.5, 4.7, and 31 mg·L⁻¹ to grow *Argyranthemum frutescens* (L.) Sch. Bip., *Symphotrichum novi-belgii* (L.) G. L. var. *novi-belgii*, and *Euphorbia pulcherrima* Willd. ex Klotzsch. They reported that significant height control was achieved between plants grown with P concentrations of 1.5 or less, and 31 mg·L⁻¹. This work indicated that current P concentrations used for bedding plant production exceed what is required.

Low P fertilization may have other benefits to growers in addition to growth control, as environmental regulations place more emphasis on reducing agricultural runoff (Majsztzik and Lea-Cox, 2013). Many municipalities and agencies are becoming aware of the issues associated with excessive nutrient application and runoff into water sources. Greenhouse and nursery growers in the Chesapeake Bay watershed have been encouraged to use improved management practices to reduce unnecessary runoff and improve the bay water quality (Majsztzik and Lea-Cox, 2013).

Determining P concentrations which lead to compact growth without the development of deficiency symptoms will provide growers an alternative method of growth control for their crops. The objective of this study was to determine if restricting P fertilization alone can result in comparable growth control to plants produced with a more commonly used concentration of P in conjunction with a PGR application.

Materials and Methods

Two cultivars each of New Guinea impatiens and angelonia (*Angelonia angustifolia*) were grown, representing crops with high and low sensitivity to PGRs, respectively. Plants were propagated and grown in a glass greenhouse at 35°N latitude in Raleigh, NC. All cuttings were stuck into 128-cell plug trays with cell dimensions of 2.7 x 2.7 x 3.8 cm (length x width x depth). Greenhouse day/night temperature set points were 23.9/18.3°C. During the course of the experiment, the maximum temperature was 35.4°C, the minimum was 15.8°C, and the mean was 23.7°C. Experiments were conducted under natural photoperiod, with 50% shade (Ludwig Svensson Inc., Charlotte, NC), drawn between 11:00 and 15:00.

The substrate used for the entire experiment was an 80:20 (v:v) mix of Canadian sphagnum peat moss (Conrad Fafard, Agawam, MA) and horticultural coarse perlite (Perlite Vermiculite Packaging Industries, Inc., North Bloomfield, OH), with added dolomitic lime at 8.875 kg/m³ (Rockydale Agricultural, Roanoke, VA) and AquaGro 2000-G Wetting Agent (Aquatrols, Cherry Hill, NJ) at 600.3 g/m³. This custom substrate was used to ensure that there was no initial charge of P, which is typically included in most commercial substrate mixes.

Phosphorus (referring to phosphate-phosphorus) fertilizer concentrations of 0, 2.5, 5, 10, or 20 mg·L⁻¹ were used, with nitrogen (N) and potassium (K) remaining constant at 150 mg·L⁻¹ each. The highest P concentration of 20 mg·L⁻¹ was similar to the amount of P that would be supplied by a common fertilizer formulation such as 20 N – 4.4 P – 16.6 K mixed at 100 mg·L⁻¹ N, or 15 N – 2.2 P – 12 K Cal – Mag mixed at 150 mg·L⁻¹ N. Fertilization began on the day of transplant, and fertilizers were custom blends of the following individual technical grade salts: Ca(NO₃)₂·4H₂O, KNO₃, KH₂PO₄, K₂SO₄, MgSO₄·7H₂O, Mg(NO₃)₂, FeDTPA, MnCl₂·4H₂O, ZnCl₂·7H₂O, CuCl₂·2H₂O, H₃BO₃, and Na₂MoO₄·2H₂O (Appendix A). Fertilizer solution was

mixed in 100 L barrels, and was applied via sump pumps (Model 1A, Little Giant Pump Co., Oklahoma City, OK) which was connected to 1.9 cm black irrigation tubing. Tubing was fitted with drip rings (Dramm USA, Manitowoc, WI) to deliver irrigation solution to each pot.

Destructive harvests were completed upon termination of each experiment. Plant height, diameter, and dry mass were recorded. Measurements were collected for plant height by measuring the highest point of the foliage with a ruler from the rim of the pot, and plant diameter by averaging the widest point and the axis perpendicular to that. Harvested plants were severed at the substrate level, and aboveground tissues were allowed to dry for at least 72 hours at 70°C. After drying, total plant dry mass was recorded.

Angelonia

A. angustifolia ‘Sungelonia Blue’ and ‘Sungelonia White’ cuttings (Dümmen Orange, Columbus, OH) were stuck on 3 May 2016 into 128-cell plug trays. Rooted cuttings were transplanted on 27 May into 5-inch azalea pots (Dillen, Middlefield, OH) with dimensions of 12.7 x 9.2 cm (diameter x depth) and a volume of 0.8 L. Sixteen plants of each cultivar were grown at each P concentration. Four weeks after transplant, eight plants of each P concentration were randomly selected and treated with a PGR, while the other eight remained untreated. A substrate drench of paclobutrazol (Piccolo 10 XC; Fine Americas, Inc., Walnut Creek, CA) was applied at a concentration of 4 mg a.i. per pot, within a total volume of 118.3 mL per pot. A final destructive harvest was conducted eight weeks after transplant.

Impatiens

I. hawkeri ‘Tamarinda Dark Red’ and ‘Pure Beauty Red on Pink’ cuttings (Dümmen Orange, Columbus, OH) were stuck on 3 May 2016. Once the cuttings were well rooted, plugs were transplanted on 24 May into 5-inch diameter pots. Sixteen plants of each cultivar were grown using each P concentration. Five weeks after transplant, eight plants of each cultivar from each P concentration were randomly selected and treated with a foliar spray of 7.5 mg·L⁻¹ paclobutrazol using a volume of 0.2 L per square meter. The remaining eight plants remained untreated. Ten weeks after transplant, the experiment was terminated and plants were destructively harvested.

Data analysis

Height, diameter, and dry mass were analyzed and used to calculate a growth index (GI) (Equation 1) which was based off the equation for GI presented by Krug et al. (2010).

Equation 1

$$GI = \frac{\text{height} + \frac{\text{diameter 1} + \text{diameter 2}}{2} + \text{dry mass}}{3}$$

Statistical analysis was conducted using SAS (version 9.4; SAS Institute, Cary, NC). Data were subjected to PROC GLM and the means were separated by least significant differences at $P \leq 0.05$. PROC REG and PROC NLIN were then used to regress the data and determine the best fit linear or quadratic model for P concentrations with and without PGR applications. For PROC REG, variables used in the models were P concentration and indicator

variables for PGR applications, to separate data from the same P concentration grown with or without a PGR. Terms significant at the 0.05 level were selected for inclusion in the model. The full models used were:

Model 1

$$\text{Growth Parameter}_{ij} = \beta_0 + \text{PGR2} + \beta_1 \text{Con} + \text{PGR2Con} + \beta_2 \text{Con}^2 + \text{PGR2Con}^2$$

Where:

Growth Parameter = height, diameter, dry mass, growth index, or percent of maximum growth index, with:

i = fertilizer P concentration

j = PGR application

Con = P concentration

β_k = estimated coefficients (k = 0 to 2)

Using the following indicator variables:

PGR2 = 1 if PGR application was paclobutrazol
0 otherwise

PGR2Con = PGR2 * Con

PGR2Con² = PGR2 * Con²

PROC NLIN was used to determine best fit quadratic plateau models (Model 2) for each growth parameter.

Model 2

$$Y_i = \begin{cases} \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \varepsilon_i & \text{if } X < X_0 \\ Y_0 + \varepsilon_i & \text{if } X \geq X_0 \end{cases}$$

Where:

$$X_0 = -\frac{\hat{\beta}_1}{2\hat{\beta}_2}, \text{ and}$$

$$Y_0 = \hat{\beta}_0 - \frac{\hat{\beta}_1^2}{4\hat{\beta}_2}$$

Quadratic models obtained from PROC REG and quadratic plateau models obtained from PROC NLIN were then compared and selected based on r^2 values. X_0 values provided for growth plateaus indicate the P concentration where each growth parameter reached its respective maximum, past which point no additional growth increase was observed.

Results

Significant effects on several aspects of plant growth, including height, diameter, and dry mass were observed with different P concentrations. Applications of paclobutrazol also resulted in significant differences in these aspects of plant growth. The maximum P concentration of 20 mg·L⁻¹ was considered a standard concentration used by growers of these two crops. As these crops are often produced with PGR applications, growth parameters were compared between plants grown with and without paclobutrazol applications. These parameters were primarily compared for plants with no PGR application and those grown with 20 mg·L⁻¹ P and a paclobutrazol application.

Impatiens

I. hawkeri ‘Pure Beauty Red on Pink’ (Figure 3.1) and ‘Tamarinda Dark Red’ (Figure 3.2) were both significantly affected by P concentration and PGR application (Table 3.1). Plants grown with and without paclobutrazol application followed similar trends in growth across P concentrations, with PGR treated plants being shorter than those produced with the same concentration of P but no PGR.

Height of ‘Pure Beauty Red on Pink’ plants grown with 20 mg·L⁻¹ P and a paclobutrazol application were similar to plants grown with 5 mg·L⁻¹ P and no PGR. Although plants grown with 5 mg·L⁻¹ P and no PGR had similar height as those grown with 20 mg·L⁻¹ P and a paclobutrazol application, they had significantly smaller diameters and produced less dry mass. Plants grown without P developed negative symptoms of P deficiency including a noticeable darkening of the lower foliage with minor necrosis, and an inhibition of flowering. Plants grown with 2.5 mg·L⁻¹ P also developed mild P deficiency symptoms on the lower foliage, including a dark purple spotting. This purple spotting was not readily apparent, as it easily blended in with the naturally dark coloration of the foliage. Canopy architecture appeared to be affected both by P concentration and PGR application. Leaves of plants grown with lower P concentrations were more upright than those of plants grown with higher P concentrations. Additionally, leaves of plants treated with paclobutrazol were not as upright as their counterparts not treated with a PGR.

When comparing plants grown with 20 mg·L⁻¹ P and a paclobutrazol application with 5 mg·L⁻¹ P and no PGR, ‘Tamarinda Dark Red’ reacted similarly to ‘Pure Beauty Red on Pink’ in regards to diameter and dry mass (Table 3.1). Height of ‘Tamarinda Dark Red’ plants grown with 5 mg·L⁻¹ P and no PGR were significantly taller, while plants grown with 2.5 mg·L⁻¹ P and

no PGR were significantly shorter than plants grown with 20 mg·L⁻¹ P and a PGR. This indicates that although plants grown with 5 mg·L⁻¹ P and no PGR are taller than plants grown with 20 mg·L⁻¹ P and a PGR, P concentrations less than 5 mg·L⁻¹, but greater than 2.5 mg·L⁻¹ can be used to control height with this cultivar. A visual comparison of plants grown with 5 mg·L⁻¹ P and no PGR application, and plants grown with 20 mg·L⁻¹ P and a paclobutrazol application may be observed in Figure 3.3. Plants that were grown without P developed detrimental symptoms of P deficiency including a prominent reddening of the lower foliage, necrosis, and inhibited flowering (Figure 3.4). The severe stunting and change in overall leaf architecture was very apparent for plants grown without P, regardless of PGR application.

Nonlinear regression models for height (Figure 3.5) illustrate how growth plateaus with increasing concentrations of P. Growth plateaus for ‘Pure Beauty Red on Pink’ indicate that plants grown with a PGR reached their maximum height with 9.46 mg·L⁻¹ P (X_0), while plants grown without PGR reached their maximum height with 10.07 mg·L⁻¹ P (Figure 3.5 A). Differences in growth plateaus for plants grown with and without a PGR application were wider for ‘Tamarinda Dark Red’. Maximum height for plants not treated with a PGR was achieved with 8.06 mg·L⁻¹ P, while plants treated with a PGR reached their maximum height with 10.99 mg·L⁻¹ P (Figure 3.5 B).

Models for plant diameter (Figure 3.6) indicate that cultivars reacted differently in terms of optimal P concentration and PGR response. Maximum diameter of ‘Pure Beauty Red on Pink’ plants treated with a PGR was achieved with 9.21 mg·L⁻¹ P, while plants not treated with a PGR reached their maximum with 14.1 mg·L⁻¹ P (Figure 3.6 A). ‘Tamarinda Dark Red’ plants exhibited very little difference among plants that were and were not treated with paclobutrazol. Maximum diameter was similar for both plants grown with and without PGR, and was reached

using concentrations of 9.65 and 10.08 mg·L⁻¹ P, respectively (Figure 3.6 B). These trends illustrate that growth parameters were different between cultivars and were significantly affected by both the concentration of P and PGR application.

Maximum dry mass did not differ greatly between plants that were and were not treated with paclobutrazol, especially when using lower concentrations of P. This is in agreement with LSDs provided in Table 3.1. ‘Pure Beauty Red on Pink’ plants that were not treated with paclobutrazol achieved maximum dry mass with 13.69 mg·L⁻¹ P, while PGR treated plants achieved maximum diameter with P concentrations of 12.38 mg·L⁻¹ (Figure 3.7 A). Maximum dry mass was achieved for ‘Tamarinda Dark Red’ using P concentrations of 12.02 and 11.52 mg·L⁻¹ for plants that did and did not receive a PGR application, respectively (Figure 3.7 B). P concentrations to reach maximum dry mass were greater than concentrations used to reach maximum height and maximum diameter in all cases, except for ‘Pure Beauty Red on Pink’ plants that did not receive a PGR application. This illustrates how plants typically continue to exhibit luxury consumption of P (Nelson et al., 2012) and accumulate additional biomass past the point at which plants reached their maximum dimensions.

Growth plateau models for GI provide the optimal maximum P concentration for each cultivar, grown with and without a PGR application (Figure 3.8). This is due to the fact that GI takes all other growth parameters into account and averages them equally to determine overall growth response over a range of P concentrations. ‘Pure Beauty Red on Pink’ plants grown without a PGR application had a greater P requirement to achieve maximum growth than plants that received a paclobutrazol application. Plants not treated with a PGR achieved maximum GI with P concentrations of 12.43 mg·L⁻¹, while plants treated with paclobutrazol required only 9.72 mg·L⁻¹ P (Figure 3.8 A). In contrast, ‘Tamarinda Dark Red’ plants achieved maximum GI using

9.64 mg·L⁻¹ P for plants that did not receive a paclobutrazol application, and 10.52 mg·L⁻¹ P for plants that did receive a paclobutrazol application (Figure 3.8 B). This cultivar required greater P concentrations to achieve maximum overall growth when paclobutrazol was applied as a spray.

Comparing GIs between cultivars demonstrates that cultivars vary in response to P concentrations and their respective PGR application. Overall, differences among means and growth models suggest that maximum heights for New Guinea impatiens were achieved with P concentrations of approximately 10 mg·L⁻¹. By observing where the PGR model plateau intersects with the untreated model (Figure 3.5), one can determine the point at which untreated plants achieved the same height as those treated with paclobutrazol. This point was between 4 – 5 mg·L⁻¹ P, depending on cultivar. As height may be considered the primary aspect to be controlled by PGR applications, using P concentrations targeted to control height are likely most useful for commercial production practices. This indicates that growers may achieve comparable growth control to PGR treated New Guinea impatiens plants by utilizing P concentrations of 4 – 5 mg·L⁻¹ P.

Angelonia

Least significant differences for *A. angustifolia* ‘Sungelonia Blue’ and ‘Sungelonia White’ illustrate overall differences in height, diameter, and dry mass (Table 3.2). The paclobutrazol application did not result in significant height control at most P concentrations for ‘Sungelonia Blue’. Plants grown with 10 mg·L⁻¹ P were the only ones with significant height control when treated with a PGR. Significant height control was still achieved with lower P concentrations, regardless of PGR. Diameters only differed between plants grown 20 mg·L⁻¹ P, with the paclobutrazol treated plants being wider. All other differences in diameter were due to

fertilizer effects, and did not vary by PGR application. Dry mass only differed between paclobutrazol treated and not treated plants grown with 10 mg·L⁻¹ P (Table 3.2).

‘Sungelonia White’ plants grown with 20 mg·L⁻¹ P and a paclobutrazol application had similar height as plants grown with 5 mg·L⁻¹ P and no PGR. This trend was in general agreement with results from New Guinea impatiens. Plant diameter reacted similarly to ‘Sungelonia Blue’, with significant differences only between plants grown with 20 mg·L⁻¹ P, with and without a paclobutrazol application. The greatest diameter for paclobutrazol treated plants was at 20 mg·L⁻¹ P, and 10 mg·L⁻¹ P for plants that were not treated. All other differences in diameter were among P concentrations. Dry mass of plants treated with paclobutrazol and P concentrations of 5 – 20 mg·L⁻¹ were less than the corresponding untreated plants (Table 3.2). At detrimentally low P concentrations of 0 and 2.5 mg·L⁻¹, dry masses were similar between each P concentration, regardless of whether a PGR was applied. This indicates that at sub-optimal P concentrations, P has a greater impact on plant growth than a PGR.

Nonlinear regression models for height and diameter were determined with data from both cultivars of angelonia combined. The models for height (Figure 3.9 A) indicate that maximum height for plants not treated with a PGR was achieved with 4.34 mg·L⁻¹ P, while plants treated with a PGR reached maximum height with 4.53 mg·L⁻¹ P. Although plants that received a paclobutrazol application achieved maximum height that was 4.3 cm less than untreated plants, all plants required a similar amount of P to achieve maximum height. This indicates that optimal P concentrations for height control of angelonia do not differ greatly depending on PGR application. The models for plant diameter (Figure 3.9 B) indicate that P concentrations to achieve maximum diameter were affected by PGR application. Maximum diameter for plants grown with and without PGR required concentrations of 10.78 and 8.85

mg·L⁻¹ P, respectively. Maximum diameter of PGR treated plants was 3.8 cm greater than plants that did not receive a PGR application, indicating that low P fertilization may provide better control for diameter than PGRs.

‘Sungelonia Blue’ plants that were not treated with a PGR reached maximum dry mass with P concentrations of 16.11 mg·L⁻¹ (Figure 3.10 A). The model for paclobutrazol treated plants was better suited for a typical quadratic regression model, since dry mass did not reach a plateau within the confines of the P concentrations used in this study. According to this model, maximum dry mass for PGR treated plants occurred at approximately 18 mg·L⁻¹ P. Maximum dry mass for ‘Sungelonia White’ was achieved using P concentrations of 15.02 mg·L⁻¹ for paclobutrazol treated plants, and 14.93 mg·L⁻¹ for untreated plants (Figure 3.10 B). Although plants that received a PGR application did not accumulate as much dry mass as untreated plants, they still required a similar amount of P to achieve maximum dry mass.

Maximum GI for ‘Sungelonia Blue’ plants not treated with paclobutrazol was reached with P concentrations 8.79 mg·L⁻¹ (Figure 3.11 A). PGR treated plants reached maximum GI with similar P concentrations of 9.09 mg·L⁻¹. ‘Sungelonia White’ plants reached maximum GI with 7.27 mg·L⁻¹ when left untreated (Figure 3.11 B). Plants that were treated with a PGR reached maximum GI with P concentrations of 8.53 mg·L⁻¹. These maximum GIs indicate that concentrations less than 7 – 9 mg·L⁻¹ P may be used to control growth.

Phosphorus concentrations for optimal height control should be considered most useful for commercial production practices. Overall, these growth models suggest that maximum height for angelonia is achieved with P concentrations of approximately 4.5 mg·L⁻¹. By observing where the PGR model plateau intersects with the untreated model (Figure 3.9 A), one can determine the point at which untreated plants achieved the same height as those treated with

paclobutrazol. This point occurs at just 2.8 mg·L⁻¹ P. This indicates that growers may achieve comparable growth control to PGR treated angelonia plants by utilizing P concentrations of 3 mg·L⁻¹ P.

Discussion

Utilizing low P concentrations provided comparable growth control to plants grown with the typically high P concentrations of 20 mg·L⁻¹ P used in greenhouse bedding plant production, in conjunction with a PGR. According to regression analysis, New Guinea impatiens grown with 4 – 5 mg·L⁻¹ P, and no PGR had comparable height to those grown with a typical high concentration of 20 mg·L⁻¹ P in conjunction with a PGR. Both cultivars of angelonia had a similar, but slightly lower P requirement than New Guinea impatiens. Angelonia grown with approximately 3 mg·L⁻¹ P, and no PGR had comparable height to PGR treated plants grown with a typical high P concentration and a PGR. These results deviate from the findings of Hansen and Nielsen (2000, 2001), as significant height control was not achieved when lowering P concentrations from 31 to 4.7 mg·L⁻¹. However, Hansen and Nielsen tested four species that did not include New Guinea impatiens or angelonia. In our study, significant growth control was achieved for both cultivars of New Guinea impatiens (Table 3.1) and angelonia (Table 3.2) when using P concentrations of 5 mg·L⁻¹ compared to 20 mg·L⁻¹.

Hansen and Nielsen (2000, 2001) found that P concentrations of 1.5 mg·L⁻¹ provided significant height control when compared with their highest concentration of 31 mg·L⁻¹ P. This is in agreement with the findings in this study; however, P concentrations of 2.5 mg·L⁻¹ led to plants that were extremely small and even developed mild symptoms of P deficiency, as was the case with *I. hawkeri* ‘Pure Beauty Red on Pink’. Plants grown with this P concentration were

significantly smaller in both height and diameter, to the point that plants did not grow sufficiently for the canopy to cover the pot (Figure 3.1). Plants grown without P had severe symptoms of P deficiency, though symptoms were more severe on ‘Tamarinda Dark Red’ (Figure 3.3). Results from Hansen and Nielsen (2000, 2001) indicated that growing plants with $1.5 \text{ mg}\cdot\text{L}^{-1}$ provided significant growth control with no detrimental effects on appearance or flowering.

The discrepancy between the findings of this study and those of Hansen and Nielsen (2000, 2001) may be explained by the differences in substrate and fertility. Hansen and Nielsen used kiln dried clay as the substrate, which was treated with a P buffer. This buffer supplied P at a constant concentration that releases steadily upon each irrigation, rather than being supplied through a liquid fertilizer (Oh et al., 2016). This P buffer likely provided a more constant P supply (Borch et al., 1998; Oh et al., 2016), thus preventing deficiency symptoms from developing even when P concentrations are very low. In fact, other studies have found that plants grown in P buffered substrates can achieve maximum growth with just $0.093 \text{ mg}\cdot\text{L}^{-1}$ P (Lynch et al., 1991). This low concentration would result in detrimental P deficiency symptoms in the peat based substrates typically used in commercial bedding plant production. In this study, a peat and perlite based substrate was used in conjunction with a constant liquid feed fertilization program, which is more typical of practices used in greenhouse production of ornamental bedding plants. As the methods in this study replicate typical production practices, results may be used directly to provide fertilizer recommendations to growers.

Much of the low P work that has been done with bedding plants has utilized a P buffer, and have suggested using P concentrations ranging from $0.093 - 1.5 \text{ mg}\cdot\text{L}^{-1}$ for compact and healthy growth (Borch et al., 1998; Hansen and Nielsen, 2000, 2001). Borch et al. (1998) used

low P to investigate the effects of growth control on *Impatiens walleriana* Hook. f. and *Tagetes patula* L.; however, P buffered substrate was compared with periotic liquid fertilization. Plants were grown using P buffer concentrations of 0.093 and 0.28 mg·L⁻¹, and compared with plants grown using liquid fertilization with a P concentration of 46.5 mg·L⁻¹. Even with these differences in P concentration, no significant height control was observed in either species (Borch et al., 1998). In this study however, significant height control was achieved for New Guinea impatiens and angelonia with several of the lower P concentrations (Table 3.1, Table 3.2).

Also of note was that low P concentrations provided significant height control in *A. angustifolia* ‘Sungelonia Blue’, while the PGR application did not (Table 3.2). This indicates that there was a cultivar effect, as PGR applications resulted in significant height control for ‘Sungelonia White’, and both cultivars of New Guinea impatiens. This implies that low P concentrations may be used to supply more significant and uniform height control than PGRs in some instances.

Conclusions

Significant differences in growth parameters occurred among plants grown within a narrow range of fertilizer P concentrations. This study demonstrated that growers can successfully control plant growth using low P fertilization. Plants grown with low P concentrations were comparable in size to plants grown with a typical P concentration in conjunction with a PGR application. Optimal P concentrations varied by species, but were lower than current recommendations for greenhouse bedding plant production. Optimal concentrations for height control in New Guinea impatiens were approximately 4 – 5 mg·L⁻¹ P, when fertilizing

at every irrigation, while angelonia appeared to require concentrations as low as 2.8 mg·L⁻¹ P. Lowering rates more than this resulted in significantly smaller plants, though they were severely stunted and also displayed varying symptoms of P deficiency, including reddening and necrosis of the lower foliage, and floral inhibition in New Guinea impatiens.

Knowing optimal P concentrations for growth control will provide growers with more options when deciding upon growth management strategies. Growth regulation using conventional PGRs and utilizing low P fertilization are not mutually exclusive. Used in conjunction, growers may be able to limit PGR overdoses while still maintaining high quality production practices. Additionally, using low P fertilization will aid in reducing P runoff and leaching into natural water supplies, limiting eutrophication, and helping growers to follow best management practices that are becoming more commonly used in environmental regulations.

Acknowledgements

We are grateful for the funding support provided by Fred C. Gloeckner Foundation, American Floral Endowment Altman Family Scholarship, and The Garden Club of America. We would also like to express our gratitude to Dümmer Orange for providing cuttings, and for peat moss provided by Sun Gro Horticulture.

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Table 3.1. Least Significant Differences for height, diameter, and dry mass of *Impatiens hawkeri* 'Pure Beauty Red on Pink' and 'Tamarinda Dark Red' plants grown with and without a plant growth regulator (PGR) and at five fertilizer P concentrations.

Phosphorus Concentration (mg·L ⁻¹)	Height (cm)		Diameter (cm)		Dry Mass (g)	
	No PGR	PGR ¹	No PGR	PGR	No PGR	PGR
Pure Beauty Red on Pink						
0	10.2 e	7.5 f	11.5 f	11.3 f	0.9 f	0.8 f
2.5	15.0 c	12.1 d	17.7 e	18.0 e	3.1 e	2.9 e
5	17.3 b	14.9 c	22.6 d	22.6 d	5.6 d	5.6 d
10	20.1 a	16.9 b	26.5 b	24.7 c	7.4 bc	7.0 c
20	20.1 a	17.4 b	29.4 a	26.5 b	8.3 a	7.7 b
LSD _{0.05}	0.87		1.08		0.42	
Tamarinda Dark Red						
0	10.1 f ²	8.6 g	7.8 f	7.3 f	0.8 f	0.7 f
2.5	14.5 d	12.0 e	15.1 e	16.8 d	3.1 e	2.9 e
5	18.8 b	15.0 d	23.2 c	21.9 c	6.3 c	5.6 d
10	18.7 b	17.8 bc	26.6 b	26.5 b	7.9 b	7.8 b
20	20.6 a	17.5 c	28.2 a	27.3 ab	8.5 a	7.8 b
LSD _{0.05}	1.09		1.29		0.52	

¹ PGR treatment was applied as a foliar spray of 7.5 mg·L⁻¹ paclobutrazol using a volume of 0.2 L per square meter.

² Significant differences among treatment means indicated by corresponding letters based on LSD *t* tests significant at $P \leq 0.05$.

Table 3.2. Least Significant Differences for height, diameter, and dry mass of *Angelonia angustifolia* 'Sungelonia Blue' and 'Sungelonia White' plants grown with and without a plant growth regulator (PGR) and at five fertilizer P concentrations.

Phosphorus Concentration (mg·L ⁻¹)	Height (cm)		Diameter (cm)		Dry Mass (g)	
	No PGR	PGR ¹	No PGR	PGR	No PGR	PGR
Sungelonia Blue						
0	18.7 e ²	18.7 e	6.1 f	6.7 f	0.2 e	0.3 e
2.5	42.2 cd	39.4 d	19.5 e	19.7 e	2.1 d	2.0 d
5	44.3 bc	41.4 cd	30.0 d	31.5 d	4.0 c	3.9 c
10	48.7 a	43.8 bc	37.6 bc	40.2 ab	7.5 a	5.0 b
20	50.9 a	47.5 ab	36.5 c	41.9 a	8.1 a	7.2 a
LSD _{0.05}	3.85		3.34		0.90	
Sungelonia White						
0	23.4 f	21.0 f	7.6 f	7.7 f	0.3 g	0.3 g
2.5	53.2 d	47.3 e	24.7 e	22.8 e	2.9 f	2.4 f
5	57.1 bc	52.4 d	40.2 cd	38.1 d	7.2 d	5.9 e
10	58.9 ab	54.6 cd	46.4 ab	46.1 b	9.7 b	8.8 c
20	61.2 a	56.0 c	42.1 c	48.9 a	11.4 a	9.9 b
LSD _{0.05}	2.72		2.55		0.75	

¹ PGR treatment was applied as a paclobutrazol substrate drench applied at a concentration of 4 mg a.i. per pot, within a total volume of 118.3 mL per pot.

² Significant differences among treatment means indicated by corresponding letters based on LSD *t* tests significant at $P \leq 0.05$.

Impatiens hawkeri 'Pure Beauty Red on Pink'

Paclobutrazol



No PGR



0

2.5

5

10

20

Phosphorus Concentration ($\text{mg}\cdot\text{L}^{-1}$)

Figure 3.1. Visual differences in plant size, canopy architecture, and overall growth for *Impatiens hawkeri* 'Pure Beauty Red on Pink' plants grown with a foliar spray of $7.5 \text{ mg}\cdot\text{L}^{-1}$ paclobutrazol (top) and without a PGR (bottom) and at five fertilizer P concentrations.

Impatiens hawkeri 'Tamarinda Dark Red'

Paclobutrazol



No PGR



0

2.5

5

10

20

Phosphorus Concentration ($\text{mg}\cdot\text{L}^{-1}$)

Figure 3.2. Visual differences in plant size, canopy architecture, and overall growth for *Impatiens hawkeri* 'Tamarinda Dark Red' plants grown with a foliar spray of $7.5 \text{ mg}\cdot\text{L}^{-1}$ paclobutrazol (top) and without any PGR (bottom) and at five fertilizer P concentrations.

Impatiens hawkeri 'Tamarinda Dark Red'

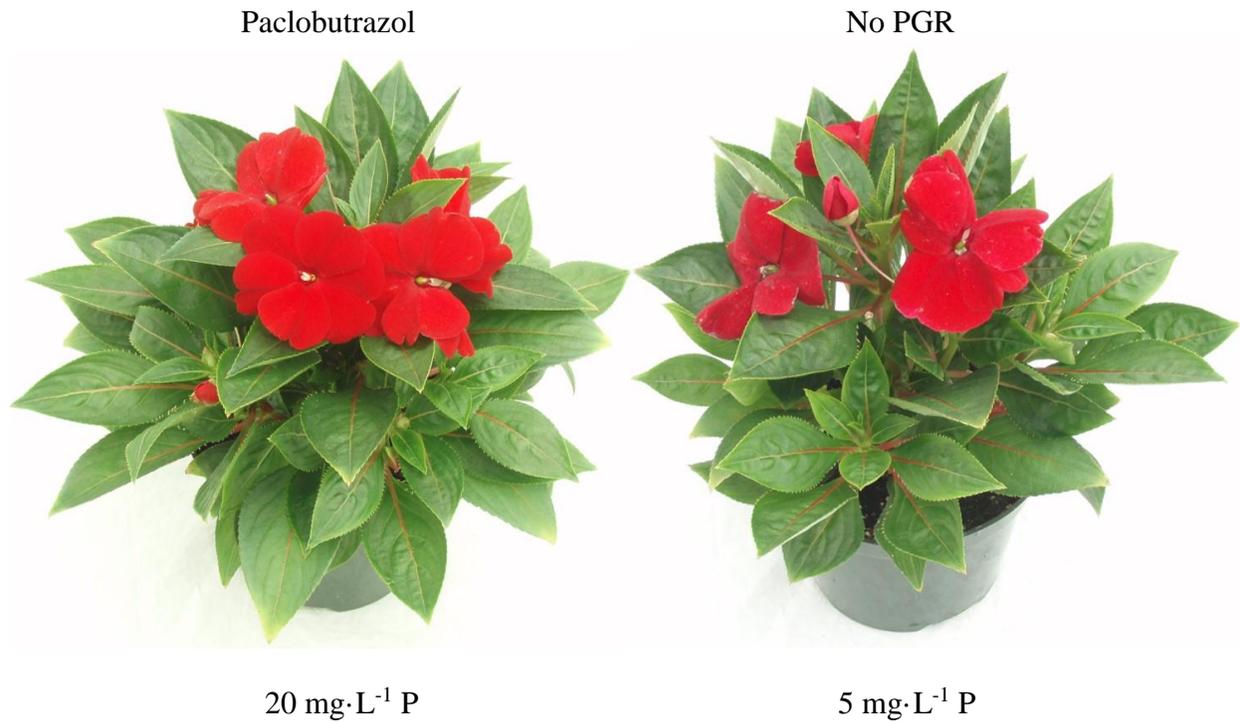


Figure 3.3. An *Impatiens hawkeri* 'Tamarinda Dark Red' plant grown with 20 mg·L⁻¹ P and a foliar spray of 7.5 mg·L⁻¹ paclobutrazol (left) compared to a plant grown with 5 mg·L⁻¹ P and no PGR application (right). Overall plant size was similar between plants of these two treatments.

Impatiens hawkeri 'Tamarinda Dark Red'



Figure 3.4. *Impatiens hawkeri* 'Tamarinda Dark Red' grown with $0 \text{ mg}\cdot\text{L}^{-1}$ P displayed symptoms of severe stunting, lower leaf reddening and necrosis. Plants grown with this concentration of P also did not flower. This photo was taken at termination of the experiment, 10 weeks after transplant.

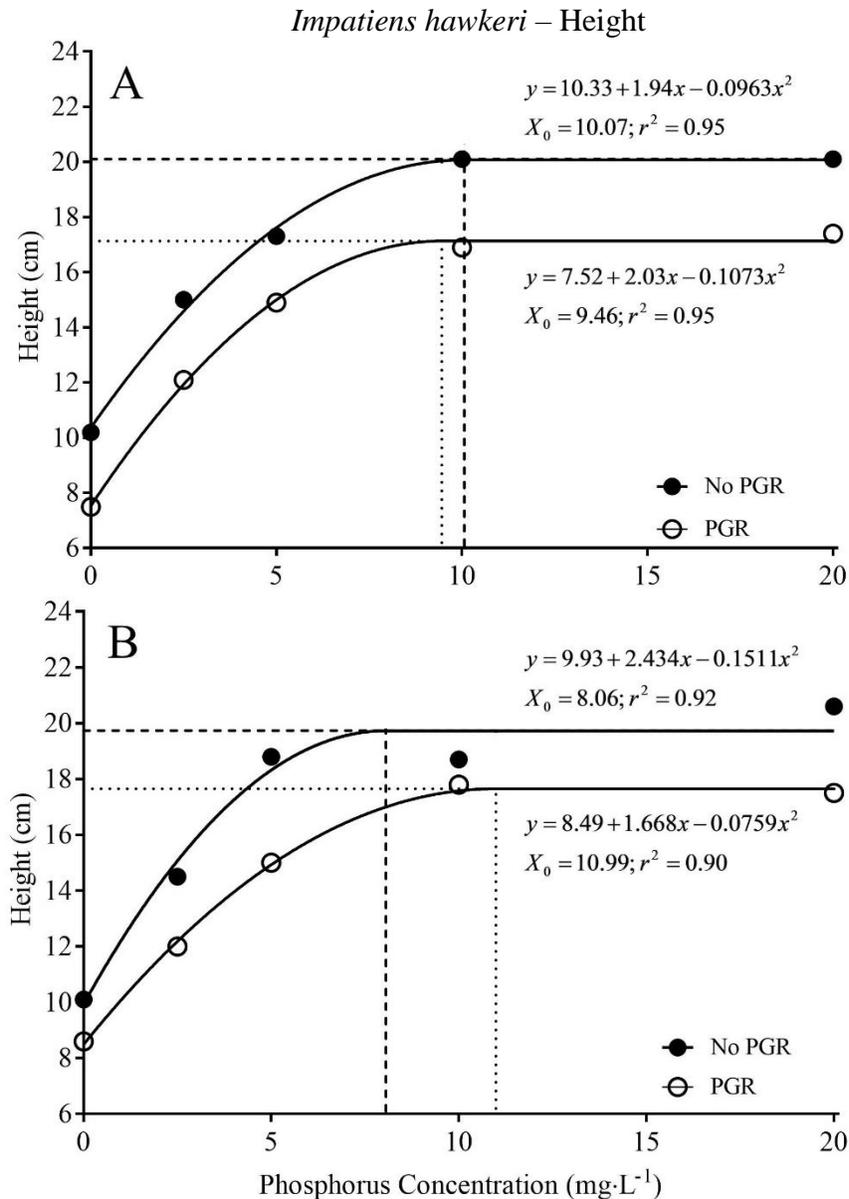


Figure 3.5. Nonlinear regression plateaus for height of *Impatiens hawkeri* ‘Pure Beauty Red on Pink’ (A) and ‘Tamarinda Dark Red’ (B). Regression lines were generated from means of each treatment ($n = 8$). PGR application was a foliar spray of $7.5 \text{ mg}\cdot\text{L}^{-1}$ paclobutrazol. Equations, r^2 , and X_0 values for plants not treated with a PGR are located above the corresponding line. Equations, r^2 , and X_0 values for plants treated with a PGR are located below the corresponding line. Dotted lines represent X_0 values, where the model plateaus, along with corresponding height. Models significant at $P \leq 0.0001$.

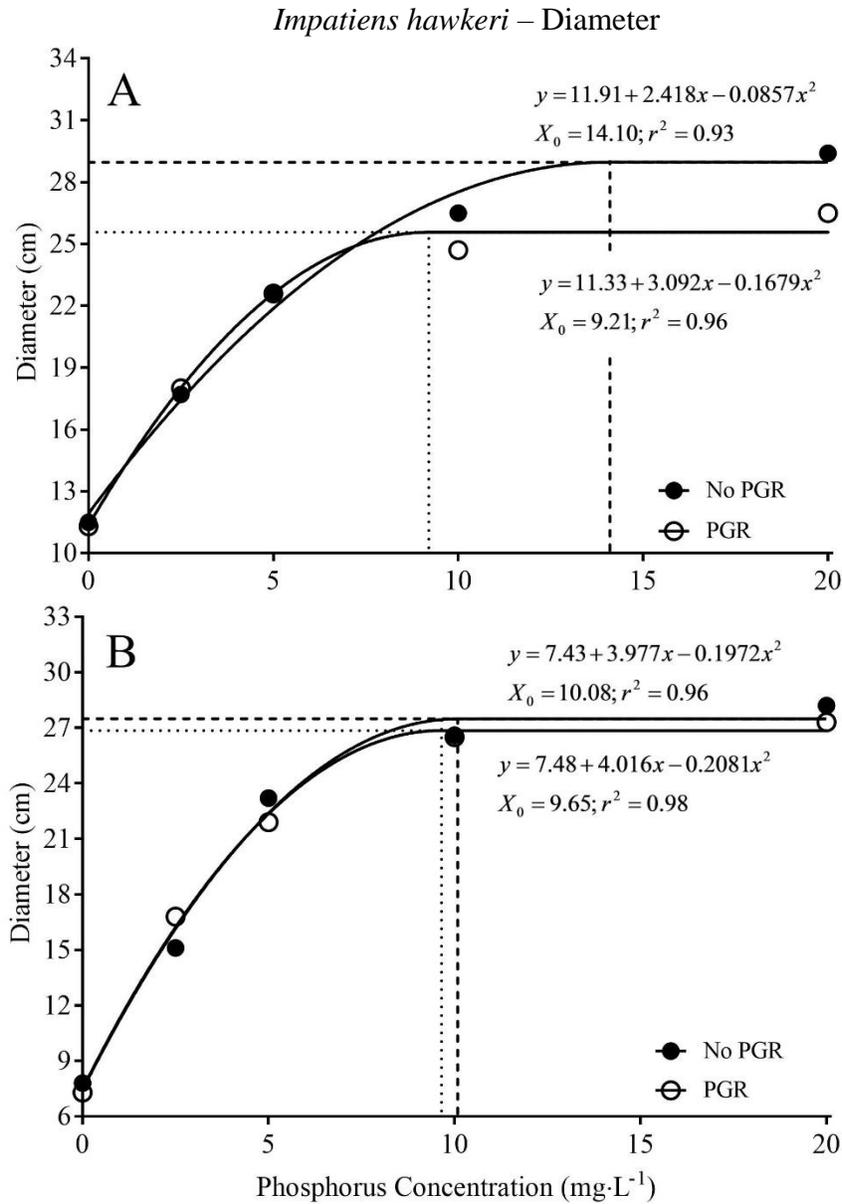


Figure 3.6. Nonlinear regression plateaus for diameter of *Impatiens hawkeri* ‘Pure Beauty Red on Pink’ (A) and ‘Tamarinda Dark Red’ (B). Regression lines were generated from means of each treatment (n = 8). PGR application was a foliar spray of 7.5 mg·L⁻¹ paclobutrazol. Equations, r^2 , and X_0 values for plants not treated with a PGR are located above the corresponding line. Equations, r^2 , and X_0 values for plants treated with a PGR are located below the corresponding line. Dotted lines represent X_0 values, where the model plateaus, along with corresponding diameter. Models significant at $P \leq 0.0001$.

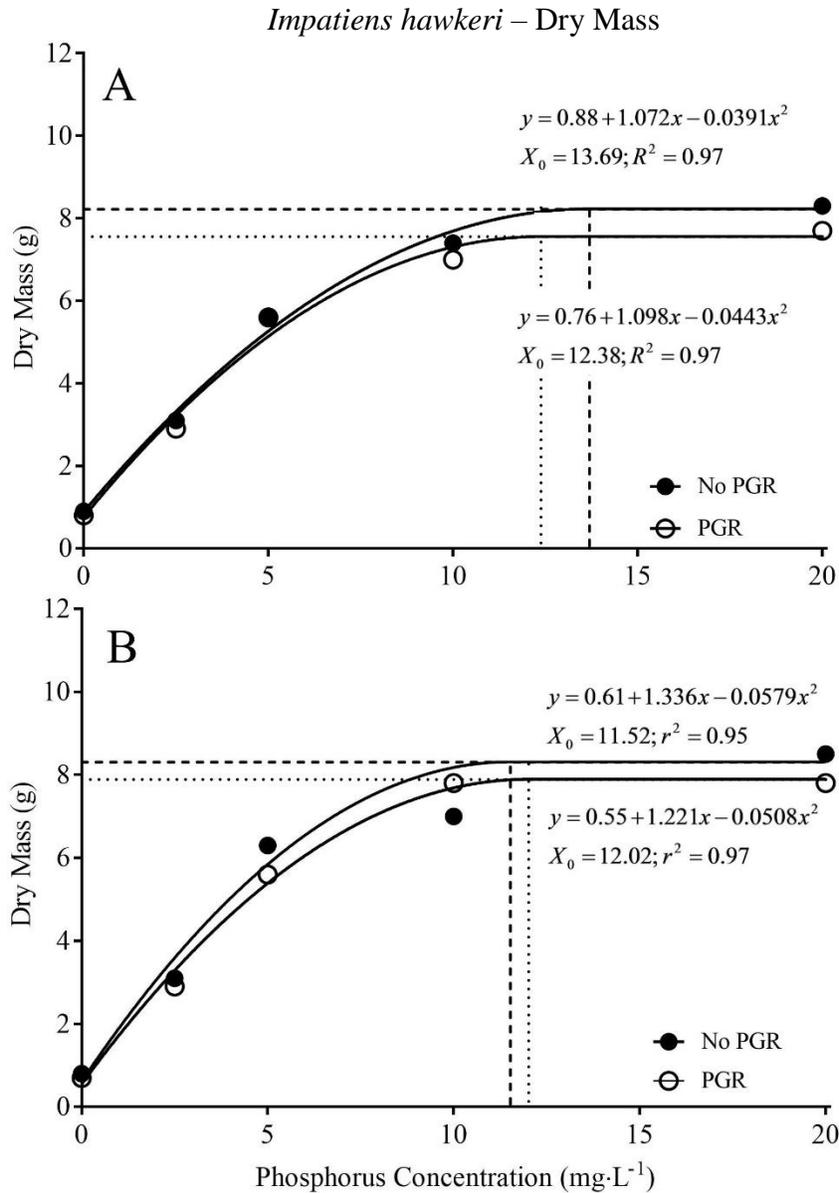


Figure 3.7. Nonlinear regression plateaus for dry mass of *Impatiens hawkeri* ‘Pure Beauty Red on Pink’ (A) and ‘Tamarinda Dark Red’ (B). Regression lines were generated from means of each treatment ($n = 8$). PGR application was a foliar spray of $7.5 \text{ mg}\cdot\text{L}^{-1}$ paclobutrazol. Equations, r^2 , and X_0 values for plants not treated with a PGR are located above the corresponding line. Equations, r^2 , and X_0 values for plants treated with a PGR are located below the corresponding line. Dotted lines represent X_0 values, where the model plateaus, along with corresponding dry mass. Models significant at $P \leq 0.0001$.

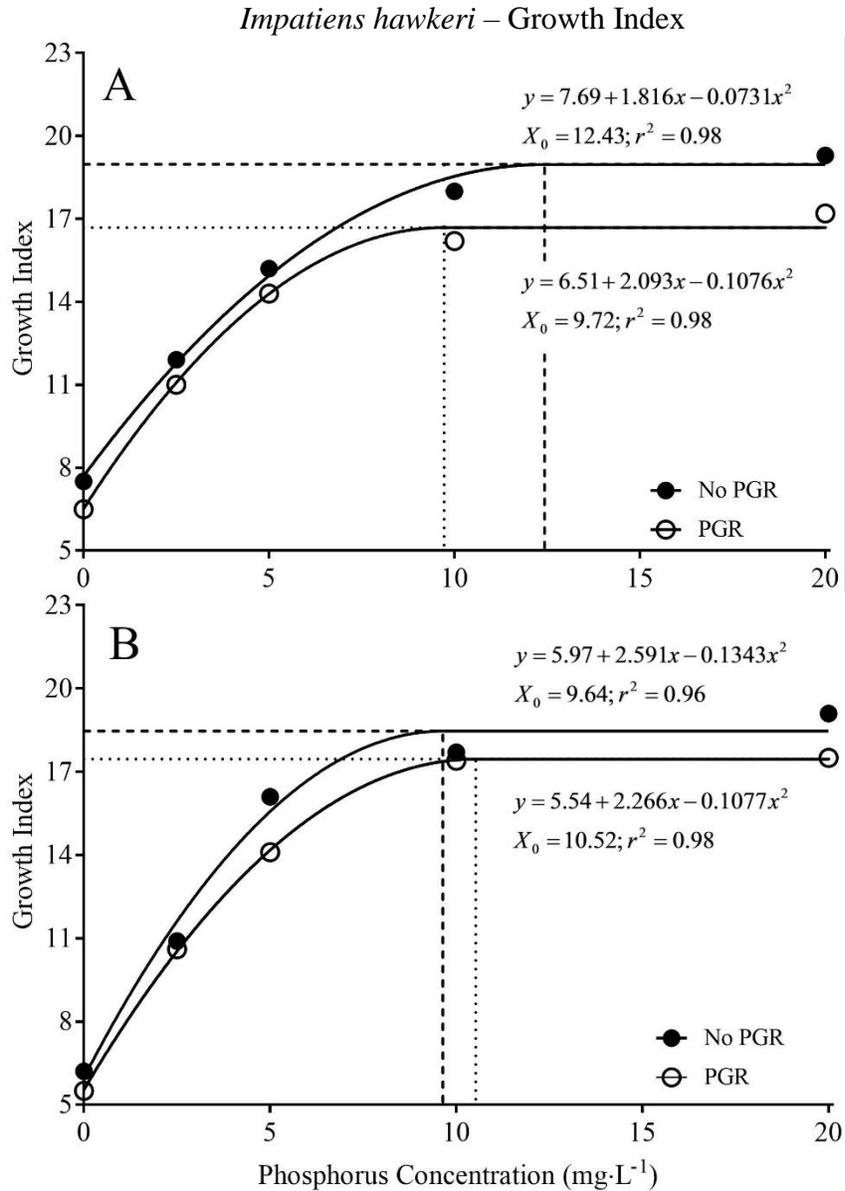


Figure 3.8. Nonlinear regression plateaus for growth index of *Impatiens hawkeri* ‘Pure Beauty Red on Pink’ (A) and ‘Tamarinda Dark Red’ (B). Regression lines were generated from means of each treatment ($n = 8$). PGR application was a foliar spray of $7.5 \text{ mg}\cdot\text{L}^{-1}$ paclobutrazol. Equations, r^2 , and X_0 values for plants not treated with a PGR are located above the corresponding line. Equations, r^2 , and X_0 values for plants treated with a PGR are located below the corresponding line. Dotted lines represent X_0 values, where the model plateaus, along with corresponding growth index. Models significant at $P \leq 0.0001$.

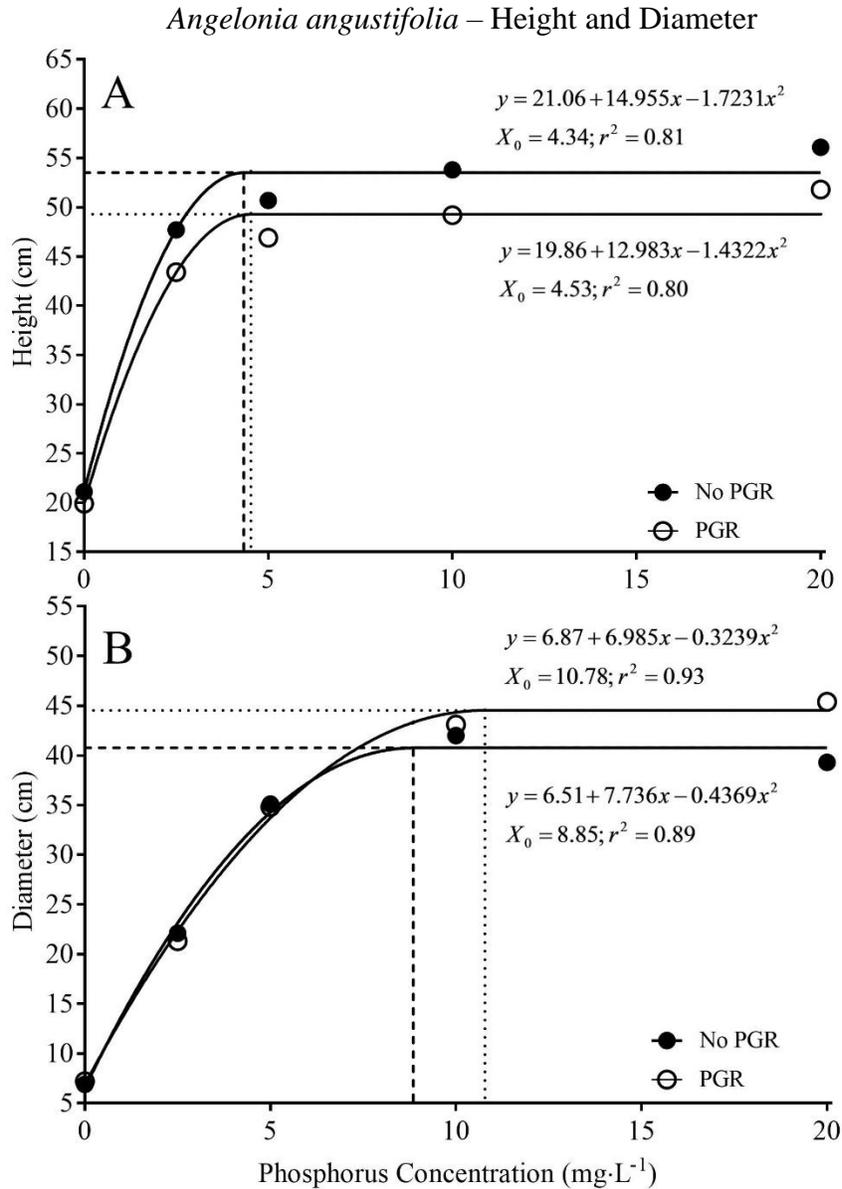


Figure 3.9. Nonlinear regression plateaus for height (A) and diameter (B) of *Angelonia angustifolia* ‘Sungelonia Blue’ and ‘Sungelonia White’ with cultivar data combined. Regression lines were generated from means of each treatment ($n = 16$). PGR application was a substrate drench at a concentration of 4 mg a.i. per pot, within a total volume of 118.3 mL per pot. Equations, r^2 , and X_0 values for plants not treated with a PGR are located above (A) or below (B) the corresponding line. Equations, r^2 , and X_0 values for plants treated with a PGR are located above (B) or below (A) the corresponding line. Dotted lines represent X_0 values, where the model plateaus, along with corresponding height or diameter. Models significant at $P \leq 0.0001$.

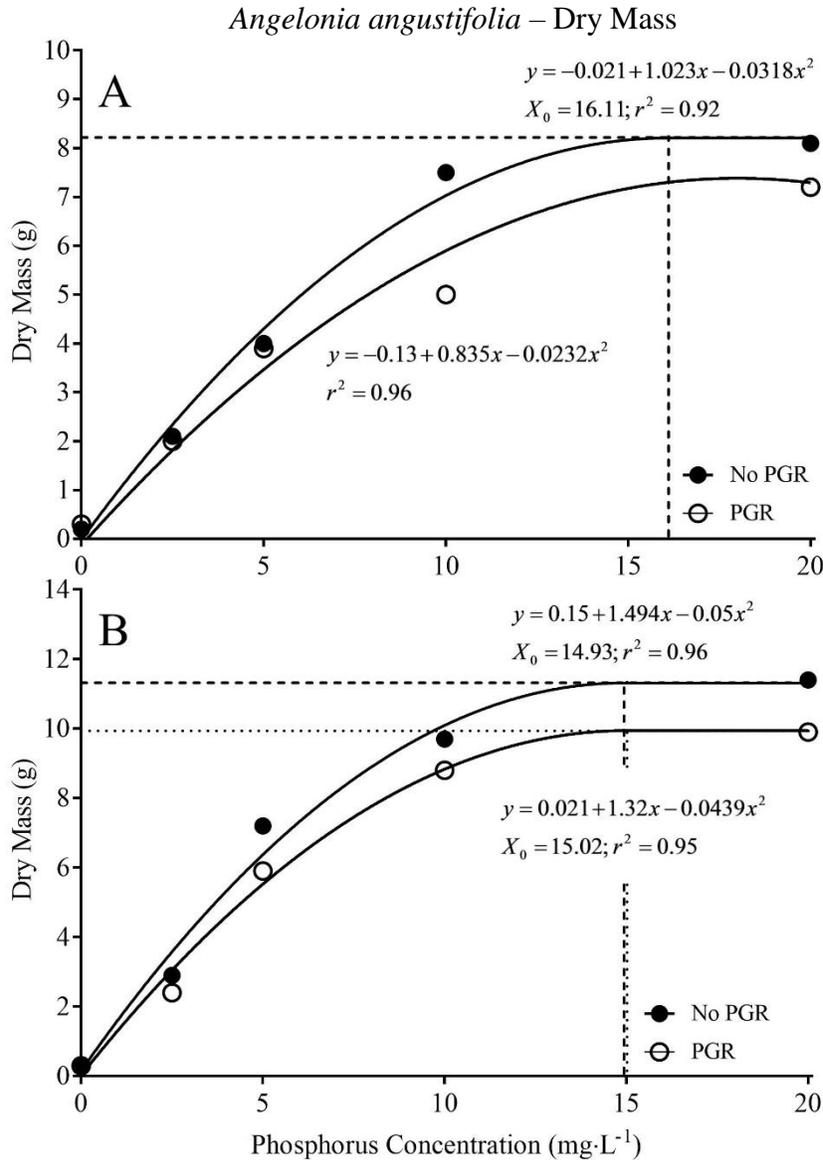


Figure 3.10. Nonlinear regression plateaus or quadratic regression model for dry mass of *Angelonia angustifolia* ‘Sungelonia Blue’ (A) and ‘Sungelonia White’ (B). Regression lines were generated from means of each treatment ($n = 8$). PGR application was a substrate drench at a concentration of 4 mg a.i. per pot, within a total volume of 118.3 mL per pot. Equations, r^2 , and X_0 values for plants not treated with a PGR are located above the corresponding line. Equation, r^2 , and X_0 values for plants treated with a PGR are located below the corresponding line for ‘Sungelonia White’. Equation and r^2 value are located below the quadratic regression line for ‘Sungelonia Blue’. Dotted lines represent X_0 values, where the model plateaus, with corresponding dry mass. Models significant at $P \leq 0.0001$.

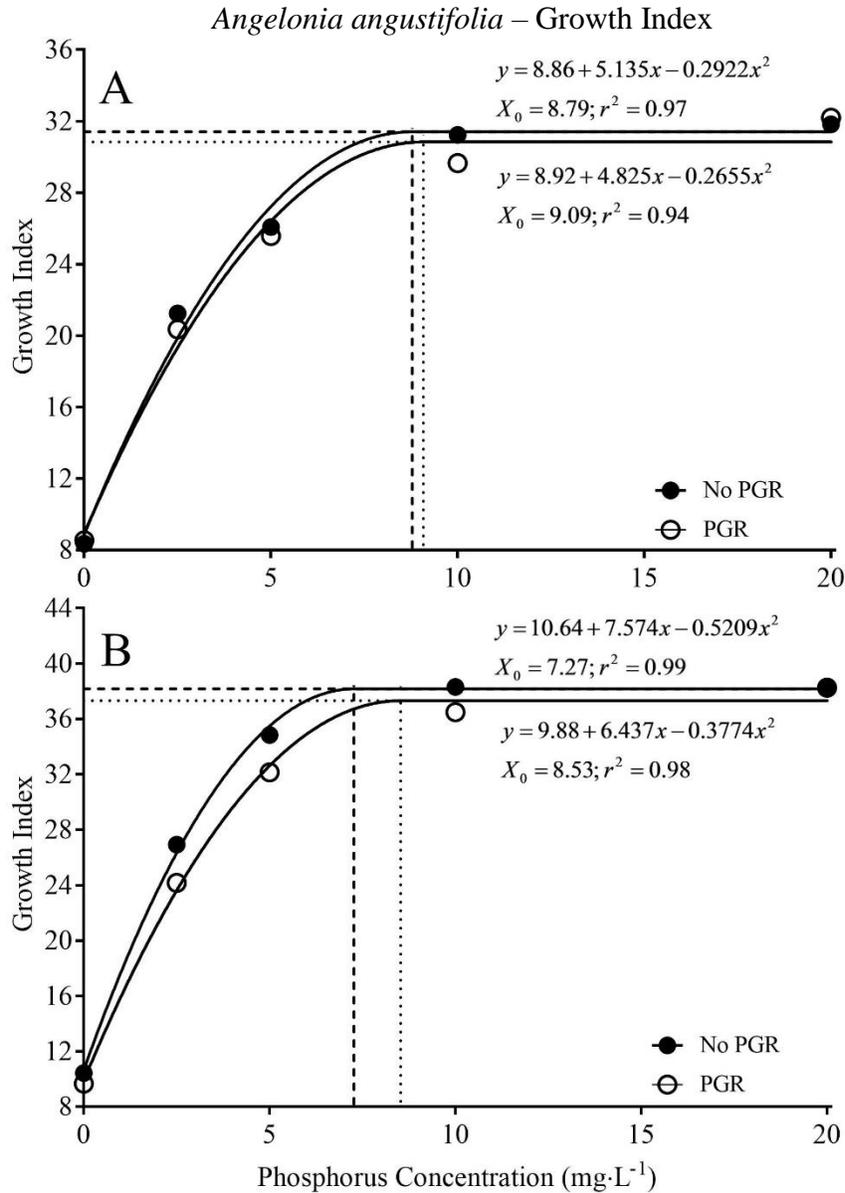


Figure 3.11. Nonlinear regression plateaus or quadratic regression model for growth index of *Angelonia angustifolia* ‘Sungelonia Blue’ (A) and ‘Sungelonia White’ (B). Regression lines were generated from means of each treatment ($n = 8$). PGR application was a substrate drench at a concentration of 4 mg a.i. per pot, within a total volume of 118.3 mL per pot. Equations, r^2 , and X_0 values are located above or below the corresponding line. Dotted lines represent X_0 values, where the model plateaus, with corresponding growth index. Models significant at $P \leq 0.0001$.

Chapter 4

Source-Sink Interactions Lead to Reproductive Stage Phosphorus Deficiency Symptoms on the Upper Foliage of Ornamental Plants

Abstract

Phosphorus (P, referring to phosphorus supplied by phosphate) restriction is becoming more common in ornamental plant production, as a means to control growth, and to reduce environmental issues associated with nutrient runoff. As low P fertilization strategies become more prevalent, growers are likely to encounter issues of P deficiency in their crops. New symptoms of a reproductive stage P deficiency have been recently reported in which symptomology occurs on the foliage directly below the flowers or fruit, as opposed to the traditional location on the older leaves. The goal of this study was to replicate these symptoms in order to describe them, and the conditions under which they occur. To induce these symptoms, ornamental peppers (*Capsicum annuum* L.) and chrysanthemums (*Chrysanthemum morifolium* Ramat.) were grown using P concentrations of 0 – 20 mg·L⁻¹. Half of the plants from each P treatment were switched to 0 mg·L⁻¹ P upon early signs of floral initiation. Plants that had P restricted developed symptoms of chlorosis, olive green spotting, and necrosis on the upper foliage below the floral structures, while central foliage remained green and non-symptomatic. The chrysanthemum cultivar ‘Crystal Misty Purple’, developed symptoms of upper leaf purpling. All plants were divided into subsections, dried, and analyzed for tissue nutrient concentrations to determine how P levels differed among plants grown with continuous or restricted P fertilization. The highest P concentrations occurred in the flowers or fruit for both species. Reproductive tissues of restricted P plants developed in the absence of an external P supply, yet it was found that up to ~80% of the total P in aboveground tissues was in the flowers and fruit. Vegetative tissue P concentrations and P content were significantly lower in symptomatic plants, indicating that large quantities of P were translocated from vegetative tissues to the developing reproductive tissues. Remobilized P from the lower tissues appeared to bypass the upper foliage

in favor of the flowers and fruit. As a result, the developing upper foliage was deprived of P and developed symptoms due to competition with the maturing flowers and fruit.

Introduction

Restriction of phosphorus (P) can be an effective method for growth control of floriculture species (Hansen and Nielsen, 2000, 2001; Nelson et al., 2012). Production of young plants (plug production) can benefit from low P fertilization (Nelson et al., 2012), but P restriction in long-term crops may be more difficult to successfully manipulate. This is especially true in greenhouse bedding crops that are typically grown in soilless substrate, which tends to have a very limited P holding capacity (Marconi and Nelson, 1984; Whipker, 2014). Utilizing low P fertilizer concentrations has become more common in the greenhouse production industry, but has caused issues for some growers (Whipker, 2014). Without adequate P application via fertilization, long-term crops have the potential to deplete the substrate of P. When a plant no longer has an external P supply, it reallocates P from older plant tissues, commonly leading to the appearance of deficiency symptoms on the lower foliage (Epstein and Bloom, 2005; Marschner, 1995; Mengel et al., 2001).

Deficiency symptoms associated with P are commonly observed when plant tissue comprises less than 0.2% or $2,000 \text{ kg} \cdot \text{L}^{-1}$ P (Mills and Jones, 1996). Typical symptoms are often described as a reddening or purpling of the lower foliage, an overall darker green coloration, stunted growth, delayed flowering, and greater root lengths (Epstein and Bloom, 2005; Marschner, 1995; Mengel et al., 2001). An additional symptom may sometimes manifest as olive green spots of the lower leaves during warmer conditions (Whipker, 2014). Foliage may also develop a dull green coloration which can later develop into chlorosis and eventual necrosis. The

occurrence of red coloration is most commonly reported and tends to occur in cool, wet conditions that are conducive to P deficiency (Whipker, 2014). In either case, deficiency symptoms initially develop on the lower leaves due to P mobility within the plant. As P becomes deficient, plants translocate P from lower plant tissue to the actively growing areas (Marschner, 1995). This illustrates why symptoms develop on the lower foliage as P concentrations decrease in those tissues.

Many factors contribute to P deficiency including available P in the substrate, pH, moisture level, substrate temperature, and root health (Mills and Jones, 1996). When there is insufficient P available in the substrate or nutrient solution, plants are unable to accumulate sufficient tissue P concentrations. Other potential factors are attributed to the roots inability to function properly regardless of the P concentration available in the substrate. In the case of excessively high or low soil moisture, the roots ability to take up P is limited, as is the case with soil temperature. Plants can experience P deficiency when roots are unhealthy, regardless of whether environmental conditions are conducive for healthy growth or P levels are sufficient in the substrate (Marschner, 1995; Whipker, 2014; Yan et al., 2012).

Common symptoms of P deficiency are well documented; however, Whipker (2014) reported symptoms occurring on chrysanthemums (*Chrysanthemum morifolium*) that have not been previously described. An upper leaf necrosis was reported to develop under P deficient conditions, when chrysanthemums were entering the reproductive growth stage. As the severity of these symptoms progressed, symptoms developed in the flower itself, as P was depleted in all plant tissues (Whipker, 2014). Conditions of sufficient P during vegetative maturation, followed by P deficient conditions upon floral development led to these symptoms. These atypical reproductive stage P deficiency symptoms on the upper foliage occur when there are two sinks

competing for limited P supplies: the flowers and developing fruit, and the actively growing upper foliage. When these sinks compete for P, the reproductive tissues take precedence, and translocated P supplies bypass the upper foliage in favor of the reproductive tissues. It has been observed that reproductive chrysanthemums and other plant species reallocate P from the foliage to the flowers, regardless of the availability of external P supplies (Epstein and Bloom, 2001; Hansen and Lynch, 1998); however, P deficiency symptoms have not been reported due to competition between the upper foliage and flowers. It has been found that remobilized N and P can account for 90% of these elements in developing flowers and fruit, indicating high P requirements to support these tissues, and high sink activity of reproductive structures (Epstein and Bloom, 2005; Marschner, 1995).

The initial hypothesis suggested by Whipker (2014) was that plants experiencing reproductive stage P deficiency reallocated P from the nearest source to the developing flowers: the upper leaves. Although this hypothesis is in agreement with the symptoms observed on chrysanthemums, it is unlikely that the upper foliage, which typically acts as a sink, would act as a source for the reproductive tissues. We hypothesize that under reproductive stage P deficiency, when plants cannot acquire P from external sources such as the substrate, stores in the lower foliage will translocate to the young developing tissues, but will preferentially accumulate in the reproductive tissues. In addition, this hypothesis requires that symptom development is dependent on the growth stage of the plant at the time P deficiency is induced. Research investigating the development of upper leaf necrosis on star gazer lilies (*Lilium* ‘Star Gazer’) found that symptom location was highly dependent on the plant growth stage (Chang and Miller, 2003). ‘Star Gazer’ lilies develop upper leaf necrosis in response to calcium (Ca) deficiency, while the lower and middle foliage remained healthy. Chang and Miller (2003) grew star gazer

lilies with normal or low Ca for one growing season, vernalized the bulbs, and then replanted them. During the second growth cycle all plants were grown with normal Ca fertilization. Plants grown with normal Ca the previous year developed upper leaf necrosis, while plants grown with low Ca the previous year developed necrosis on the lower to middle leaves. This study indicated that Ca deficiency symptoms were dependent on the external supply of Ca as well as internal stores of Ca (Chang and Miller, 2003). It is likely that a similar concept may be applied to investigate the development of reproductive stage P deficiency on the upper foliage.

Although reproductive stage symptomology appears to be less common than typical P deficiency symptoms, it may become more of an issue as growers utilize low P fertilization growing practices. Growers can obtain numerous benefits from P restriction including growth control of their crops (Hansen and Nielsen 2000, 2001) and environmental benefits from reduced nutrient leaching. Excessive applications of agricultural nutrients including P have resulted in water quality issues (Boesch et al., 2001). Algae blooms in Lake Erie due to excessive P are one example of these problems (Wines, 2014). Many municipalities and agencies are also becoming aware of the issues associated with eutrophication. Greenhouse and nursery growers in the Chesapeake Bay watershed have been encouraged to use improved management practices to reduce unnecessary runoff and improve the bay water quality (Boesch et al., 2001; Majsztzik and Lea-Cox, 2013). This trend will likely continue into the future, making knowledge and awareness of these unusual symptoms crucial for ornamental growers.

The objective of this study was to replicate the symptoms reported to be P deficiency on the upper foliage, and to determine the specific conditions under which they occur. In addition, the results of this study can be used to provide descriptions and recommendations to benefit growers who utilize low P fertilization strategies.

Materials and Methods

A custom substrate was used for all experiments, consisting of an 80:20 (v:v) mix of Canadian sphagnum peat moss (Conrad Fafard, Agawam, MA) and horticultural coarse perlite (Perlite Vermiculite Packaging Industries, Inc., North Bloomfield, OH), with added dolomitic lime at 8.875 kg/m^3 (Rockydale Agricultural, Roanoke, VA) and AquaGro 2000-G Wetting Agent (Aquatrols, Cherry Hill, NJ) at 600.3 g/m^3 . This custom substrate was used to ensure that there was no initial charge of P.

Fertilization treatments began on the day of transplant, and were applied at each irrigation. Fertilizers were custom blends of the following individual technical grade salts: $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, KNO_3 , KH_2PO_4 , K_2SO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, $\text{Mg}(\text{NO}_3)_2$, FeDTPA, $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$, $\text{ZnCl}_2 \cdot 7\text{H}_2\text{O}$, $\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$, H_3BO_3 , and $\text{Na}_2\text{MoO}_4 \cdot 2\text{H}_2\text{O}$ (Appendix A). Phosphorus (referring to phosphate-phosphorus) concentrations of 0, 2.5, 5, 10, or $20 \text{ mg} \cdot \text{L}^{-1}$ were used in experiment 1. In experiments 2 and 3, initial concentrations of 0 and $2.5 \text{ mg} \cdot \text{L}^{-1}$ P were not included because they resulted in the development of typical lower leaf symptomology. In addition, 35% sulfuric acid was used in experiments 2 and 3 in order to control pH. Sulfuric acid at $5 \text{ mL}/100 \text{ L}$ was added to the fertilizer solution to obtain a solution pH of 5.8. Nitrogen (N) and potassium (K) remained constant at $150 \text{ mg} \cdot \text{L}^{-1}$ each. Fertilizer solution was mixed in 100 L barrels, and was applied via sump pumps (Model 1A, Little Giant Pump Co., Oklahoma City, OK) which were connected to 1.9 cm black irrigation tubing fitted with drip rings.

Destructive harvests were conducted upon termination of each experiment. At each harvest, measurements were collected for plant height by measuring the highest point of the foliage with a ruler from the rim of the pot, and plant diameter by averaging the widest point and the axis perpendicular to it. Substrate pH and electrical conductivity (EC) were obtained using

the Pour-Thru method (Cavins et al., 2005), and recorded using a HI 9813-6 portable meter (Hanna Instruments, Woonsocket, RI). Leachate was poured into 50 mL centrifuge vials and submitted for analysis at the North Carolina Department of Agriculture & Consumer Services (NCDA&CS, Raleigh, NC). Tissue samples were collected and methods varied for each experiment. These tissue samples were rinsed initially with deionized water, then washed in a solution of 0.5 M HCl, followed by a rinse of deionized water. Tissue samples were dried for at least 72 hours at 70°C, and total plant dry mass was recorded. Tissue samples were then ground using a Thomas Wiley® Mini-Mill (Thomas Scientific, Swedesboro, NJ), and analyzed for nutrient content by AgSource Laboratories (Lincoln, NE). Total N was processed by Kjeldahl digestion, and determined via flow injection analysis (FIA). Extractable K was processed by 2% acetic acid digestion, and determined via inductively coupled plasma mass spectrometry (ICP-MS). Total P and all other plant minerals were processed by nitric acid/hydrogen peroxide digestion, and determined via ICP-MS.

Experiment 1

Capsicum annuum ‘Tango Red’ seeds (Fred C. Gloeckner & Co., Inc., Harrison, NY), were sown on 6 Apr. 2016 into 1204 flat inserts with cell dimensions of 5.7 x 3.8 x 5.4 cm (length x width x depth) and propagated in a glass greenhouse at 35°N latitude in Raleigh, NC. Fertilization of seedlings began after cotyledons fully expanded, with a fertilizer consisting of 75N–0P–75K (mg·L⁻¹), with all other essential nutrients provided with a combination of the previously listed fertilizer salts. No P was supplied until the plants began their fertilizer treatments upon transplant on 13 May. All seedlings were transplanted into 5.5-inch diameter pots (Dillen, Middlefield, OH) with dimensions of 13.7 x 11.6 cm (diameter x depth) and a

volume of 1.28 L. After six weeks, half the plants grown with each non-zero P concentration were restricted to 0 mg·L⁻¹ P, while the other half remained on their initial P fertilization regiment. There were six single plant replicates for each of nine total P treatments.

There was one destructive harvest that occurred upon termination of the study. Plants were divided into three sections, consisting of the flowers and fruit, upper stems and leaves, and lower stems and leaves. The upper stems and leaves and the lower stems and leaves each constituted roughly half of the vegetative tissue. Dividing the plants was done to determine how tissue P content changed among plants grown with different P concentrations, within these different tissue types.

Experiment 2

Methods were refined based on observations from experiment 1. Due to the fact that plants grown with initial concentrations of P less than 10 mg·L⁻¹ P did not develop the desired symptoms, initial P concentrations were narrowed to include 10, 15, and 20 mg·L⁻¹ P.

Chrysanthemum morifolium ‘Little Rock’, ‘Swiftly Yellow’, and ‘Crystal Misty Purple’ cuttings (Dümmen Orange, Columbus, OH) were stuck on 9 Aug. 2016 into 72 round cell plug trays with cell dimensions of 3.8 x 6.4 cm (diameter x depth). Cuttings were rooted under mist, and were removed once root growth had reached the cell wall. At that time, plants were fertilized with a custom fertilizer solution consisting of 75N–5P–75K (mg·L⁻¹), with all other essential nutrients provided with a combination of the previously listed fertilizer salts. This fertilizer solution consisted of nutrient concentrations equal to half of the lowest initial P fertilizer treatment used in this experiment.

Chrysanthemums were transplanted on 31 Aug. into 5-inch diameter pots (Dillen, Middlefield, OH) with dimensions of 12.7 x 9.2 cm (diameter x depth) and a volume of 0.8 L. Fertilizer treatments began the same day the cuttings were transplanted. After four weeks, half of the plants grown with each P concentration were restricted to 0 mg·L⁻¹ P, while the other half remained on their initial P fertilization regiment. There were five two-plant replicates each of six total P treatments. Each cultivar was terminated based on when symptoms were observed. ‘Little Rock’ was terminated on 4 Nov., ‘Swiftly Yellow’ on 7 Nov., and ‘Crystal Misty Purple’ on 10 Nov.

Upon termination of each cultivar, tissue samples were harvested from five two plant replicates of each treatment. Harvested plants were divided into six sections. Moving sequentially down the plant, these sections consisted of the flowers, upper third stems and leaves, reproductive middle third stems and leaves, vegetative middle third stems and leaves, reproductive lower third stems and leaves, and vegetative lower third stems and leaves. “Vegetative stems and leaves” refer to the older tissues, and leaves that were mature and had heavily lobed margins. These leaves were distinctly different from the “reproductive” leaves located below the flowers which were young and often had fewer or no lobes whatsoever. Branches emerging from the lower third and middle third sections of the plant had portions with both vegetative leaves and reproductive leaves with flowers. The leaf tissues from the middle third and bottom third of the plant were divided this way so that reproductive and vegetative leaves were analyzed separately. This division was done to better distinguish tissues by age and location, rather than location alone.

Experiment 3

C. annuum 'Tango Red' seeds were sown on 25 Aug. 2016 using the same methods as experiment 1. Fertilization of seedlings began after cotyledons fully expanded, with a fertilizer consisting of 75N–5P–75K ($\text{mg}\cdot\text{L}^{-1}$), with all other essential nutrients provided with a combination of the previously listed fertilizer salts. Peppers were transplanted into 5.5-inch diameter pots on 19 Sept. and moved to a polyethylene greenhouse for the remainder of the study. Initial fertilizer concentrations of 10, 15, and 20 $\text{mg}\cdot\text{L}^{-1}$ P were used in this experiment. After six weeks, half the plants grown with each P concentration were restricted to 0 $\text{mg}\cdot\text{L}^{-1}$ P, while the other half remained on their initial P fertilization regiment. There were five single plant replicates each of six total P treatments.

There was one destructive harvest that occurred upon termination of the study, on 21 Dec 2016. Plants were divided into four sections, consisting of the flowers and fruit, upper third stems and leaves, middle third stems and leaves, and lower third stems and leaves. Vegetative tissues were divided into three sections as evenly as possible. This further division of the plants, compared to experiment 1, was done to better differentiate and determine how P movement occurred in plants grown with different P concentrations.

Data Analysis

Statistical analysis was conducted using SAS (version 9.4; SAS Institute, Cary, NC). Data for plant height, diameter, tissue and leachate nutrient concentrations, and percent of total plant P content within each harvested part were subjected to PROC GLM and the means were separated by Tukey's honestly significant differences (HSD) at $P \leq 0.05$. Minimum significant differences were determined among plants grown with different P concentrations and across

different plant tissues. This was done to better compare how growth differed among plant tissues using various fertilizer P concentrations. Percent of total plant P content within each harvested plant part was analyzed only among plants grown with the same initial concentration of P. This was done to better compare how P movement differentiated among plants that initially received the same P concentration.

Results and Discussion

Symptoms of P deficiency were successfully induced on the upper foliage of peppers and chrysanthemums in these experiments. Under specific fertilization regimens, both species developed initial deficiency symptoms on the upper foliage, just below the maturing reproductive tissues. These symptoms ranged from general chlorosis and necrosis, to a dark purpling of leaf and upper stems, to an olive green leaf spotting. Some plants only developed one or two of these symptoms, while other plants were observed with all symptoms. In fact, there were instances of all symptoms occurring on a single leaf (Figure 4.1). A thorough description of each symptom and the conditions under which they occurred is described here.

Plants grown with continuous P fertilization will be referred to as having been grown with continuous P. In contrast, plants grown with restricted P will be referred to as having been grown with restricted P, or an “initial concentration”, implying that the P concentration was switched to 0 mg·L⁻¹ P later in production.

Experiment 1

A variety of symptoms were observed on peppers grown in the first experiment. Pepper plants grown with initial concentrations of 0 and 2.5 mg·L⁻¹ P developed what was considered

typical P deficiency symptoms on the lower foliage. These symptoms began on the lowest foliage and spread up the plant until ~75% of the foliage abscised. Leaves became chlorotic with olive green leaf spots and necrotic spotting. Peppers grown with an initial concentration of 5 mg·L⁻¹ P developed symptoms primarily on the lower foliage, but also on the upper foliage. This led to a more sudden decline and collapse of the foliage than in plants grown with higher P concentrations. Plants grown with initial concentrations of 10 – 20 mg·L⁻¹ P developed symptoms first on the upper foliage, just below the flowers and fruit (Figure 4.2). Central and lower foliage initially remained green while upper foliage became chlorotic with necrotic spots. This symptomatic foliage quickly curled and abscised from the plant. Approximately half of the symptomatic leaves developed olive green spotting in addition to chlorosis and necrotic spotting, and olive green spots were less commonly observed than necrotic spots. Symptoms then began to develop on the lower foliage, which quickly spread over the plant and led to the majority of the foliage abscising in a matter of days.

A number of trends were observed across all plants in terms of tissue P concentration (Table 4.1). Within each fertilization treatment, pepper reproductive tissues had significantly higher P concentrations than the upper or lower foliage and stems. Although the tissue P concentration varied significantly by fertilizer P concentration, reproductive tissues always had greater P concentration than the corresponding vegetative tissues. Similarly, within treatments all plants grown with continuous P concentrations had significantly higher tissue P concentrations in the upper foliage and stems than in the lower foliage and stems. This was not the case for plants grown with restricted P. Plants grown with initial fertilizer P concentrations of 5 and 20 mg·L⁻¹ P had similar tissue P concentrations in the lower foliage and stems as in the upper foliage and stems. Trends may also be observed in Figure 4.3. Among plants grown with restricted P,

vegetative tissue P concentrations were similar for tissues from the same plant part (Figure 4.3 A & B). Reproductive tissues, however, had significantly higher P concentrations in plants grown with an initial fertilizer P concentration of 20 mg·L⁻¹ than in plants grown with 5 or 10 mg·L⁻¹ P (Figure 4.3 C).

Phosphorus content of different tissues, expressed as a percentage of the total, was compared only between plants grown with the same initial P concentration that was later restricted or remained constant (Table 4.2). All plants had a significantly higher percentage of total plant P in the flowers and fruit than in the upper or lower foliage and stems. The percentage of total plant P content in the flowers and fruit was similar between plants grown with the same initial fertilizer P concentration for 5 and 10 mg·L⁻¹ P; however, plants grown with continuous 20 mg·L⁻¹ P had a lower percentage of the total P content in the reproductive tissues than plants grown with an initial concentration of 20 mg·L⁻¹ P. Plants grown with 5 mg·L⁻¹ P had a similar percentage of total plant P in the upper and lower vegetative tissues, regardless of whether P was restricted. Conversely, plants grown with continuous concentrations of 10 or 20 mg·L⁻¹ P had higher percentages of total plant P in the upper foliage and stems than in the lower foliage and stems, compared to restricted P plants grown with the same initial P concentration.

Leachate solution analysis demonstrated trends in substrate P concentrations over the course of the experiment (Table 4.3). Six weeks after transplant, P concentrations in the leachate solution were the highest for plants grown with 20 mg·L⁻¹ P. Plants grown with 0 or 2.5 mg·L⁻¹ P had significantly lower P concentrations in the solution. Nine weeks after transplants, P concentration in the solution of plants fertilized with 20 mg·L⁻¹ P remained significantly greater than any other fertilizer treatment. This indicates that plants grown with continuous concentrations of 10 mg·L⁻¹ P or less, or any restricted concentration were absorbing a majority

of the P available in the substrate. Eleven weeks after transplant, P concentrations in the leachate followed a similar trend to what was observed at week nine. The substrate of plants grown with 20 mg·L⁻¹ P had significantly higher P concentrations in the leachate than all other treatments. These trends illustrate how plants limited by P have low levels of P in the substrate, as they utilize a majority of the available P supply.

Experiment 2

Chrysanthemums developed similar P deficiency symptoms on the upper foliage as peppers. Symptoms first developed on plants grown with initial concentrations of 10 mg·L⁻¹ P, with symptoms developing later on plants grown with initial concentrations of 15 and 20 mg·L⁻¹ P. Symptoms also developed on the lower leaves simultaneously, however they were primarily covered by the healthy, central foliage. Symptoms on ‘Swiftly Yellow’ developed on the upper foliage first, after 28 days without P. Marginal chlorosis with necrotic margins were the primary initial symptoms (Figure 4.4 A). These symptoms quickly progressed to necrotic curling of the margins (Figure 4.4 B). ‘Little Rock’ developed symptoms soon after ‘Swiftly Yellow’, by day 30 without P. Chlorosis and necrosis were more pronounced on the larger leaves of this cultivar, and symptoms developed over the majority of the plant, including the lower foliage.

Symptomology on the lower foliage supports our hypothesis that P was translocated out of the lower foliage and into the flowers. As the upper foliage developed symptoms, the flowers initially remained healthy and continued to mature. This evidence supports our hypothesis that P bypassed the upper foliage in favor of the flowers.

‘Crystal Misty Purple’ developed symptoms after 32 days without P. Initially, a purple coloration developed on the leaf petioles of the upper foliage on all plants that were restricted to

0 mg·L⁻¹ P. This coloration then developed on the stems, but was completely absent on plants grown with a constant supply of P. A deep purple coloration of the stem was also described by Whipker and Cloyd (1998) on P deficient chrysanthemums. A dark purple coloration then developed on the upper foliage of symptomatic plants (Figure 4.5). This purpling was likely due to the accumulation of anthocyanins in the foliage and stems. Anthocyanins are water-soluble pigments found in vacuoles of all plant parts, both above and below ground (Gould et al., 2009). The link between P deficiency and anthocyanin production has long been established (Mengel et al., 2001; Ulrychová and Sosnová, 1970), and increased foliar anthocyanins are one of the primary symptoms associated with P deficiency in plants (Sarker and Karmoker, 2011). This leads to a reddening or purpling that often accompanies P deficiency. Plants grown with an initial concentration of 10 mg·L⁻¹ P also began to develop symptoms of purpling on the lower foliage, after initial symptoms developed on the upper foliage. Lower leaf symptoms were not as severe on plants grown with initial concentrations of 15 or 20 mg·L⁻¹ P (Figure 4.6). Although this cultivar was the only one in this study to develop red or purple coloration, it demonstrated that this symptom can develop on the upper or lower foliage in response to P deficiency.

Prior to termination of the experiment, the purple coloration became highly pronounced, especially on leaves that were fully exposed to the sun. Symptomatic leaves that were shaded by higher leaves were green or chlorotic in the shaded region. In fact, the shaded area had non-purple coloration in the precisely defined shape of the shading leaf (Figure 4.7). This indicates that the development of purple symptoms relies on exposure to light. This evidence is supported by the fact that anthocyanin production is upregulated by light, and many species exhibit an absolute light requirement in order to synthesize anthocyanins (Gould et al., 2009). A range of symptoms was observed on the leaves just below the flowers, which develop the typical

reproductive shape, being narrower with few or no lobes compared to the multi-lobed leaves that develop during vegetative maturation. These symptoms included chlorosis, necrotic margins, necrotic spotting, olive green leaf spotting, and purpling, and were found individually or in combination with several symptoms (Figure 4.8). Upper leaf symptoms were different than what was observed in previously reported studies of chrysanthemums. In fact, Hansen and Lynch (1998) described P deficiency on chrysanthemums as the typical symptoms of stunted, darker green leaves with a red coloration on the lower stem. Whipker and Cloyd (1998) also describe typical P deficiency symptoms on chrysanthemums as a reddening or yellowing of the lower foliage with necrosis spreading from the leaf tip.

The number of days that it took to flower was similar across treatments within each of the three chrysanthemum cultivars. On average, it took 47 days for 'Swifty Yellow' flowers to open, 58 days for 'Little Rock', and 64 days for 'Crystal Misty Purple' (data not shown). Additionally, heights were similar within each cultivar, averaging 27.8 cm for 'Swifty Yellow', 32.5 cm for 'Little Rock', and 29.6 cm for 'Crystal Misty Purple' (data not shown). This indicates that plants were able to attain similar heights and flowering date was not affected by restricted P fertilization. In addition, because no differences in height were observed among plants of the same cultivar, it was likely that the P concentration required to attain maximum height was $\leq 10 \text{ mg}\cdot\text{L}^{-1}$ P. This is in agreement with other studies which found that herbaceous ornamentals attain maximum height with P concentrations of 5.5 to 13.1 $\text{mg}\cdot\text{L}^{-1}$ (J. Henry, unpublished data).

Phosphorus tissue concentrations varied significantly among 'Little Rock' plants grown with the same continuous or initial fertilizer P concentrations (Table 4.4). All tissue types had higher tissue P concentrations for plants grown with continuous P fertilization than plants grown with the same initial P concentration. Within every treatment except continual 20 $\text{mg}\cdot\text{L}^{-1}$ P,

flower tissue had higher P concentrations than any individual vegetative tissue. For all continuous P fertilization regiments, the upper foliage and stems had higher tissue P concentrations than the lower reproductive or vegetative foliage and stems. In contrast, tissue P concentrations were similar among all foliage and stems within each restricted P regiment. Among all foliage and stem types from plants grown with continuous fertilizer P concentrations, tissue P concentrations ranged from 0.23 – 0.60%. These values fall within the P sufficiency range for reproductive chrysanthemums of 0.23 – 0.7% (Mills and Jones, 1996). In contrast, tissue P concentrations ranged from 0.03 – 0.09% in ‘Little Rock’ chrysanthemums grown with restricted P. These tissue values were similar to what was observed during the first report of reproductive stage P deficiency, with tissue P concentrations of 0.07% and 0.11% (Whipker, 2014). Tissue values of 0.20 – 0.22% are considered low for chrysanthemums, indicating that restricting P half way through the production cycle resulted in severe P deficiency in all vegetative tissues (Mills and Jones, 1996).

The tissue P concentrations of restricted P ‘Little Rock’ plants demonstrate that P in the vegetative tissues was uniformly low, regardless of location (Table 4.4). When comparing continuous and restricted P plants of the same fertilizer P concentration, differences between P concentrations in the upper foliage and stems were greater than the differences in lower vegetative foliage and stems. The fact that tissue concentrations were less in the lower vegetative foliage and stems of continuous P plants indicated that even under sufficient P conditions, the lower leaves had a lower P concentration than the middle or upper foliage and stems. It is known that P in the younger leaves is partially supplied from mature foliage (Mengel and Kirkby, 2001). This illustrates how P requirements were greater in the young maturing foliage, and how P is preferentially remobilized to this younger growth, regardless of whether the plant is experiencing

P deficiency. This finding was in agreement with research on castor bean (*Ricinus communis* L.), which found that P concentrations were higher in tissues found closer to the terminal growing point (Jeschke et al., 1997). Additionally, P is known to translocate in high quantities from vegetative to reproductive tissues during reproductive maturation (Mengel and Kirkby, 2001). Within the tissues of restricted P plants, P concentrations were highest in the flowers, and the upper foliage and stems had similar P concentrations to the lower foliage and stems. Although the flowers and upper foliage both act as P sinks from internal sources, this study demonstrated that P translocates preferentially to reproductive tissues under conditions of reproductive stage P deficiency.

Preferential movement of P to reproductive tissues was previously observed in chrysanthemums grown with deficient ($0.03 \text{ mg}\cdot\text{L}^{-1}$), adequate ($3.1 \text{ mg}\cdot\text{L}^{-1}$), and high ($155 \text{ mg}\cdot\text{L}^{-1}$) P concentrations (Hansen and Lynch, 1998). In fact, Hansen and Lynch stated that, “phosphorus allocated to developing flowers was predominantly lost from the leaves”. Although Hansen and Lynch looked at the movement of P within chrysanthemums grown with varying P concentrations, they only observed P deficiency symptoms on the lower foliage and stems of plants grown with a constant concentration of $0.03 \text{ mg}\cdot\text{L}^{-1}$ P. This provides additional evidence that reproductive stage P deficiency symptoms on the upper foliage require that plants were initially grown with sufficient P that is later restricted at the onset of floral initiation.

Development of P deficiency symptoms are highly dependent on the growth stage of the plant. The fact that P movement from foliage to flowers has been observed in P sufficient and deficient plants further illustrates the importance and strength of floral sink activity in chrysanthemums, and supports the findings observed in our study.

Floral tissues of restricted P ‘Little Rock’ plants had significantly lower tissue P concentrations than plants grown with continuous P fertilization (Table 4.4); however, the percentage of total plant P in the flowers of restricted P plants was higher than in continuous P plants (Table 4.5). In fact, plants grown with an initial concentration of $10 \text{ mg}\cdot\text{L}^{-1}$ P had an average of 81.3% of total plant P content in the flowers, while continuous P fertilization plants contained 59%. For plants grown with continuous or initial concentrations of 10 and $15 \text{ mg}\cdot\text{L}^{-1}$ P, the majority of P accumulated in the flowers, but a greater percentage of P content was observed in the flowers of restricted P plants. Additionally, restricted P plants had a significantly lower percent of total P content in the upper foliage and stems when compared to plants grown with the same continuous P concentration. In fact, the percent of total P content in the upper foliage and stems of restricted P plants was less than half that of continuous P plants. These trends illustrate P movement through the plant after the external supply of P was restricted to $0 \text{ mg}\cdot\text{L}^{-1}$ P. Additionally, these trends further support the hypothesis that P is translocated preferentially to the flowers under conditions of reproductive stage P deficiency, depriving the newly developing upper foliage and leading to deficiency symptoms manifesting on the upper foliage prior to the mature foliage.

‘Swiftly Yellow’ tissue P concentrations followed similar overall trends as ‘Little Rock’ (Table 4.6). All tissue types from continuous P plants had significantly higher P concentrations than any tissue type of the restricted P plants. The lower reproductive foliage and stems had similar tissue P values as the flowers of all restricted fertilization regiments. Additionally, the middle and lower vegetative foliage and stems had similar P concentrations to the flowers of plants grown with an initial concentration of $20 \text{ mg}\cdot\text{L}^{-1}$ P. Plants that were grown with continuous P fertilization all had similar upper foliage and stem tissue P concentrations as the

flowers. In contrast, upper foliage and stem tissue P concentrations were significantly lower than flower tissue P concentrations in plants grown with restricted P fertilization, indicating that the upper foliage was P deficient in restricted P plants. Among all foliage and stem types from plants grown with continuous fertilizer P concentrations, tissue P concentrations ranged from 0.32 – 0.51%. These values were within the P sufficiency range for reproductive chrysanthemums of 0.23 – 0.7% (Mills and Jones, 1996). In contrast, foliage and stem tissue P concentrations ranged from 0.05 – 0.14% in ‘Swiftly Yellow’ chrysanthemums grown with restricted P. Tissue P concentrations of 0.20 – 0.22% are considered low for chrysanthemums, indicating that restricting P resulted in severe P deficiency in all vegetative tissues (Mills and Jones, 1996). ‘Swiftly Yellow’ tissue P concentrations further support the evidence that P was reallocated to the flowers, rather than the upper foliage.

Total P content, as expressed as a percentage of the total, for ‘Swiftly Yellow’ plants was highest for the flowers of plants grown with restricted P (Table 4.7). This is in agreement with what was observed with ‘Little Rock’. Additionally, the flowers of all fertilizer treatments had greater than half of all aboveground P content. This again illustrates the significance of the reproductive tissues as a sink in chrysanthemums, regardless of whether the plant is experiencing P deficiency conditions. The percentages of total P content in the upper foliage and stems of restricted P plants were less than in the upper foliage and stems of plants grown with the same continuous P concentration. The tissue P concentration and percent of total P content in the upper foliage and stems both demonstrate that these tissues were P deficient when compared to healthy plants grown with a continuous fertilizer P concentration.

Phosphorus concentrations remaining in the substrate were determined from the leachate solution (Table 4.8). All three cultivars had the highest P concentration in the substrate of plants

grown with a continuous concentration of 20 mg·L⁻¹ P. Each cultivar had lower P concentrations in the substrate of plants grown with 15 mg·L⁻¹ P, but ‘Crystal Misty Purple’ had similar P concentrations in the substrate of plants grown with 10 mg·L⁻¹ P. ‘Little Rock’ and ‘Swiftly Yellow’ had significantly lower leachate P concentrations for plants grown with 10 mg·L⁻¹ P compared to those grown with 15 mg·L⁻¹ P. All plants grown with continuous P concentrations had significantly higher leachate P concentrations than plants that were grown with restricted P fertilization. Additionally, all plants grown with restricted P fertilization had similar leachate P concentrations. This indicates that plants that were restricted to 0 mg·L⁻¹ P absorbed the majority of what P was available in the substrate, and there were excess P concentrations remaining in the substrate of plants grown with continuous P fertilization.

Experiment 3

Symptoms on peppers during experiment 3 took longer to develop than in experiment 1. This was likely because these plants grew slower in the fall and winter with shorter days and cooler temperatures. Symptoms first developed four weeks after P was restricted. These symptoms occurred on the upper leaves of plants grown with an initial concentration of 10 mg·L⁻¹ P. Early symptoms were similar to those observed in experiment 1 (Figure 4.2), although they did not advance as quickly, and leaf abscission was minimal. After initial symptoms developed, it took an additional two weeks for symptoms to develop on plants grown with initial concentrations of 15 or 20 mg·L⁻¹ P. Symptoms were allowed to progress for one more week prior to termination of the study.

Plant tissue P concentrations were greater for almost all plants grown with continuous P compared to their restricted counterparts grown with the same initial fertilizer P concentration.

Central (Figure 4.9 C) and lower vegetative tissues (Figure 4.9 D) of plants grown with an initial concentration of $10 \text{ mg}\cdot\text{L}^{-1}$ P had similar tissue P concentrations between plants grown with continuous or restricted P fertilization. Plants grown with continuous concentrations of 10 and $15 \text{ mg}\cdot\text{L}^{-1}$ P had significantly higher reproductive tissue P concentrations (Figure 4.9 A) compared with upper foliage and stem tissue from plants of the same restricted fertilizer P concentration (Figure 4.9 B). All plants grown with restricted P had significantly greater tissue P concentrations in the flowers and fruit (Figure 4.9 A) than in the upper foliage and stems (Figure 4.9 B) of plants grown with the same initial P concentration. Plants grown with $20 \text{ mg}\cdot\text{L}^{-1}$ P continuously had similar tissue P concentrations in the reproductive tissues (Figure 4.9 A) and the upper vegetative tissues (Figure 4.9 B). Additionally, leachate P concentrations obtained at harvest illustrated how P was highest in the substrate of plants grown with $20 \text{ mg}\cdot\text{L}^{-1}$ P, and was lowest for all plants grown with restricted P fertilization (data not shown).

Considering experiments 1 and 3, reproductive stage P deficiency symptoms occurred most readily when plants were initially supplied with $10 \text{ mg}\cdot\text{L}^{-1}$ P and restricted to no P halfway through production. These symptoms were similar to those described by Whipker (2014) occurring on chrysanthemums. This type of upper leaf symptomology in response to P deficiency is highly unusual, and confirming that these symptoms also occur on peppers was an important step to better understanding this unique form of P deficiency. Gibson et al. (2007) describe P deficiency in vegetative ornamental peppers as an initial darkening of the upper leaves, followed by development of an overall dull green coloration and leaf curling. Curling of the upper leaves also occurred in response to reproductive stage P deficiency, though leaf coloration was significantly different. Additionally, no dull green coloration was observed on the plants grown

in this study. Symptomatic leaves quickly progressed from a healthy green appearance to severe chlorosis in a matter of days.

Conclusions

Symptoms of a reproductive stage P deficiency were induced under specific fertility conditions with low but adequate P concentrations early on that are restricted to no P after floral initiation. Plants were vegetative when P was restricted to 0 mg·L⁻¹ P, demonstrating that the reproductive tissues grew and matured without an external P supply. This indicates that all of the P in the reproductive tissues was obtained from internal sources, or any P remaining in the substrate; however, it was found that up to ~80% of the total P in aboveground tissues was located in the reproductive tissues. This illustrates the preferential reallocation of P to the flowers and fruit, which resulted in bypassed P translocation past the upper foliage. This bypass led to P deprivation in the upper foliage and resulted in symptoms of reproductive stage P deficiency. These symptoms were highly unusual when compared to the typical symptoms of P deficiency which occur on the lower foliage. This indicates that plants grown with reduced or eliminated P later in the fertilization program could develop these symptoms. This was in agreement with observations of chrysanthemums that were moved to an outside growing area and received no P for the remainder of the crop cycle (Whipker, 2014). The severity of these symptoms resulted in a significant economic loss to these growers, totaling approximately \$130,000 worth of affected plants.

The fact that all plants grown with restricted P developed deficiency symptoms in this study demonstrates the need to provide P at a minimal level throughout the entire production cycle. The necessity for continuous P fertilization was further supported by observing P values in

the leachate. Peppers grown with a high concentration of $20 \text{ mg}\cdot\text{L}^{-1}$ P had a significantly lower P concentration in the substrate after three weeks of withholding P. This P was lost from a combination of absorption by the plant and leaching from the substrate. Because floriculture crops are typically grown in soilless substrate with limited P adsorption capacity (Marconi and Nelson, 1984), it is imperative that P is provided via fertilization. Symptoms were avoided by providing continuous concentrations of $10 \text{ mg}\cdot\text{L}^{-1}$ P for all cultivars grown in this study. Growers of these crops should be able to avoid these symptoms by implementing a constant feed P fertilization program with $10 \text{ mg}\cdot\text{L}^{-1}$ P.

The symptoms observed on the two species grown in this study were similar and included chlorosis, necrosis, olive green spotting, and in the case of peppers, leaf abscission. However, due to purple coloration only forming on ‘Crystal Misty Purple’ chrysanthemums, the symptom development may be dependent on the plants innate ability to synthesize anthocyanins. ‘Crystal Misty Purple’ has purple flowers, while the other two cultivars did not exhibit purple coloration in their flowers or foliage. This indicates that ‘Crystal Misty Purple’ develops purple P deficiency symptoms because it can synthesize anthocyanins. Shaded symptomatic leaves developed symptoms of chlorosis similar to the other two cultivars. Additional research is required to determine the interaction of environmental and genetic factors on the development of red P deficiency symptoms.

As research and recommendations for growers to utilize low P fertilization progresses, issues associated with P deficiency are likely to become more prevalent (Whipker, 2014). This research provides growers with the information, descriptions, and recommendations needed to recognize and avoid these symptoms, in order to prevent losses and produce healthy floriculture crops.

Acknowledgements

We are grateful for the funding support provided by Fred C. Gloeckner Foundation, American Floral Endowment Altman Family Scholarship, and The Garden Club of America. We would also like to express our gratitude to Dümmer Orange for providing cuttings, and for peat moss provided by Sun Gro Horticulture.

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Table 4.1. Phosphorus concentration within different tissue types for *Capsicum annuum* ‘Tango Red’ plants grown with continuous or restricted phosphorus fertilization.

Plant Part	Phosphorus Concentration (mg·L ⁻¹)					
	Continuous			Restricted ¹		
	5	10	20	5	10	20
Flowers and Fruit	0.21 e ²	0.31 c	0.50 a	0.18 ef	0.20 e	0.26 d
Upper Stems and Foliage	0.16 fg	0.19 ef	0.37 b	0.11 hi	0.13 gh	0.12 ghi
Lower Stems and Foliage	0.12 hi	0.11 hi	0.27 d	0.089 i	0.087 i	0.095 hi

¹ Restricted phosphorus plants were grown with initial concentrations of 5, 10, or 20 mg·L⁻¹ P for six weeks, that was then restricted to 0 mg·L⁻¹ P for five weeks.

² Lower case letters signify minimum significant differences among phosphorus content across all phosphorus concentrations and tissue types. Means with different letters are significantly different at $P \leq 0.05$.

Table 4.2. Phosphorus content within different tissues, expressed as a percentage of the total, for *Capsicum annuum* ‘Tango Red’ plants grown with continuous or restricted phosphorus fertilization.

Plant Part	Phosphorus Concentration (mg·L ⁻¹)					
	5		10		20	
	Continuous	Restricted ¹	Continuous	Restricted	Continuous	Restricted
Flowers and Fruit	66% a ²	68% a	67% a	65% a	61% b	71% a
Upper Stems and Foliage	20% b	17% b	23% b	18% c	23% c	15% d
Lower Stems and Foliage	14% b	17% b	10% d	16% c	16% d	15% d

¹ Restricted phosphorus plants were grown with initial concentrations of 5, 10, or 20 mg·L⁻¹ P for six weeks, that was then restricted to 0 mg·L⁻¹ P for five weeks.

² Lower case letters signify minimum significant differences among percent of total plant phosphorus content, among all parts of plants grown with the same initial phosphorus concentration. Means with different letters are significantly different at $P \leq 0.05$.

Table 4.3. Leachate phosphorus concentrations at three different weeks for *Capsicum annuum* ‘Tango Red’ grown with continuous or restricted phosphorus fertilization.

Fertilization Regime ¹	Phosphorus Concentration (mg·L ⁻¹)	Leachate Phosphorus Concentration (mg·L ⁻¹)		
		Week 6	Week 9	Week 11
Continuous	0	0.4 c ²	0.4 b	0.3 b
	2.5	1.2 c	0.4 b	0.7 b
	5	1.9 bc	0.7 b	0.9 b
	10	4.5 b	1.3 b	1.4 b
	20	10.5 a	8.1 a	4.8 a
Restricted	2.5	-	0.8 b	0.3 b
	5	-	0.4 b	0.4 b
	10	-	0.3 b	0.3 b
	20	-	1.0 b	0.3 b
	MSD ³	2.64	2.02	1.84

¹ Continuous phosphorus plants were grown with continuous concentrations of 0, 2.5, 5, 10, or 20 mg·L⁻¹ P. Restricted phosphorus plants were grown with initial concentrations of 0, 2.5, 5, 10, or 20 mg·L⁻¹ P for six weeks, later restricted to 0 mg·L⁻¹ P for five weeks.

² Lower case letters signify minimum significant differences among leachate phosphorus concentrations within each sampled week. Means are significant at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for the leachate phosphorus concentrations for each week.

Table 4.4. Phosphorus concentration within different tissue types for *Chrysanthemum morifolium* ‘Little Rock’ plants grown with continuous or restricted phosphorus fertilization.

Plant Part	Phosphorus Concentration (mg·L ⁻¹)					
	10		15		20	
	Continuous	Restricted ¹	Continuous	Restricted	Continuous	Restricted
Flowers	0.52% a ²	0.26% c	0.54% a	0.30% de	0.54% ab	0.31% de
Upper Foliage & Stems	0.35% b	0.05% d	0.46% b	0.06% f	0.60% a	0.08% f
Middle Reproductive Foliage & Stems	0.27% c	0.05% d	0.36% cd	0.06% f	0.47% bc	0.06% f
Middle Vegetative Foliage & Stems	0.29% bc	0.04% d	0.42% bc	0.06% f	0.60% a	0.09% f
Lower Reproductive Foliage & Stems	0.24% c	0.05% d	0.33% d	0.05% f	0.39% cd	0.06% f
Lower Vegetative Foliage & Stems	0.23% c	0.03% d	0.25% e	0.04% f	0.30% e	0.07% f
MSD ³	0.067%		0.072%		0.091%	

¹ Restricted phosphorus plants were grown with initial concentrations of 10, 15, or 20 mg·L⁻¹ P for four weeks, later restricted to 0 mg·L⁻¹ P for four weeks.

² Lower case letters signify minimum significant differences among tissue phosphorus concentrations among all parts of plants grown with the same initial phosphorus concentration. Means are significant at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for each phosphorus concentration.

Table 4.5. Phosphorus content within different tissues, expressed as a percentage of the total, for *Chrysanthemum morifolium* ‘Little Rock’ plants grown with continuous or restricted phosphorus fertilization.

Plant Part	Phosphorus Concentration (mg·L ⁻¹)					
	10		15		20	
	Continuous	Restricted ¹	Continuous	Restricted	Continuous	Restricted
Flowers	59.0% b ²	81.3% a	52.6% b	79.4% a	46.0% b	74.1% a
Upper Foliage & Stems	13.9% c	6.0% ef	13.9% c	6.4% de	16.8% c	8.3% d
Middle Reproductive Foliage & Stems	4.4% fgh	3.0% gh	4.2% ef	3.4% ef	5.9% def	3.4% fg
Middle Vegetative Foliage & Stems	10.8% d	5.2% fg	16.3% c	5.5% def	19.1% c	7.0% de
Lower Reproductive Foliage & Stems	3.3% fgh	1.6% h	4.1% ef	1.8% f	4.5% efg	2.2% g
Lower Vegetative Foliage & Stems	8.6% de	2.9% gh	8.9% d	3.5% ef	7.7% de	5.0% defg
MSD ³	2.9%		4.0%		3.4%	

¹ Restricted phosphorus plants were grown with initial concentrations of 10, 15, or 20 mg·L⁻¹ P for four weeks, later restricted to 0 mg·L⁻¹ P for four weeks.

² Lower case letters signify minimum significant differences among percent of total plant phosphorus content among all parts of plants grown with the same initial phosphorus concentration. Means are significant at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for each phosphorus concentration.

Table 4.6. Phosphorus concentration within different tissue types for *Chrysanthemum morifolium* ‘Swiftly Yellow’ plants grown with continuous or restricted phosphorus fertilization.

Plant Part	Phosphorus Concentration (mg·L ⁻¹)					
	10		15		20	
	Continuous	Restricted ¹	Continuous	Restricted	Continuous	Restricted
Flowers	0.39% ab ²	0.14% c	0.40% a	0.17% b	0.43% b	0.17% c
Upper Foliage & Stems	0.41% b	0.06% d	0.42% a	0.08% c	0.46% ab	0.09% d
Middle Reproductive Foliage & Stems	0.45% a	0.06% d	0.43% a	0.08% c	0.50% a	0.09% d
Middle Vegetative Foliage & Stems	0.32% b	0.05% d	0.37% a	0.07% c	0.45% ab	0.10% cd
Lower Reproductive Foliage & Stems	0.43% a	0.08% cd	0.43% a	0.10% bc	0.51% a	0.14% cd
Lower Vegetative Foliage & Stems	0.33% b	0.05% d	0.37% a	0.08% c	0.40% b	0.11% cd
MSD ³	0.075%		0.094%		0.069%	

¹ Restricted phosphorus plants were grown with initial concentrations of 10, 15, or 20 mg·L⁻¹ P for four weeks, later restricted to 0 mg·L⁻¹ P for four weeks.

² Lower case letters signify minimum significant differences among tissue phosphorus concentrations among all parts of plants grown with the same initial phosphorus concentration. Means are significant at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for each phosphorus concentration.

Table 4.7. Total plant phosphorus content, expressed as a percentage of the total, within different tissue types for *Chrysanthemum morifolium* ‘Swift Yellow’ plants grown with continuous or restricted phosphorus fertilization.

Plant Part	Phosphorus Concentration (mg·L ⁻¹)					
	10		15		20	
	Continuous	Restricted ¹	Continuous	Restricted	Continuous	Restricted
Flowers	52.2% b ²	69.4% a	52.4% b	68.8% a	51.1% b	63.3% a
Upper Foliage & Stems	11.1% c	7.0% def	12.6% c	8.3% def	12.6% c	8.0% def
Middle Reproductive Foliage & Stems	11.0% c	5.7% f	9.6% cd	4.9% f	9.5% cde	5.8% f
Middle Vegetative Foliage & Stems	9.1% cde	6.1% f	9.6% cd	5.8% ef	10.4% cd	8.3% def
Lower Reproductive Foliage & Stems	6.6% ef	5.2% f	6.4% def	4.8% f	6.7% ef	5.7% f
Lower Vegetative Foliage & Stems	9.8% cd	6.6% ef	9.5% cde	7.4% def	9.7% cde	8.9% def
MSD ³	2.9%		3.7%		3.6%	

¹ Restricted phosphorus plants were grown with initial concentrations of 10, 15, or 20 mg·L⁻¹ P for four weeks, later restricted to 0 mg·L⁻¹ P for four weeks.

² Lower case letters signify minimum significant differences among percent of total plant phosphorus content among all parts of plants grown with the same initial phosphorus concentration. Means are significant at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for each phosphorus concentration.

Table 4.8. Leachate phosphorus concentrations collected upon termination of each cultivar for three cultivars of *Chrysanthemum morifolium* grown with continuous or restricted phosphorus fertilization.

Fertilizer Regiment ¹	Fertilizer Phosphorus Concentration (mg·L ⁻¹)	Leachate Phosphorus Concentration (mg·L ⁻¹)		
		Little Rock	Swiftly Yellow	Crystal Misty Purple
Continuous	10	1.95 c ²	4.03 c	8.74 b
	15	7.71 b	10.06 b	9.22 b
	20	12.28 a	15.77 a	18.82 a
Restricted	10	0.11 d	0.13 d	0.16 c
	15	0.12 d	0.11 d	0.18 c
	20	0.12 d	0.21 d	1.10 c
	MSD ³	1.66	3.38	7.47

¹ Continuous phosphorus plants were grown with continuous concentrations of 10, 15, or 20 mg·L⁻¹ P. Restricted phosphorus plants were grown with initial concentrations of 10, 15, or 20 mg·L⁻¹ P for four weeks, later restricted to 0 mg·L⁻¹ P for four weeks.

² Lower case letters signify minimum significant differences among leachate phosphorus concentrations within each cultivar. Means are significant at $P \leq 0.05$.

³ Minimum significant differences (MSD) are listed for the leachate phosphorus concentrations for each cultivar.



Figure 4.1. Symptoms of reddening, olive green spotting, and necrotic spotting were all present simultaneously on this *Chrysanthemum morifolium* 'Crystal Misty Purple' leaf.



Figure 4.2. Symptoms of upper leaf chlorosis, necrotic spotting, and leaf abscission occurring on *Capsicum annuum* 'Tango Red' after six weeks of phosphorus fertilization at $20 \text{ mg}\cdot\text{L}^{-1}$, and four weeks with fertilization restricted to $0 \text{ mg}\cdot\text{L}^{-1}$ P.

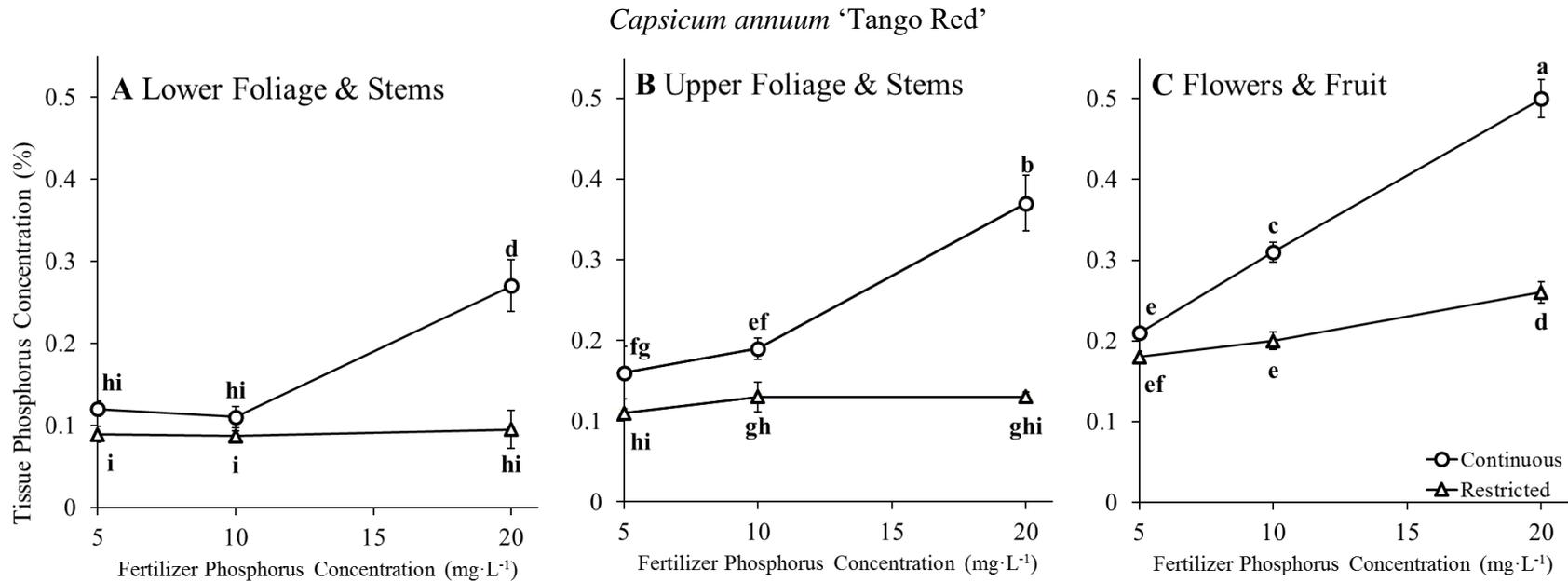


Figure 4.3. Plotted means for *Capsicum annuum* 'Tango Red' lower foliage and stems (A), upper foliage and stems (B), and flowers and fruit (C) with minimum significant differences (MSDs) signified by lower case lettering. Means with different letters are significantly different at $P \leq 0.05$. MSDs were determined across both fertilizer phosphorus concentration and plant part. Line labels "Continuous" and "Restricted" refer to whether the phosphorus fertilization regiment remained the same or was restricted to 0 mg·L⁻¹ P after six weeks.



Figure 4.4. Symptoms of marginal chlorosis and necrosis (A), and necrotic curling (B) of the upper leaves on *Chrysanthemum morifolium* 'Swifty Yellow'.



Figure 4.5. Symptoms of upper leaf purpling and chlorosis, as well as upper stem purpling on *Chrysanthemum morifolium* 'Crystal Misty Purple'.



10

15

20

Initial Phosphorus Concentration (mg·L⁻¹)

Figure 4.6. *Chrysanthemum morifolium* 'Crystal Misty Purple' plants grown with initial concentrations of 10, 15, or 20 mg·L⁻¹ P, restricted to 0 mg·L⁻¹ P after four weeks.



Figure 4.7. Symptoms of purpling on *Chrysanthemum morifolium* 'Crystal Misty Purple' were only present on leaf surfaces that were exposed to light. Here, a leaf was partially covering a symptomatic leaf, resulting in the shaded area being devoid of purple coloration.

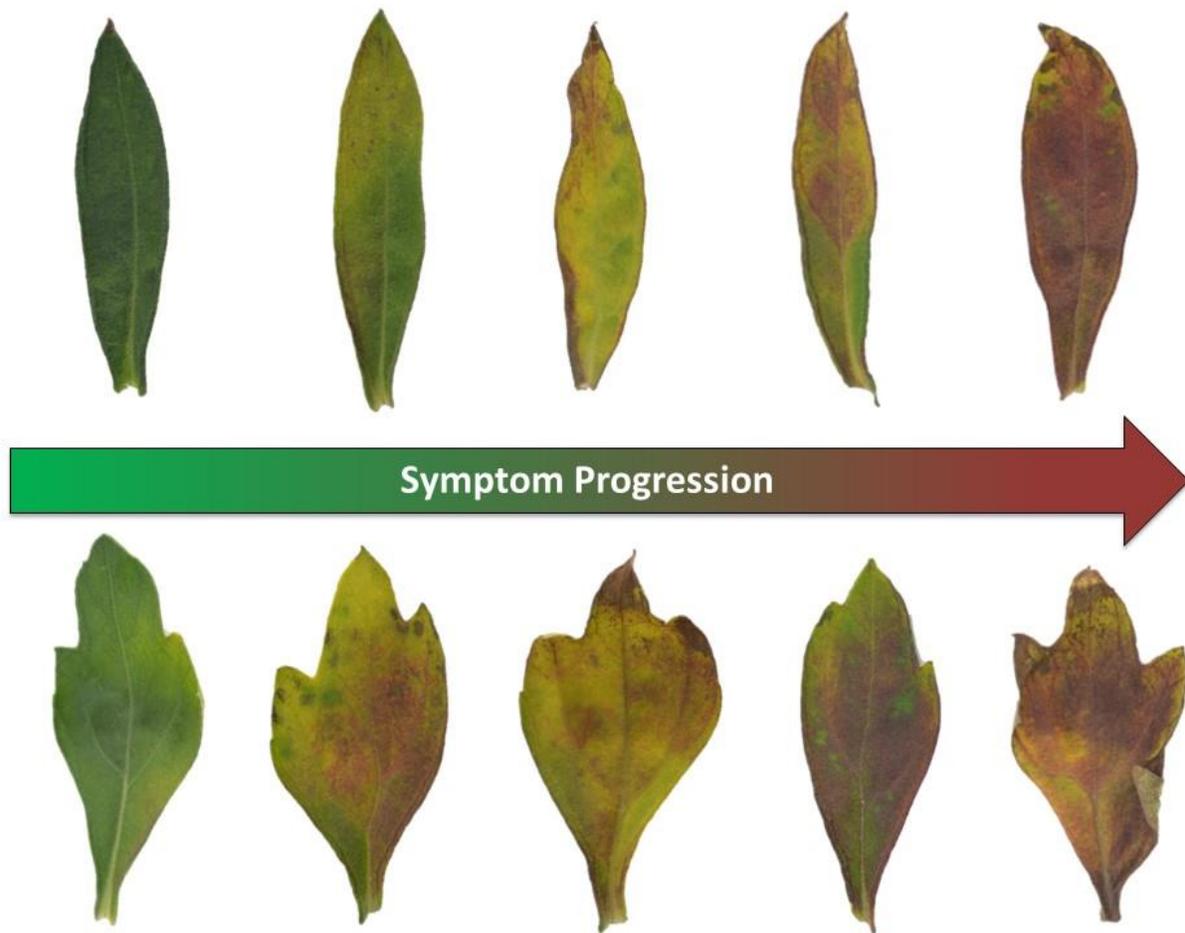


Figure 4.8. Upper leaf symptomology of chlorosis, necrotic margins, necrotic spotting, olive green leaf spotting, and purpling was observed on *Chrysanthemum morifolium* 'Crystal Misty Purple'. Progression of symptoms are displayed for two distinct upper leaf shapes: top row (reproductive leaves) and bottom row (intermediate vegetative/reproductive leaves).

Capsicum annuum 'Tango Red' – Tissue Phosphorus Concentrations

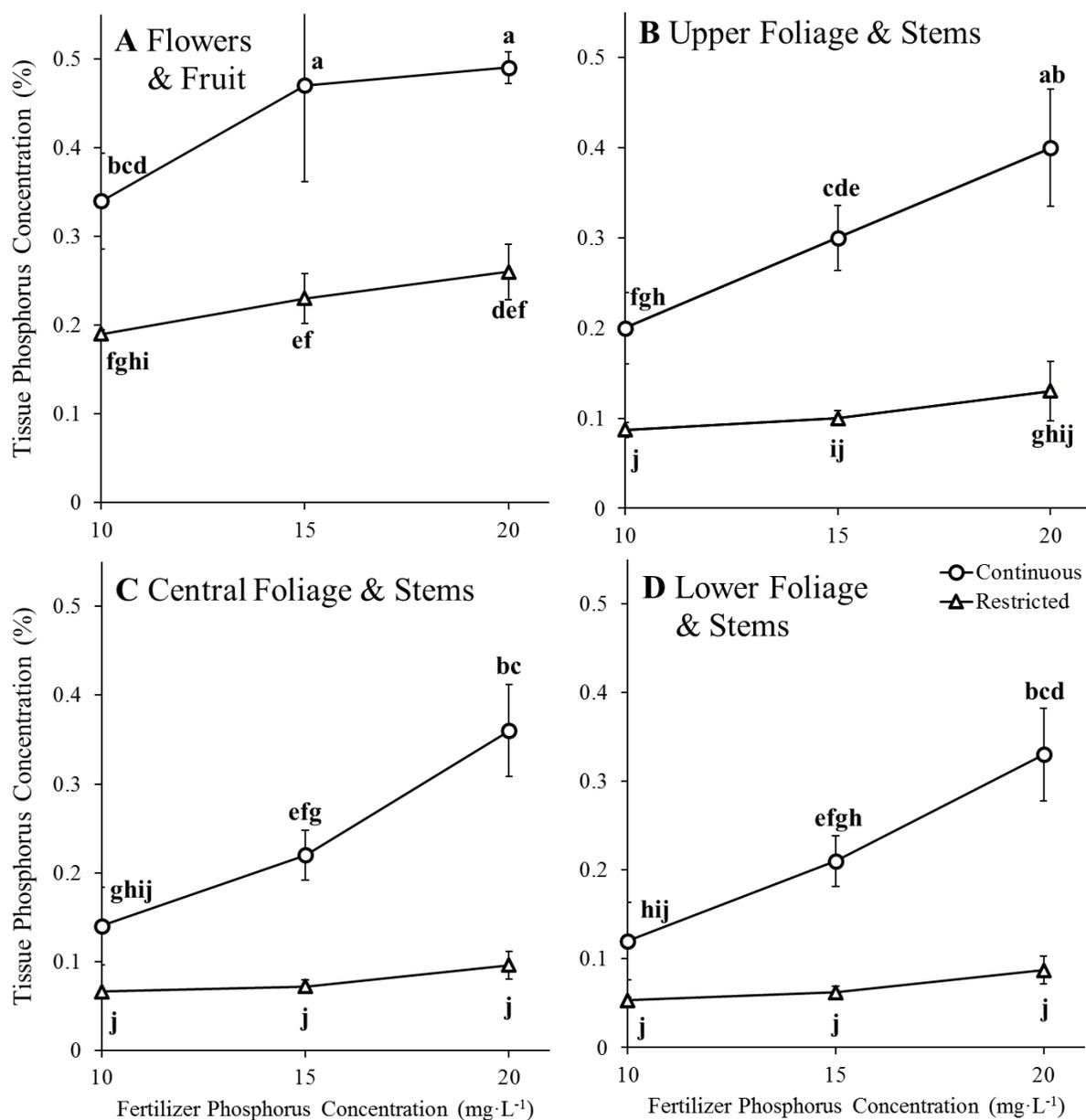


Figure 4.9. Plotted means of tissue P concentrations for *Capsicum annuum* 'Tango Red' flowers and fruit (A), upper foliage and leaves (B), central foliage and leaves (C), and lower foliage and stems (D) with minimum significant differences (MSD) signified by lower case lettering. Means with different letters are significantly different at $P \leq 0.05$. MSDs were determined among all fertilizer phosphorus concentrations and plant parts. Line labels "Continuous" and "Restricted" refer to whether the phosphorus fertilization regiment remained the same or was restricted to 0 mg·L⁻¹ P after six weeks.

Chapter 5

Low Phosphorus Fertilization Enhances Coloration of Red Leafed Ornamental Species

Abstract

Phosphorus (P, referring to phosphorus supplied by phosphate) deficiency commonly leads to the development of a red to purple foliage coloration in many species. This coloration is the result of increased production of anthocyanins in deficient tissues. Symptoms can be pronounced in plants with naturally green foliage, which reduces the quality and marketability of ornamental bedding and potted crops. A number of ornamental species are grown for their naturally red leaf coloration. Zonal geraniums (*Pelargonium x hortorum* L. H. Bailey) are valued for the anthocyanin rich dark band or “zone” located on the upper leaf surface, and many species of alternanthera are grown for their deep red foliage. This study was conducted to determine if a low P fertilization strategy would enhance the red coloration in these crops. *Pelargonium x hortorum* ‘Bullseye Red’ and *Alternanthera brasiliana* (L.) Kuntze ‘Purple Prince’ were grown using P concentrations of 0, 2.5, 5, 10, and 20 mg·L⁻¹ P. After four weeks, half of the ‘Purple Prince’ plants grown with 2.5, 5, 10, and 20 mg·L⁻¹ P had their P fertilization restricted to 0 mg·L⁻¹ P. Similarly, ‘Bullseye Red’ plants grown with 5, 10, and 20 mg·L⁻¹ P were restricted to 2.5 mg·L⁻¹ P after eight weeks. Growth parameters including height, diameter, dry mass, branch development, and flowering were recorded and compared to ensure plants were not stunted or delayed. Color parameters were measured using a handheld colorimeter, and L*a*b* color space was used to determine chroma and hue angle. Upon termination of the study, most recently matured leaves were harvested and analyzed for foliar nutrient concentrations. Significant differences were compared among the P concentrations and regiments (continuous or restricted) to determine optimal P concentrations to enhance coloration. Initial concentrations of 5 mg·L⁻¹ P restricted to either 0 mg·L⁻¹ P for alternanthera or 2.5 mg·L⁻¹ P for geranium resulted in plants with the reddest coloration, without negative aspects of P deficiency such as excessive stunting.

These plants also had significantly higher saturation, indicating that they had a more pronounced red coloration than plants grown with higher concentrations that are typical of many commercial fertilizers. Growers may use initial P concentrations of 2.5 to 5 mg·L⁻¹ to enhance the coloration of naturally red leafed crops while gaining the added benefit of moderate growth control.

Introduction

Anthocyanins are water-soluble pigments found in vacuoles of all plant parts, both above and below ground (Gould et al., 2009). For ornamental plants, anthocyanins in the leaves and flowers are valued for their aesthetic appeal, and contribute a wide range of colors making them desirable for consumers. Some plants have naturally high levels of foliar anthocyanins that contribute to their red coloration regardless of health status. Many other plant species are known to increase anthocyanin production in response to stress. According to Gould et al. (2009), these conditions can include “strong light, UV-B radiation, high and low temperature extremes, drought, ozone, nitrogen (N) and phosphorus (P) deficiencies, bacterial and fungal infections, wounding, herbivory, herbicides, and various pollutants”. An increase of foliar anthocyanins is one of the primary symptoms associated with P deficiency in plants (Sarker and Karmoker, 2011). This leads to a reddening or purpling that typically occurs on older foliage, and also accounts for the “darker green” coloration that sometimes accompanies P deficiency.

Vacuoles of the leaf epidermal cells hold accumulated anthocyanins, which are thought to provide a defense role against excessive sunlight (Hernández and Munné-Bosch, 2015). This photoprotective role would depend on the leaf position in the canopy, leaf thickness, and the location of anthocyanin concentration within the leaf tissue (Henry et al., 2012). Anthocyanins would likely play a photoprotective role when located in the leaf epidermis and not in the

mesophyll (Henry et al., 2012). Although the exact purposes of anthocyanins are currently unknown, it is widely accepted that they play a variety of protective roles.

Limited supply of both P and N leads to such conditions that limit growth and increase anthocyanin production (Gould et al., 2009). It is believed that because P deficiency stress limits plant growth, the number of cells that produce anthocyanins and the amount those cells produce both increase (Gould et al., 2009). As growth becomes limited, primary metabolism decreases, and secondary metabolism, such as anthocyanin production increases. There is evidence that indicates certain molecules and precursors normally used for primary metabolic processes, such as phenylalanine, are used instead for secondary metabolic processes including anthocyanin synthesis when growth is less (Gould et al., 2009). An increase in sugar accumulation due to P deficiency may be another factor that leads to higher production of anthocyanins (Hammond et al., 2011; Sarker and Karmoker, 2011).

Past research demonstrates how low P increases foliar anthocyanin content. Tomato (*Solanum lycopersicum* L.) plants grown without P develop anthocyanin levels up to five times greater than the levels found in plants grown with adequate P (Ulrychová and Sosnová, 1970). It was also noted that both light quantity and temperature have an additive effect on anthocyanin production regardless of cultivar and P concentration in the nutrient solution (Ulrychová and Sosnová, 1970). Anthocyanin levels in carrot (*Daucus carota* L.) callus tissue grown in cell culture were significantly higher under both low P and N stress when compared to the control (Rajendran et al., 1992). When limiting available P, anthocyanin content as a percent of total dry weight was about 2.25 times greater than the control (Rajendran et al., 1992).

Chinese kale (*Brassica alboglabra* L.H. Bailey) plants that are normally green may become mauve in color when P is limited (Chen et al., 2013). Chen et al. (2013) provided

continuous P concentrations of 30 (normal), 7.5 (low) and 0 mg·L⁻¹ (deficient) to observe the effects on plant growth and pigmentation using a green and a mauve Chinese kale cultivar. Height was only significantly less as P decreased from 7.5 to 0 mg·L⁻¹, though fresh weight was significantly lower with every reduction of P concentration. Additionally, anthocyanin content was not significantly different between normal and low P concentrations, but was significantly greater when no P was supplied (Chen et al., 2013). This may indicate that additional P concentrations must be used within this range of 0 and 7.5 mg·L⁻¹ to better determine if anthocyanin content can increase without detrimentally decreasing mass accumulation.

The link between P deficiency and anthocyanin production has long been established (Mengel et al., 2001; Ulrychová and Sosnová, 1970), though there has been little research explaining the use of low P fertilization concentrations to beneficially enhance pigmentation. There is the possibility that plants naturally high in anthocyanins such as coleus [*Plectranthus scutellarioides* (L.) R. Br.] and red cabbage (*Brassica oleracea* L. var. *capitata* f. *rubra*) may not change significantly in anthocyanin concentration regardless of P fertilization concentration or tissue P concentration (Boldt, 2013). Still, P deficient plants with naturally red foliage typically display a more intense red coloration when compared to P sufficient plants. Although most studies indicate increased anthocyanin production and therefore overall coloration changes, they also indicate significant control of plant growth in response to low P (Chen et al., 2013).

There are three hypotheses being tested in this study. The first hypothesis is that low P fertilization can beneficially control growth and enhance foliar coloration of red leafed plant species. Popular bedding plants such as *Pelargonium x hortorum* have the potential to benefit from lower P application due to increased anthocyanin accumulation in the foliar bands. Conversely, P stress may also lead to undesirable factors such floral delay (Baas et al., 1995).

For this reason, lower P regiments may be more beneficial for plants grown for their foliage rather than their flower, though effectiveness certainly varies by species. The second hypothesis is that issues related to excessive stunting and delay of flowering may be overcome by providing adequate P during the initial period of growth, and then restricting P later in the production cycle.

Though anthocyanins are the most common red pigment found in plants, betalains are another class of red pigments that can occur. Betacyanins are a category of betalain which cause red plant pigmentation found only in plants from the order Caryophyllales (Tanaka et al., 2008). This order includes a number of ornamental species such as *Dianthus* sp. and amaranths such as *Alternanthera* and *Iresine* sp. *Alternanthera* and *Iresine* are grown for their deep red foliage coloration. As these species do not produce anthocyanins, it is of interest to see if low P fertilization can affect the coloration of these plants. Betalains and anthocyanins do not occur in the same species, and it is unknown whether P deficiency leads to increased betalain synthesis or accumulation (Tanaka et al., 2008). The third hypothesis of this study is that low P fertilization will increase foliar betalain concentration, leading to enhanced red coloration.

This study was conducted to determine if low P concentrations enhance the red coloration of geranium (*Pelargonium x hortorum*), an anthocyanin producing species, and alternanthera (*Alternanthera brasiliana*), a betacyanin producing species. In addition, higher initial P concentrations that were later restricted were used to determine if excessive stunting could be avoided.

Materials and Methods

P. x hortorum 'Bullseye Red' and *A. brasiliana* 'Purple Prince' were the two plants used in this study. The substrate used for the entire experiment was an 80:20 (v:v) mix of Canadian

sphagnum peat moss (Conrad Fafard, Agawam, MA) and horticultural coarse perlite (Perlite Vermiculite Packaging Industries, Inc., North Bloomfield, OH), with added dolomitic lime at 8.875 kg/m³ (Rockydale Agricultural, Roanoke, VA) and AquaGro 2000-G Wetting Agent (Aquatrols, Cherry Hill, NJ) at 600.3 g/m³. This custom substrate was used to ensure that there was no initial charge of P.

Fertilizers were custom blends of the following individual technical grade salts: Ca(NO₃)₂·4H₂O, KNO₃, KH₂PO₄, K₂SO₄, MgSO₄·7H₂O, Mg(NO₃)₂, FeDTPA, MnCl₂·4H₂O, ZnCl₂·7H₂O, CuCl₂·2H₂O, H₃BO₃, and Na₂MoO₄·2H₂O (Appendix A). Phosphorus (referring to phosphate-phosphorus) concentrations of 0, 2.5, 5, 10, or 20 mg·L⁻¹ were used for growing both species, and were supplied entirely in the form of potassium phosphate (KH₂PO₄). Nitrogen and potassium (K) were held constant at 150 mg·L⁻¹, with all other essential microelements remaining constant. Fertilizer solution was mixed in 100 L barrels, and was applied at each irrigation through 1.9 cm black irrigation tubing fitted with drip rings. Each species had plants that remained on their initial P fertilization regiment, and others that were restricted to a lower P concentration later in production. This restriction varied by species, with nine total P treatments for alternanthera and eight total P treatments for geranium.

The experiment was completely randomized with sixteen single plant replicates of each fertilization treatment. Biweekly measurements were collected for plant height by measuring the highest point of the foliage with a ruler from the rim of the pot, and plant diameter was recorded by averaging the widest point and the axis perpendicular to that. Relative chlorophyll content was also determined from the most recently matured leaves on a biweekly basis using a SPAD Chlorophyll Meter (SPAD-502; Konica Minolta Sensing, Inc., Osaka, Japan). Two readings were collected from each plant and averaged at each measurement.

A destructive harvest was conducted upon termination of each species. Eight of the single plant replicates were measured for height and diameter. The number of branches was counted for each plant and categorized as primary branches (branches which emerge directly from the main shoot), secondary branches (branches which emerge from the primary branches), and tertiary branches (branches which emerge from the secondary branches) when applicable. Substrate pH was obtained using the Pour-Thru method (Cavins et al., 2005), and recorded using a HI 9813-6 portable meter (Hanna Instruments, Woonsocket, RI). L*a*b* color space readings were collected from the most recently matured foliage of each plant using a ColorTec PCM/PSM colorimeter (ColorTec, Clinton, NJ). L* is lightness ranging 0 (dark) to 100 (light), a* is the range from green (negative 100) to red (positive 100), and b* is the range from blue (negative 100) to yellow (positive 100). L*a*b* color readings were used to calculate chroma (C*, Equation 1) and hue (h°, Equation 2), which indicates the hue on a 360° spectrum (0° is red) (McGuire, 1992).

Equation 1

$$C^* = \sqrt{a^{*2} + b^{*2}}$$

Equation 2

$$h^{\circ} = \arctangent \ b^*/a^*$$

Tissue samples were obtained by harvesting the most recently matured foliage from each of these eight replicates. Samples were rinsed initially with deionized water, then washed in a solution of 0.5 N HCl, and followed with a rinse of deionized water. Remaining stems and leaves

were harvested as well to record dry mass of the vegetative and floral portions of the plants. Tissue samples and remaining tissue were allowed to dry for at least 72 hours at 70°C, and total plant dry mass was recorded. Tissue samples from five single plant replicates were then ground using a Thomas Wiley® Mini-Mill (Thomas Scientific, Swedesboro, NJ), and analyzed for nutrient content by AgSource Laboratories (Lincoln, NE). Total N was processed by Kjeldahl digestion, and determined via flow injection analysis (FIA). Extractable K was processed by 2% acetic acid digestion, and determined via inductively coupled plasma mass spectrometry (ICP-MS). Total P and all other plant minerals were processed by nitric acid/hydrogen peroxide digestion, and determined via ICP-MS.

Experiment 1

A. brasiliana 'Purple Prince' cuttings were obtained on 12 Aug. 2016 and stuck in 72 round cell plug trays with cell dimensions of 3.8 x 6.4 cm (diameter x depth). Cuttings were rooted under mist, and were removed from the mist once root growth had reached the cell wall. After being removed from the mist, plants were fertilized with a custom fertilizer solution consisting of 75N–5P–75K (mg·L⁻¹), with all other essential nutrients provided with the previously listed fertilizer salts. Cuttings were transplanted on 31 Aug. into 5-inch diameter pots (Dillen, Middlefield, OH) with dimensions of 12.7 x 9.2 cm (diameter x depth) and a volume of 0.8 L. Plants were initially grown with P concentrations of either 0, 2.5, 5, 10, or 20 mg·L⁻¹. Four weeks after transplanting, half of the plants being grown with 2.5, 5, 10, or 20 mg·L⁻¹ P were switched to 0 mg·L⁻¹ P, while the other half remained on their initial concentrations. Following this switch, there were sixteen single-plant replicates of each fertilizer treatment.

Plants were destructively harvested eight weeks after transplant. Eight plants were used for tissue nutrient analysis. The remaining eight single plant replicates of each treatment of *alternanthera* were harvested for betalain determination. Fresh tissue was collected, weighed, and shredded to increase total surface area. The tissue was then placed into bags and immediately put on ice. After harvest was completed, bags were frozen at -80°C . Frozen tissue was weighed and homogenized in 0.05 M phosphate buffer solution (von Elbe, 2001) using a vortex mixer. Solution was then centrifuged at 3000 rpm for 5 minutes. Supernatant was extracted and used to quantify betacyanins using a spectrophotometer (UV-2450, Shimadzu, Tokyo, Japan) set to absorb at 536 and 650 nm. Betacyanin was determined based on the methods of Cai et al. (1998), using the molar extinction factor for amaranthine (5.66×10^4). Absorbance values at 650 nm were subtracted from the 536 values to account for impurities in the solution (von Elbe, 2001).

Experiment 2

P. x hortorum 'Bullseye Red' seeds (Fred C. Gloeckner & Co., Inc., Harrison, NY) were sown on 7 Sept. 2016 into 128-cell plug trays with cell dimensions of 2.7 x 2.7 x 3.8 cm. Fertilization began on 14 Sept. using the same fertilizer solution as *alternanthera*. Seedlings were transplanted on 21 Sept. Plants were initially grown with P concentrations of 0, 2.5, 5, 10, or 20 $\text{mg}\cdot\text{L}^{-1}$. Eight weeks after transplant, half of the plants being grown with concentrations of 5, 10, or 20 $\text{mg}\cdot\text{L}^{-1}$ P were restricted to 2.5 $\text{mg}\cdot\text{L}^{-1}$ P, while the other half remained on their initial concentrations. Plants grown initially with 0 and 2.5 $\text{mg}\cdot\text{L}^{-1}$ P remained on the same fertilization regiment. Twelve weeks after transplant, the experiment was terminated and plants were destructively harvested.

Data Analysis

Statistical analysis was conducted using SAS (version 9.4; SAS Institute, Cary, NC). Data for final plant height, diameter, dry mass, branch number, substrate pH, tissue nutrient concentrations, SPAD readings, L*a*b*, chroma, and hue angle were subjected to PROC GLM and the means were separated by Tukey's honestly significant differences (HSD) at $P \leq 0.05$. Minimum significant differences were determined among plants grown with different P concentrations and fertilization regiments for each individual parameter.

Results and Discussion

Plants grown with the highest concentration of $20 \text{ mg} \cdot \text{L}^{-1}$ P were considered control plants, as these concentrations were similar to those provided by many commercial fertilizers mixed using recommended concentrations. Both species in this study exhibited a redder coloration than control plants when grown with lower P concentrations. Restricting P later in production resulted in plants with optimal size and coloration for both species. Whether P was restricted or remained continuous will be referred to as the fertilization regiment. When the term "initial" is used, it will refer only to the restricted-P fertilization treatments and specifically to the P concentrations applied during the first half of production of each crop. Plant size, color, and overall appearance in terms of deficiency symptoms all were considered when determining optimal P concentrations and fertilization regiments.

Experiment 1

A. brasiliana 'Purple Prince' plants varied little in substrate pH across P fertilizer regimes, which ranged from 6.46 to 6.61 (Table 5.1). Height was significantly affected by

different P concentrations (Table 5.1). When P was completely withheld, plants were stunted, averaging 20.7 cm in height. Plants grown with continuous or initial concentrations of 10 and 20 mg·L⁻¹ P, or a continuous concentration of 5 mg·L⁻¹ P were the tallest. Plants grown with continuous 10 mg·L⁻¹ P, or either 20 mg·L⁻¹ P regiment had the greatest diameters, while plants grown without P were narrowest (Table 5.1). Initial concentrations of 5 and 10 mg·L⁻¹ P resulted in moderate control of diameter. These differences in height and diameter indicate that initial concentrations of 5 and 10 mg·L⁻¹ P may be used as part of a growth control management strategy.

Fertilizer recommendations for *A. brasiliense* suggest using a constant feed fertilization program with a complete fertilizer that is low in P (PanAmerican Seed Co., 2017). Specific recommendations state using 15N–2.2P–12.5K fertilizer mixed at a concentration of 175 – 225 ppm mg·L⁻¹ N. This fertilization program would provide 25.7 – 33.0 mg·L⁻¹ P, which far exceeds the optimal concentrations determined in this study. Growth control with 5 – 10 mg·L⁻¹ P demonstrates that current low P recommendations exceed what was determined to be optimal in this study.

Branching was also affected by P concentration (Table 5.1). The number of primary branches emerging from the main stem ranged from 10 to 32. Plants grown with either 20 mg·L⁻¹ P regiment, or 10 mg·L⁻¹ P continuously had the greatest number of branches, while plants grown without P had fewest. Plants grown with initial concentrations of 5 and 10 mg·L⁻¹ P had a moderate number of primary branches. The number of secondary branches emerging from primary branches also increased with P concentration, though the range was much greater. There were 0 secondary branches on plants grown without P and up to 135 secondary branches on plants grown with continuous 20 mg·L⁻¹ P. Dry mass followed a nearly identical trend, as plants

grown with $20 \text{ mg}\cdot\text{L}^{-1}$ P had the greatest dry mass and plants grown with $0 \text{ mg}\cdot\text{L}^{-1}$ P accumulated the least (Table 5.1).

Color was greatly affected by P concentration, and was determined with several different measurements (Table 5.2). Hue may be considered the most important parameter, by providing a value that may be directly compared to a corresponding color. Plants grown with continuous P concentrations of $20 \text{ mg}\cdot\text{L}^{-1}$ had significantly higher hue (less red) than plants grown with continuous concentrations of $0 - 5 \text{ mg}\cdot\text{L}^{-1}$ P, or initial P concentrations of $2.5 - 10 \text{ mg}\cdot\text{L}^{-1}$. Other low P concentrations resulted in similar hue, although these plants were excessively stunted. A fertilizer regiment of $5 \text{ mg}\cdot\text{L}^{-1}$ P restricted to $0 \text{ mg}\cdot\text{L}^{-1}$ P also had the highest average a^* value, indicating it was closest to red (Table 5.2). Additionally, chroma values were highest for plants grown with initial concentrations of $5 - 10 \text{ mg}\cdot\text{L}^{-1}$ P, indicating these plants had the most vivid or saturated coloration (Table 5.2). Average SPAD values (indicating relative chlorophyll) were lowest for plants grown with an initial concentration of $5 \text{ mg}\cdot\text{L}^{-1}$ P as well. SPAD values were greatest for plants supplied with high or continuous concentrations of P, while plants grown with low or restricted P concentrations had the lowest SPAD readings (Table 5.2).

Tissue betacyanin concentration differed among plants grown with varying P regiments (Figure 5.1). Plants grown with continuous concentrations of 0 and $2.5 \text{ mg}\cdot\text{L}^{-1}$ P or initial concentrations of 5 and $10 \text{ mg}\cdot\text{L}^{-1}$ P had the highest levels of betacyanin. Using either $20 \text{ mg}\cdot\text{L}^{-1}$ P fertilization regiment or continuous concentrations of $10 \text{ mg}\cdot\text{L}^{-1}$ P, resulted in plants with the lowest betacyanin concentration. Mean betacyanin concentration of plants grown with continuous P exhibited an inverse relationship with increasing P concentrations. Conversely, plants grown with restricted P exhibited increasing betacyanin concentration as initial concentrations increased from $2.5 \text{ mg}\cdot\text{L}^{-1}$ P to $10 \text{ mg}\cdot\text{L}^{-1}$ P. Plants grown with initial

concentrations of 20 mg·L⁻¹ P did not follow this increasing trend, and were not significantly different from plants grown with continuous concentrations of 20 mg·L⁻¹ P. Although there were no differences between plants grown with either 20 mg·L⁻¹ P regiment, plants grown with either 10 mg·L⁻¹ P regiment were significantly different from each other. In fact, plants grown with initial concentrations of 10 mg·L⁻¹ P had betacyanin concentration fifteen times greater than plants grown with a continuous concentration of 10 mg·L⁻¹ P. Restricting P did not result in increased betacyanins in plants grown with an initial concentration of 2.5 mg·L⁻¹ P. These plants had significantly lower betacyanin concentration compared to plants grown with a continuous concentration of 2.5 mg·L⁻¹ P. Overall, fertilizer P concentrations greatly influenced not only the visible coloration of alternanthera, but also the development of betalain pigments within the foliage.

Significant differences in betacyanin concentration have previously been observed in *A. brasiliiana* when exposed to different stresses and stimuli (Silva et al., 2005). For instance, plants had greater betacyanin accumulation when cultured on media containing tyrosine, an important precursor to betacyanins. Conversely, betacyanin accumulation was less when cultured on media containing the common herbicide 2,4 dichlorophenoxyacetic acid (2,4-D) (Silva et al., 2005). Additionally, differences in betacyanin concentration have been observed in *Amaranthus* sp. based on genotype, growth stage, and plant part (Cai et al., 1998). Betacyanin concentration in the leaves of *Amaranthus cruentus* L. were found to decrease from 150 mg/100 g fresh weight to 15 mg/100 g fresh weight over the course of ten weeks as the plants matured (Cai et al., 1998). Although these changes in betacyanin concentration have been documented, little has been published regarding the impact of P fertilization on betacyanin accumulation. This work suggests

that P nutrition greatly influences betacyanin concentration in plants, and additional research is required to determine the mechanisms behind this relationship.

Tissue nutrient analysis indicated that a number of significant trends occurred as P concentration changed (Table 5.3). As expected, tissue P concentrations were positively correlated with fertilizer P concentration. Plants grown with a continuous supply of 20 mg·L⁻¹ P had the highest foliar P concentrations (0.40% P). Plants grown with continuous concentrations of 5 – 20 mg·L⁻¹ P had higher foliar P concentrations than those grown with the same initial fertilizer concentration restricted after four weeks. Fertilizer concentrations of 0 mg·L⁻¹ P or 2.5 – 5 mg·L⁻¹ P restricted to 0 mg·L⁻¹ P had similar, low tissue P concentrations. In fact, the recommended P fertilization regiment of 5 mg·L⁻¹ P restricted after four weeks had foliar P concentrations of 0.035%, which was ten times lower than the typical sufficient range of 0.3 – 0.5% for most crops (Marschner, 1995). Only the continuous 20 mg·L⁻¹ P regime resulted in values within the sufficiency range. This suggests that for growers whose objective it is to control growth and enhance coloration, significantly lower tissue standards for P should be developed.

Tissue N and K concentrations followed a similar trend as tissue P, where plants grown with higher and continuous fertilizer P concentrations had higher foliar N and K concentrations. N was lowest in plants grown with 0 mg·L⁻¹ P or initial concentrations of 2.5 – 10 mg·L⁻¹ P. Similarly, foliar K concentration was lowest in plants grown with 0 mg·L⁻¹ P or initial fertilizer concentrations of 5 – 20 mg·L⁻¹ P. Foliar iron (Fe) concentration was also greater when plants were fertilized with higher P concentrations. Foliar zinc (Zn) concentration was highest when P was withheld from the plant, and decreased as fertilizer P concentration increased. This

interaction is commonly observed in plants, and high P concentrations are known to induce Zn deficiencies (Marschner, 1995).

Although plants grown with 5 mg·L⁻¹ P had tissue P values within the deficient range, these plants developed optimal color based on several measured parameters. The extent of growth control provided by this fertilizer regiment may also be considered beneficial, while lower concentrations ≤ 2.5 mg·L⁻¹ P resulted in excessive stunting. Compared to plants grown with continuous P concentrations of 10 – 20 mg·L⁻¹, *A. brasiliana* ‘Purple Prince’ plants grown with initial concentrations of 5 mg·L⁻¹ P later restricted to 0 mg·L⁻¹ P developed optimal coloration while remaining visually healthy and compact. Visual differences among fertilization treatments may be observed in Figure 5.2.

Overall, these results support the first and third hypotheses proposed for this study. Plants grown with concentrations of 5 mg·L⁻¹ P later restricted to 0 mg·L⁻¹ P were simultaneously more compact and had enhanced red coloration than control plants grown with 20 mg·L⁻¹ P. Additionally, plants were able to attain a commercially acceptable size by being grown with adequate P concentrations during the first half of production, prior to having P restricted to 0 mg·L⁻¹ P. Plants with P restricted later in production were significantly larger than those grown without P for the entirety of this study. Lastly, *alternanthera* exhibited increased betacyanins in response to low P concentrations. This suggests that low P concentrations enhance the development of these pigments and the overall coloration of the plant.

Experiment 2

Significant differences were observed among the P concentrations for all growth and color parameters measured in this study. Differences in growth may be visually observed in

Figure 5.3. Low P concentrations resulted in shorter plants with significantly redder foliar zones. Continuous P fertilization with concentrations of 0 and 2.5 mg·L⁻¹ P inhibited flowering within the time frame of this study. Plants grown with 2.5 mg·L⁻¹ P developed flower stalks, though they were shorter than any other P concentration. Plants grown with 0 and 2.5 mg·L⁻¹ P were also significantly shorter in height and smaller in diameter than plants grown with any other P fertilization regiment (Table 5.4). Additionally, plants grown with 0 mg·L⁻¹ P developed severe necrosis of the lower leaves. The overall stunting, delay of flowering, and adverse P deficiency symptoms that occurred with 0 and 2.5 mg·L⁻¹ P would render these plants unmarketable, thus negating any benefits from enhanced red coloration.

According to a study by Behe et al. (1999), consumer preferences for geranium indicate that flowers were most important to consumers, followed by leaf color patterns. This demonstrates that a delay in flowering due to nutrient stress is not acceptable, but increased zonal coloration is still considered an important aspect in purchasing decisions. Additionally, consumers prefer leaves with dark zones or that are entirely green, over plants with white margins. Limiting P resulted in darker leaves with more pronounced zonal bands, which are both traits preferred by consumers.

Plants grown with 5 mg·L⁻¹ P for eight weeks and later restricted to 2.5 mg·L⁻¹ P had foliar zones with a hue similar to plants grown with continuous concentrations of 0 or 2.5 mg·L⁻¹ P (Table 5.5). Plants from these three P fertilization regiments had the lowest hues and were closest to 0° (pure red). Plants grown with any higher P concentrations had significantly higher hues, and thus, were not as close to red. The highest average chroma were observed in plants grown with continuous 0 – 5 mg·L⁻¹ P, and initial concentrations of 5 mg·L⁻¹ P (Table 5.5). These high chroma values demonstrate that the foliar zones of these plants were highly saturated

in color. Continuous P concentrations of 0 or 2.5 mg·L⁻¹, and initial concentrations of 5 mg·L⁻¹ P resulted in leaves with the lowest L* values, indicating that they were darker than leaves from other fertilizer regiments (Table 5.5). Similarly, b* values for these three regiments were also the lowest, indicating that leaves were closer to blue than those grown with other regiments (Table 5.5). Conversely, a* values were highest for these three P regiments, again demonstrating that they were closest to red (Table 5.5). For each of these color measurements, plants grown with 20 mg·L⁻¹ P were lighter, closer to green and yellow in pigment, and less saturated than other P fertilization regiments (Table 5.5). Visual differences in leaf coloration may be observed in Figure 5.4.

In addition to being more red than plants grown with higher P concentrations, plants grown with initial concentrations of 5 mg·L⁻¹ P were significantly more compact in terms of both height and diameter than plants grown with either fertilization regiment using 20 mg·L⁻¹ P, or continuous fertilization with 10 mg·L⁻¹ P (Table 5.4). This growth control provided by initial concentrations of 5 mg·L⁻¹ P led to the development of healthy and compact plants that would be considered high quality from a production standpoint. This fertilization regiment of 5 mg·L⁻¹ P restricted to 2.5 mg·L⁻¹ P developed a similar number of branches to plants grown with all higher P concentrations. These plants also flowered similarly to plants receiving the highest continuous P concentration. This is especially significant, as the flower is still the most important aspect of geranium consumer preferences (Behe et al., 1999). All of these parameters indicate that plants grown with initial concentrations of 5 mg·L⁻¹ P provide the optimal level of growth control and zone color enhancement for *P. x hortorum* 'Bullseye Red'.

Foliar P concentration varied greatly based on P fertilizer regiment and concentration (Table 5.6). Plants grown with an initial concentration of 5 mg·L⁻¹ P had some of the lowest

foliar P values, averaging 0.15%. This foliar P concentration is approximately half of what is considered sufficient for most crops (Marschner, 1995). Foliar tissue analysis of healthy seed grown geranium provided by Dole and Wilkens (2005) list recommended foliar P concentrations between 0.4 – 0.7%. These ranges all indicate that P was deficient in plants grown with initial concentrations of 5 mg·L⁻¹ P; however, a study by Krug et al. (2010) suggested optimal foliar P concentrations for dark leafed geranium range from 0.19 – 0.43%. This range is closer to what was found in this study, though still somewhat higher. Plants grown in this study with optimal P fertilization for coloration and size control did not display any negative aspects associated with P deficiency. Given that this study attempted to utilize P restriction to enhance coloration, the fact that tissue values were lower than the optimal range should be expected.

Plants grown with continuous 20 mg·L⁻¹ P had the highest foliar P concentration, averaging 0.47%, which was within the sufficiency range. Plants grown without P had the lowest foliar P concentration, averaging only 0.05% (Table 5.6). Foliar N concentration followed a similar trend, where plants grown with the highest fertilizer concentrations of P accumulated the highest levels of N. Plants grown without P had significantly lower foliar N concentrations compared to all other fertilization regiments (Table 5.6). Foliar K concentration was lowest for plants grown with restricted P regiments, which were significantly lower than each respective continuous P regiment (Table 5.6). Unlike what was observed with alternanthera, there were no significant difference in foliar Zn or Fe concentration among plants grown with varying P fertilizer concentrations or fertilization regiments (data not shown).

Results from this species supported the first and second hypotheses, as lower P fertilization led to the development of a redder coloration, and plants initially grown with 5 mg·L⁻¹ P later restricted to 2.5 mg·L⁻¹ P did not have delayed flowering compared to control

plants grown with $20 \text{ mg}\cdot\text{L}^{-1} \text{ P}$. This optimal fertilization regiment is in agreement with recommendations for growth control provided by McMahon (2011), stating that $5 - 10 \text{ mg}\cdot\text{L}^{-1} \text{ P}$ may be used to keep plants compact. Nevertheless, fertilization recommendations specifically for geranium production provided by McMahon (2011) suggested using much higher P concentrations. A fertilizer program with application at each irrigation alternating between $20\text{N}-4.4\text{P}-16.6\text{K}$ and $15\text{N}-0\text{P}-12.5\text{K}$, mixed at $250 - 300 \text{ mg}\cdot\text{L}^{-1} \text{ N}$ was recommended, which averages to $27.5 - 33 \text{ mg}\cdot\text{L}^{-1} \text{ P}$, which is much higher than the optimal concentrations determined in this study.

This initial fertilization regiment of $5 \text{ mg}\cdot\text{L}^{-1} \text{ P}$ later restricted to $2.5 \text{ mg}\cdot\text{L}^{-1} \text{ P}$ also bridges the gap between normal, healthy plants, and severely deficient plants observed by other studies. For instance, Chen et al. (2013) found that growth and anthocyanin concentration of Chinese kale were only significantly different between concentrations of 0 and $7.5 \text{ mg}\cdot\text{L}^{-1} \text{ P}$, and not between concentrations of 7.5 and $30 \text{ mg}\cdot\text{L}^{-1} \text{ P}$. This indicated that optimal P concentrations for growth control and foliar coloration enhancement was within this comparatively narrow range of 0 and $7.5 \text{ mg}\cdot\text{L}^{-1} \text{ P}$. This finding was supported by the results of this study, indicating that there is a very narrow range at which P fertilization provided sufficient P to prevent deficiency, but not enough to attain maximum growth parameters. For both species investigated in this study, these optimal concentrations fell within this range of 0 and $7.5 \text{ mg}\cdot\text{L}^{-1} \text{ P}$ observed by Chen et al. (2013).

Additional Benefits

There are a number of other added benefits growers may encounter by utilizing the low P recommendations discussed here. In addition to growing more compact plants with enhanced

coloration, growers using low P fertilization have the potential to improve their marketing strategies. Consumer preferences for sustainable growing practices may be able to aid in marketing (Campbell et al., 2015), and using low P fertilization enables growers to follow better management practices (BMPs). Excessive applications of agricultural nutrients including P have resulted in numerous water quality issues in recent years (Boesch et al., 2001). Many municipalities and agencies are also becoming more aware of issues associated with excessive nutrient application and runoff into water sources. For instance, greenhouse and nursery growers in the Chesapeake Bay watershed have been encouraged to use BMPs to reduce unnecessary runoff and improve the bay water quality (Boesch et al., 2001; Majsztrik and Lea-Cox, 2013). In the Florida Everglades, the concentration of P can be up to 60 times the maximum level determined to be safe for the unique organisms that live there (Liu et al., 2015). With the various environmental issues associated with excessive P application, growers will need to lower their fertilization regimens in order to reduce runoff.

Conclusions

Low P fertilization can successfully be used to enhance the foliar coloration found in *A. brasiliana* ‘Purple Prince’ and *P. x hortorum* ‘Bullseye Red’. For both species, providing a low initial P concentration of $5 \text{ mg}\cdot\text{L}^{-1}$ that was later restricted provided optimal color enhancement and growth control. ‘Purple Prince’ was restricted to $0 \text{ mg}\cdot\text{L}^{-1}$ P, while ‘Bullseye Red’ was restricted to $2.5 \text{ mg}\cdot\text{L}^{-1}$. Although ‘Purple Prince’ plants grown without P were excessively stunted, they did continue to grow over time and did not develop symptoms of lower leaf necrosis as was observed with ‘Bullseye Red’ plants grown without P. This may indicate that ‘Bullseye Red’ requires greater concentrations of P than ‘Purple Prince’; however, this

difference in response may have been due to the different propagule types. Due to the fact that 'Purple Prince' was propagated from vegetative cuttings, they may have had greater stores of P compared to the comparatively small seeds of 'Bullseye Red'. This demonstrates that there may be significant differences in plant response to low P by species and propagation method.

Regardless of species, restricting P later in production resulted in plants with redder, more saturated coloration compared to plants grown with continuous P fertilization, even at a low P concentration. By providing sufficient P for the first half to two-thirds of the production cycle, plants are able to adequately develop and become established, prior to being subjected to deficient levels of P. This enables plants to attain sufficient size for marketing. Plants grown with these low P concentrations gain the added benefit of growth control, as plants grown with higher P concentrations were significantly larger.

Refinement of this fertilization regiment should be considered when growing other crops, as unintended P deficiency symptoms could render a crop unmarketable. Although 'Purple Prince' plants were restricted to $0 \text{ mg}\cdot\text{L}^{-1}$ P, it may be better if P is not completely withheld. Restricting to $2.5 \text{ mg}\cdot\text{L}^{-1}$ P appears to provide enough P to prevent the negative aspects of P deficiency while still enhancing coloration. Growers of crops with red foliage can utilize P concentrations of $5 \text{ mg}\cdot\text{L}^{-1}$ restricted to $2.5 \text{ mg}\cdot\text{L}^{-1}$ P in the latter half of production to enhance coloration and keep plants compact.

Acknowledgements

We are grateful for the funding support provided by Fred C. Gloeckner Foundation, American Floral Endowment Altman Family Scholarship, and The Garden Club of America. We would

also like to express our gratitude to Sun Gro Horticulture for providing the peat moss used in these studies.

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Table 5.1. Means and minimum significant differences in growth parameters of *Alternanthera brasiliana* ‘Purple Prince’ grown with continuous or restricted concentrations of phosphorus.

Fertilization Regime ¹	Phosphorus Concentration (mg·L ⁻¹) ²	Growth Parameter					
		Height (cm)	Diameter (cm)	Primary Branch Number	Secondary Branch Number	Dry Mass (g)	Substrate pH
Continuous	0	20.7 e ³	12.0 f	9.5 d	0.0 f	1.57 f	6.56 ab
	2.5	29.4 cd	29.0 d	19.6 c	18.1 e	6.17 de	6.53 ab
	5	31.2 abcd	42.6 bc	24.4 b	66.4 c	11.84 c	6.61 a
	10	32.9 abc	46.9 ab	30.6 a	102.5 b	14.65 b	6.61 a
	20	33.4 ab	48.8 a	31.9 a	135.1 a	17.30 a	6.50 b
Restricted	2.5	28.0 d	24.3 e	16.0 c	1.9 f	4.95 e	6.53 ab
	5	29.6 bcd	30.9 d	18.9 c	9.5 ef	7.79 d	6.46 b
	10	32.8 abc	38.9 c	24.6 b	49.4 d	10.84 c	6.50 b
	20	33.9 a	46.6 ab	29.8 a	104.0 b	16.54 a	6.51 ab
	MSD ⁴	3.76	4.58	4.38	14.46	1.64	0.11

¹ Restricted phosphorus plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for four weeks, that was then restricted to 0 mg·L⁻¹ P for four weeks.

² Phosphorus concentrations remained the same for plants in the continuous fertilization regime, and were used initially for plants grown in the restricted fertilization regime.

³ Lower case letters signify minimum significant differences among all phosphorus concentrations for each growth parameter. Means with different letters are significantly different at $P \leq 0.05$.

⁴ Minimum significant differences (MSD) are listed for each growth parameter.

Table 5.2. Means and minimum significant differences in color parameters of *Alternanthera brasiliana* ‘Purple Prince’ grown with continuous or restricted concentrations of phosphorus.

Fertilization Regime ¹	Phosphorus Concentration (mg·L ⁻¹) ²	Color Parameter ³					
		Lightness (L*)	Green to Red Range (a*)	Blue to Yellow Range (b*)	Chroma (C*)	Hue Angle (h°)	SPAD
Continuous	0	34.13 b ⁴	59.19 bc	23.43 c	63.66 c	21.60 c	38.5 cd
	2.5	34.12 b	59.15 bc	23.82 bc	63.77 c	21.93 bc	44.8 ab
	5	34.14 b	59.54 b	23.86 bc	64.14 bc	21.84 bc	47.5 a
	10	34.41 b	59.00 bc	24.71 ab	63.96 bc	22.73 ab	46.7 a
	20	34.98 a	58.52 c	25.17 a	63.70 c	23.28 a	45.4 a
Restricted	2.5	34.33 b	59.94 ab	23.75 bc	64.47 bc	21.61 c	36.6 cd
	5	34.45 b	60.88 a	23.98 bc	65.45 a	21.50 c	34.9 d
	10	34.41 b	59.97 ab	24.43 abc	64.75 ab	22.16 bc	40.2 bc
	20	34.46 b	59.57 b	24.45 abc	64.39 bc	22.31 abc	46.7 a
	MSD ⁵	0.43	1.02	1.03	0.85	1.04	4.79

¹ Restricted phosphorus plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for four weeks, that was then restricted to 0 mg·L⁻¹ P for four weeks.

² Phosphorus concentrations either remained the same for plants in the continuous fertilization regime, or were used initially for plants grown in the restricted fertilization regime.

³ L* is lightness ranging 0 (dark) to 100 (light), a* is the range from green (negative 100) to red (positive 100), b* is the range from blue (negative 100) to yellow (positive 100), C* is chroma, and h° is hue angle (Ranging 0 – 360°, 0° is red).

⁴ Lower case letters signify minimum significant differences among all phosphorus concentrations for each color parameter. Means with different letters are significantly different at $P \leq 0.05$.

⁵ Minimum significant differences (MSD) are listed for each color parameter.

Table 5.3. Means and minimum significant differences in tissue nutrient concentrations of *Alternanthera brasiliana* ‘Purple Prince’ grown with continuous or restricted fertilizer concentrations of phosphorus.

Fertilization Regime ¹	Phosphorus Concentration (mg·L ⁻¹) ²	Plant Nutrient				
		N (%)	P (%)	K (%)	Zn (mg·L ⁻¹)	Fe (mg·L ⁻¹)
Continuous	0	3.02 h ³	0.028 f	4.58 bc	108.2 a	44.6 bc
	2.5	3.68 de	0.059 e	5.39 a	47.8 cd	40.2 c
	5	4.06 bc	0.111 c	5.39 a	44.8 d	54.1 b
	10	4.33 ab	0.233 b	5.45 a	42.4 d	67.8 a
	20	4.56 a	0.401 a	5.14 a	46.9 cd	70.7 a
Restricted	2.5	3.14 gh	0.029 f	5.07 ab	76.2 b	38.3 c
	5	3.36 fg	0.035 f	4.43 c	62.8 bc	37.9 c
	10	3.51 ef	0.048 e	4.39 c	55.4 cd	43.0 bc
	20	3.80 cd	0.082 d	4.54 bc	56.0 cd	47.2 a
	MSD ⁴	0.27	0.012	0.56	17.79	12.54

¹ Restricted phosphorus plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for four weeks, that was then restricted to 0 mg·L⁻¹ P for four weeks.

² Phosphorus concentrations either remained the same for plants in the continuous fertilization regime, or were used initially for plants grown in the restricted fertilization regime.

³ Lower case letters signify minimum significant differences among all phosphorus concentrations for each individual nutrient. Means with different letters are significantly different at $P \leq 0.05$.

⁴ Minimum significant differences (MSD) are listed for each nutrient.

Table 5.4. Means and minimum significant differences in growth parameters of *Pelargonium x hortorum* ‘Bullseye Red’ grown with continuous or restricted concentrations of phosphorus.

Fertilization Regiment ¹	Phosphorus Concentration (mg·L ⁻¹) ²	Growth Parameter				
		Height (cm)	Diameter (cm)	Branch Number	Dry Mass (g)	Substrate pH
Continuous	0	2.6 e ³	6.1 e	0.0 c	0.28 f	6.31 d
	2.5	15.1 d	17.5 d	4.3 b	3.64 e	6.46 bc
	5	26.5 abc	24.1 bc	10.4 a	8.46 cd	6.69 a
	10	27.3 ab	25.5 ab	10.9 a	10.39 ab	6.55 b
	20	29.5 a	27.4 a	10.5 a	11.71 a	6.38 cd
Restricted	5	25.1 bc	22.8 c	10.0 a	7.86 d	6.44 c
	10	24.3 c	24.3 bc	10.0 a	9.32 bcd	6.41 cd
	20	27.4 ab	25.2 ab	10.6 a	9.67 bc	6.38 cd
	MSD ⁴	2.97	2.16	1.91	1.47	0.10

¹ Restricted phosphorus plants were grown with initial concentrations of 5, 10, or 20 mg·L⁻¹ P for eight weeks, that was then restricted to 2.5 mg·L⁻¹ P for four weeks.

² Phosphorus concentrations remained the same for plants in the continuous fertilization regiment, and were used initially for plants grown in the restricted fertilization regiment.

³ Lower case letters signify minimum significant differences among all phosphorus concentrations for each growth parameter. Means with different letters are significantly different at $P \leq 0.05$.

⁴ Minimum significant differences (MSD) are listed for each growth parameter.

Table 5.5. Means and minimum significant differences in color parameters of *Pelargonium x hortorum* ‘Bullseye Red’ grown with continuous or restricted concentrations of phosphorus.

Fertilization Regime ¹	Phosphorus Concentration (mg·L ⁻¹) ²	Color Parameter ³				
		Lightness (L*)	Green to Red Range (a*)	Blue to Yellow Range (b*)	Chroma (C*)	Hue Angle (h°)
Continuous	0	26.34 e ⁴	79.80 a	10.00 e	80.43 a	7.14 d
	2.5	26.31 e	79.80 a	10.10 e	80.44 a	7.22 d
	5	27.31 cd	78.45 bc	11.32 cd	79.26 ab	8.21 bc
	10	28.16 b	76.89 d	12.18 ab	77.85 c	9.00 a
	20	28.95 a	75.34 e	12.69 a	76.30 d	9.58 a
Restricted	5	26.85 de	78.97 ab	10.61 de	79.68 ab	7.65 cd
	10	27.59 bc	77.81 bcd	11.48 bc	78.65 bc	8.39 b
	20	27.51 c	77.69 cd	11.27 cd	78.50 bc	8.26 b
	MSD ⁵	0.63	1.26	0.78	1.23	0.59

¹ Restricted phosphorus plants were grown with initial concentrations of 5, 10, or 20 mg·L⁻¹ P for eight weeks, that was then restricted to 2.5 mg·L⁻¹ P for four weeks.

² Phosphorus concentrations remained the same for plants in the continuous fertilization regime, and were used initially for plants grown in the restricted fertilization regime.

³ L* is lightness ranging 0 (dark) to 100 (light), a* is the range from green (negative 100) to red (positive 100), b* is the range from blue (negative 100) to yellow (positive 100), C* is chroma, and h° is hue angle (Ranging 0 – 360°, 0° is red).

⁴ Lower case letters signify minimum significant differences among all phosphorus concentrations for each color parameter. Means with different letters are significantly different at $P \leq 0.05$.

⁵ Minimum significant differences (MSD) are listed for each color parameter.

Table 5.6. Means and minimum significant differences in tissue nutrient concentrations of *Pelargonium x hortorum* ‘Bullseye Red’ grown with continuous or restricted fertilizer concentrations of phosphorus.

Fertilization Regiment ¹	Phosphorus Concentration (mg·L ⁻¹) ²	Plant Nutrient		
		N (%)	P (%)	K (%)
Continuous	0	1.88 f ³	0.05 f	2.83 bcd
	2.5	2.85 e	0.12 e	3.26 a
	5	3.60 c	0.23 d	3.27 a
	10	4.00 ab	0.35 b	3.23 ab
	20	4.08 a	0.47 a	3.08 abc
Restricted	5	3.11 d	0.15 e	2.65 d
	10	3.78 bc	0.21 d	2.68 cd
	20	4.01 a	0.31 c	2.54 d
	MSD ⁴	0.22	0.028	0.40

¹ Restricted phosphorus plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for four weeks, that was then restricted to 0 mg·L⁻¹ P for four weeks.

² Phosphorus concentrations either remained the same for plants in the continuous fertilization regiment, or were used initially for plants grown in the restricted fertilization regiment.

³ Lower case letters signify minimum significant differences among all phosphorus concentrations for each individual nutrient. Means with different letters are significantly different at $P \leq 0.05$.

⁴ Minimum significant differences (MSD) are listed for each nutrient.

Alternanthera brasiliana 'Purple Prince'

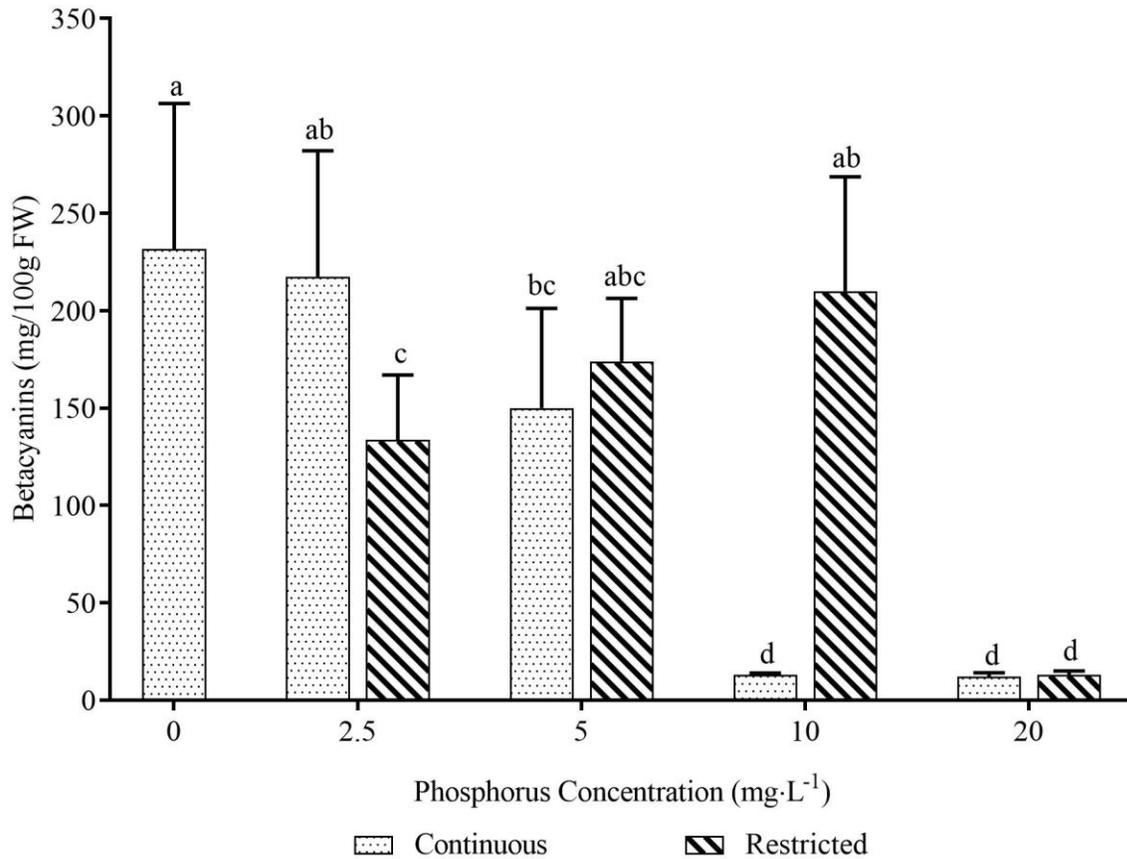


Figure 5.1. Mean betacyanins concentration for *Alternanthera brasiliana* 'Purple Prince' reported in mg/100 g fresh weight. Phosphorus concentrations either remained the same for plants in the continuous fertilization regiment, or were used initially for plants grown in the restricted fertilization regiment. Restricted phosphorus plants were grown with initial concentrations of 2.5, 5, 10, or 20 mg·L⁻¹ P for four weeks, that was then restricted to 0 mg·L⁻¹ P for four weeks. Lower case letters signify minimum significant differences among all phosphorus concentrations. Means with different letters are significantly different at $P \leq 0.05$.

Alternanthera brasiliana 'Purple Prince'

Continuous Phosphorus Fertilization



Restricted Phosphorus Fertilization



0 2.5 5 10 20

Phosphorus Concentration ($\text{mg}\cdot\text{L}^{-1}$)

Figure 5.2. *Alternanthera brasiliana* 'Purple Prince' plants grown with P concentrations of 0, 2.5, 5, 10, and 20 $\text{mg}\cdot\text{L}^{-1}$ continuously (top), or 2.5, 5, 10, and 20 $\text{mg}\cdot\text{L}^{-1}$ for four weeks, restricted to 0 $\text{mg}\cdot\text{L}^{-1}$ P for an additional four weeks (bottom). The 0 $\text{mg}\cdot\text{L}^{-1}$ plant was the same in both pictures. Visual differences may be seen among plants grown with different P concentrations, and between plants grown with continuous or restricted fertilization regimens. Note how plants grown with restricted P regimens have fewer green leaves at the growing tips.

Pelargonium x hortorum 'Bullseye Red'

Continuous Phosphorus Fertilization Regime



Restricted Phosphorus Fertilization Regime



0 2.5 5 10 20
Phosphorus Concentration ($\text{mg}\cdot\text{L}^{-1}$)

Figure 5.3. *Pelargonium x hortorum* 'Bullseye Red' plants grown with P concentrations of 0, 2.5, 5, 10, and 20 $\text{mg}\cdot\text{L}^{-1}$ continuously (top), or 5, 10, and 20 $\text{mg}\cdot\text{L}^{-1}$ for eight weeks, restricted to 2.5 $\text{mg}\cdot\text{L}^{-1}$ P for an additional four weeks (bottom). The 0 and 2.5 $\text{mg}\cdot\text{L}^{-1}$ plants were the same in both pictures. Visual differences may be seen among plants grown with different P concentrations, and between plants grown with continuous or restricted fertilization regimens.

Pelargonium x hortorum 'Bullseye Red'

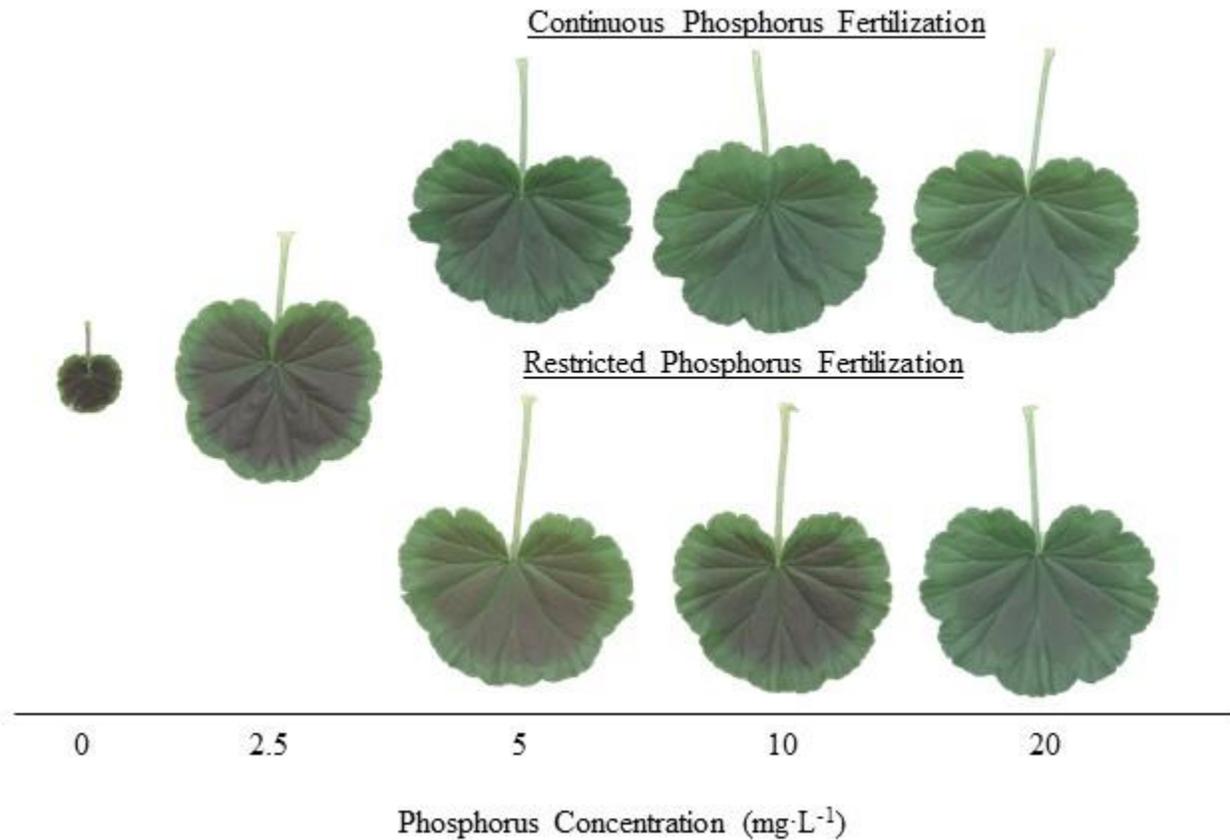


Figure 5.4. *Pelargonium x hortorum* 'Bullseye Red' plants grown with P concentrations of 0, 2.5, 5, 10, and 20 mg·L⁻¹ continuously (top), or 5, 10, and 20 mg·L⁻¹ for eight weeks, restricted to 2.5 mg·L⁻¹ P for an additional four weeks (bottom). The 0 and 2.5 mg·L⁻¹ plants were the same in continuous and restricted fertilization treatments. Visual differences may be seen among plants grown with different P concentrations, and between plants grown with continuous or restricted fertilization regimens.

Chapter 6

Conclusions: Phosphorus Nutrition of Floriculture Species

Introduction

Several aspects of phosphorus (P, referring to phosphorus supplied by phosphate) nutrition of floriculture species were investigated over the course of these studies. Low P fertilization was found to have a number of beneficial effects on the overall growth and development of greenhouse grown ornamentals. Past research investigated the growth controlling effects of low P fertilization (Hansen and Nielsen, 2000, 2001), but did not provide recommendations applicable to commercial production practices. In addition, no work had been done to directly compare the efficacy of low P fertilization to chemical plant growth retardants (PGRs), which are the most commonly used method of growth control in floriculture production (Whipker, 2017). Determining plant growth response to a range of P concentrations using a number of different species was done to fill this knowledge gap.

When using low P fertilization, growers have a greater potential to encounter advanced symptoms of P deficiency. A new type of P deficiency symptom was recently described by Whipker (2014), in which growers restricted P later in the production cycle. These atypical reproductive stage P deficiency symptoms occurred on the upper foliage of reproductive mums, and led to significant economic losses. A study was conducted to better describe these symptoms and determine the conditions under which they occur.

Symptoms of a red coloration are often associated with P deficiency, and are due to increased production of anthocyanins (Epstein and Bloom, 2005; Sarker and Karmoker, 2011). Betacyanins are a different red plant pigment which occur in some species, but have not been documented to increase in response to P deficiency. A study was done to investigate whether plants with naturally red foliage exhibit enhanced coloration when exposed to low P fertilization or by restricting P later in production.

Discussion

Low P fertilization was successfully used to control growth in several floriculture species. Growth plateaus were developed to determine the P concentration plants require to maximize growth. Table 6.1 lists the optimal P concentrations determined to achieve maximum growth for six species. No significant increases in growth were achieved with P concentrations greater than those provided in the table. These optimal concentrations ranged from 5.5 – 13.1 mg·L⁻¹ P, indicating that current fertilizer recommendations provide more P than required by most species. The highest P requirement was observed in ornamental peppers (*Capsicum annuum* L.), while the lowest was observed in alternanthera [*Alternanthera brasiliana* (L.) Kuntze]. The difference in P requirements was likely due to reproduction, as peppers developed a high number of flowers and fruit, while alternanthera remained vegetative. It has been found that N and P translocated from internal sources can account for 90% of these elements in developing flowers and fruit, indicating that P requirements to support these reproductive structures are high (Epstein and Bloom, 2005; Marschner, 1995).

A number of commercial fertilizers can be used by growers to supply optimal P concentrations in the range of 5 – 15 mg·L⁻¹. For instance, 13N–0.9P–10.8K Cal Mag mixed at 75 – 225 mg·L⁻¹ N, or 15N–2.2P–12.5K Cal Mag mixed at 50 – 100 mg·L⁻¹ N could be used to supply 5 – 15 mg·L⁻¹ P. Additionally, growers may alternate between two commercial fertilizers to apply an average within this optimal range of P. For instance, a high P fertilizer formulation such as 20N–4.4P–16.6K mixed at 100 mg·L⁻¹ N could be used in conjunction with a low P fertilizer such as 13N–0.9P–10.8K Cal Mag.

Table 6.1. Summary of phosphorus concentrations to achieve maximum growth for several floriculture species, based on growth index plateaus.

Plant Species 'Cultivar'	Phosphorus Concentration (mg·L ⁻¹)
<i>Alternanthera brasiliana</i>	
'Brazilian Red'.....	5.50
<i>Angelonia angustifolia</i>	
'Sungelonia Blue'.....	8.78
'Sungelonia White'.....	7.27
<i>Capsicum annuum</i>	
'Tango Red'.....	13.1
<i>Catharanthus roseus</i>	
'Cora Burgundy'.....	6.74
'Pacifica XP Blush'.....	6.43
<i>Impatiens hawkeri</i>	
'Pure Beauty Red on Pink'.....	12.4
'Tamarinda Dark Red'.....	9.64
<i>Petunia atkinsiana</i>	
'Potunia Neon'.....	9.08
'Surprise Sky Blue'.....	8.72

Low P fertilization was also used to compare growth control with conventional PGRs. In this study, P concentrations of 0 – 20 mg·L⁻¹ were compared with an application of paclobutrazol for angelonia (*Angelonia angustifolia* Benth.) and New Guinea impatiens (*Impatiens hawkeri* W. Bull). Growth plateaus were used to determine optimal P concentrations which provided growth control similar to paclobutrazol in conjunction with a normal P concentration of 20 mg·L⁻¹ P. Optimal concentrations for height control in New Guinea impatiens were approximately 4 – 5 mg·L⁻¹ P, while angelonia required concentrations as low as 2.8 mg·L⁻¹ P. Lowering P concentrations below these values resulted in significantly smaller plants, though they were

severely stunted and also exhibited unacceptable symptoms of P deficiency. These symptoms included reddening and necrosis of the lower foliage, and floral inhibition in New Guinea impatiens.

In chapter 4, a reproductive stage P deficiency was successfully induced on ornamental peppers and mums (*Chrysanthemum morifolium* Ramat.). Both species developed symptoms on the upper foliage of plants that were in flower or developing fruit. These symptoms occurred when plants were grown with a low but adequate P concentration supplied in the fertilizer for the first half of production, that was later restricted to no P during reproductive maturation. Initial concentrations of 10 – 20 mg·L⁻¹ P led to the development of upper leaf symptoms of chlorosis, marginal necrosis, necrotic spotting, and olive green spotting on both species. Additionally, peppers experienced significant leaf abscission that resulted in plants losing the majority of their foliage. One mum cultivar, ‘Crystal Misty Purple’, also developed symptoms of severe purpling that were not observed on peppers or the other mum cultivars (Figure 6.1).



Figure 6.1. Symptoms of reddening, chlorosis, necrotic spotting, and olive green spotting on an upper leaf of *Chrysanthemum morifolium* ‘Crystal Misty Purple’.

To avoid these symptoms of reproductive stage P deficiency, growers should use caution not to lower their P fertility too greatly. Current nutrition recommendations for species including ornamental peppers and mums suggest lowering fertility upon flower or fruit set (Dole and Wilkins, 2005). This is recommended because vegetative growth is nearly done during reproductive development and maturation (Dole and Wilkins, 2005). Lowering fertilizer concentrations near the end of production is unlikely to result in reproductive stage P deficiency when using traditional high P commercial fertilizers; however, growers utilizing fertilizers that are low or devoid of P are more likely to encounter these symptoms. From what was observed in this study, it is recommended that growers always maintain P fertility $\geq 5 - 10 \text{ mg}\cdot\text{L}^{-1}$ P to avoid reproductive stage P deficiency symptoms.

Low P fertilization was also successful in enhancing the foliar coloration found in alternanthera ‘Purple Prince’ and geranium (*Pelargonium x hortorum* L. H. Bailey) ‘Bullseye Red’ (Figure 6.2). For both species, providing a low initial P concentration of $5 \text{ mg}\cdot\text{L}^{-1}$ that was later restricted provided optimal color enhancement and growth control. ‘Purple Prince’ was restricted to $0 \text{ mg}\cdot\text{L}^{-1}$ P, while ‘Bullseye Red’ was restricted to $2.5 \text{ mg}\cdot\text{L}^{-1}$. Although ‘Purple Prince’ plants grown without P were excessively stunted, they continued to grow over time and did not develop symptoms of lower leaf necrosis as was observed with ‘Bullseye Red’ plants grown without P. This may indicate that ‘Bullseye Red’ requires greater concentrations of P than ‘Purple Prince’; however, this difference in response may have been due to the different propagule types. Due to the fact that ‘Purple Prince’ was propagated from vegetative cuttings, they may have had greater stores of P compared to the comparatively small seeds of ‘Bullseye Red’. This demonstrates that there are significant differences in plant response to low P by species and propagation method.

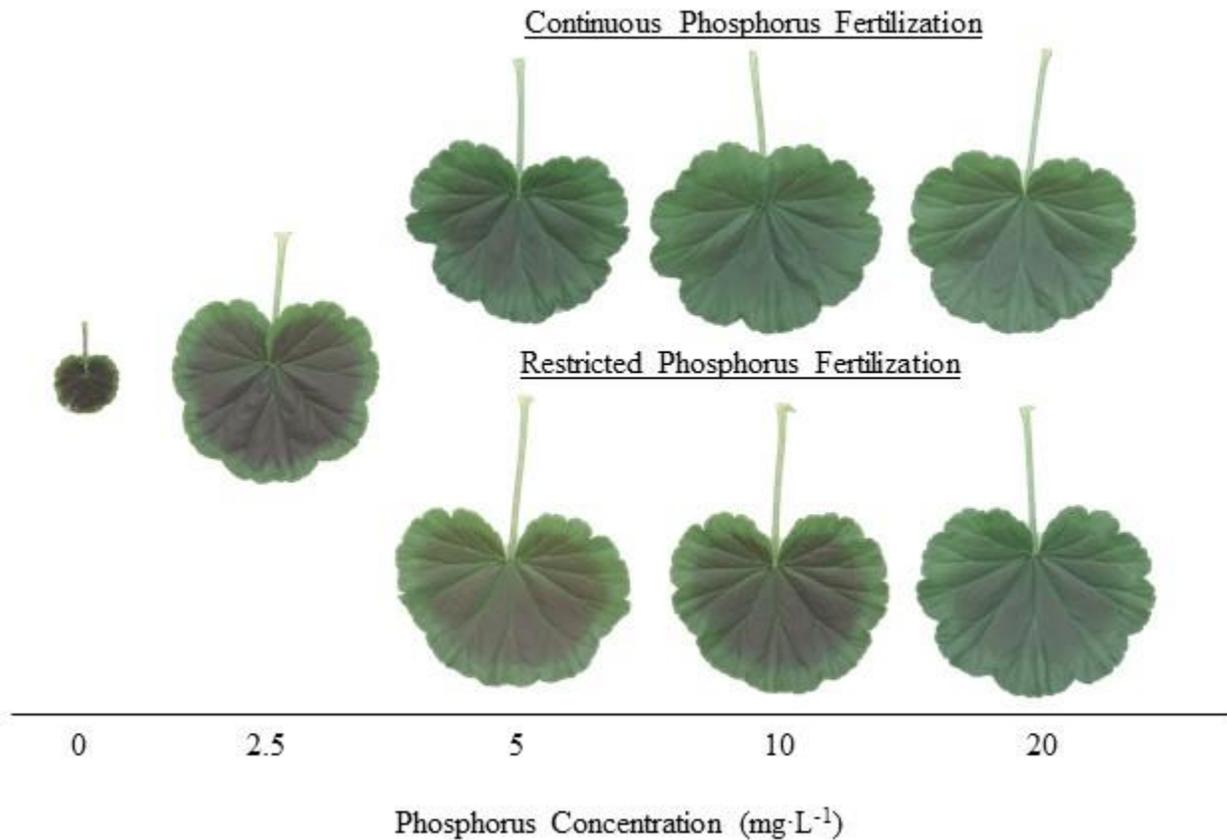


Figure 6.2. Enhanced coloration in the leaves of *Pelargonium x hortorum* grown with low and restricted P concentrations.

Regardless of species, restricting P later in production resulted in plants with redder, more saturated coloration compared to plants grown with continuous P fertilization, even at a low P concentration. By providing sufficient P for the first half to two-thirds of the production cycle, plants were able to adequately develop and become established, prior to being subjected to deficient levels of P. This enabled plants to attain sufficient size for marketing. Plants grown with these low P concentrations also had the added benefit of growth control, as plants grown with higher P concentrations were significantly larger. It was determined that growers of red leafed plant species can utilize P concentrations of 5 mg·L⁻¹ restricted to 2.5 mg·L⁻¹ P in the latter half of production to enhance coloration and keep plants compact.

Conclusion

Low P fertilization successfully enhanced several beneficial aspects of plant growth in a variety of different species. Growth control was achieved by using P concentrations of 5 – 15 mg·L⁻¹, and concentrations of 3 – 5 mg·L⁻¹ P provided growth control comparable to that of a conventional PGR. Low P fertilization also was successful in enhancing the foliage coloration of red leafed species. This color enhancement was achieved using initial P concentrations of 5 mg·L⁻¹ restricted to 2.5 mg·L⁻¹ P in the latter half of production. Although low P fertilization provided a number of benefits for plant growth, continuous P concentrations \leq 2.5 mg·L⁻¹ often resulted in severe stunting, delayed or inhibited flowering, and foliar P deficiency symptoms including chlorosis, necrosis, reddening, and olive green spotting. These symptoms were also observed on the upper foliage of peppers and mums grown with sufficient P during vegetative maturation, that was later restricted to no P during reproductive maturation. This demonstrated that P deficiency symptom development was highly dependent on the plant growth stage at the time P deficient conditions were induced. Growers can avoid these symptoms by providing a constant P concentration of at least 5 – 10 mg·L⁻¹ through the entire production cycle.

This research can be used to provide improved P nutrition recommendations to growers in order to produce healthy and attractive ornamentals.

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APPENDIX

Appendix. Fertilizer salts used to formulate each stock solution, with their respective molecular weight, the amount of each used per liter of stock solution, and the amount of stock solution used for each phosphorus fertilizer treatment. Microelements were mixed to create a single stock solution, and this formula is in the secondary table below.

Fertilizer Salt	Molecular Weight	Grams per Liter	Phosphorus Fertilizer Concentration (mg·L ⁻¹)							
			0	1.25	2.5	5	10	20	40	80
			Amount of Stock Solution Used (ml/100 L)							
Ca(NO ₃) ₂ ·4H ₂ O	236.15	354.24	213.9	213.9	213.9	213.9	213.9	213.9	213.9	213.9
KNO ₃	101.10	128.52	196.9	196.9	196.9	234.8	276.4	251.0	200.2	98.6
MgSO ₄ ·7H ₂ O	246.48	295.80	96.9	96.9	96.9	117.0	150.0	140.0	140.0	140.0
KH ₂ PO ₄	136.09	81.66	—	6.7	13.4	26.9	53.8	107.6	215.2	430.5
K ₂ SO ₄	174.26	100.00	116.2	112.7	110.0	60.2	—	—	—	—
Mg(NO ₃) ₂	256.41	256.41	89.4	89.4	89.4	65.3	38.8	55.0	87.3	151.9
FeDTPA	40.00	40.00	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
Microelement Mix	—	—	45.0	45.0	45.0	45.0	45.0	45.0	45.0	45.0

Microelement Mix		
Fertilizer Salt	Molecular Weight	Grams per Liter
MnCl ₂ ·4H ₂ O	197.91	7.92
ZnCl ₂ ·7H ₂ O	136.28	0.90
CuCl·2H ₂ O	170.48	1.13
H ₃ BO ₃	61.83	6.18
Na ₂ MoO ₄ ·2H ₂ O	241.9	0.05