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# Phosphorus requirement for biomass accumulation is higher compared to photosynthetic biochemistry for three ornamental shrubs

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ABSTRACT

Ornamental nursery producers grow a variety of plant taxa in soil-less substrates and rely on frequent water and nutrient applications to maximize plant growth and quality. This study was performed to understand the morpho-physiological basis of plant response to phosphorus (P) additions and to identify the optimum P concentration required for three common woody ornamental taxa: *Hydrangea quercifolia* 'Queen of Hearts', *Cornus obliqua* 'Powell Gardens' and *Physocarpus opulifolius* 'Seward'. In a greenhouse experiment, all plants were watered with a complete nutrient solution that varied in P concentration (0, 0.7, 1.3, 2.5, 3.7, 6.5 mg $L^{-1}$). Optimum P concentration for photosynthetic biochemistry was dependent on taxa and ranged between 2.5 and 3.7 mg $L^{-1}$. For total dry biomass, the optimum P concentration was approximately 4.0 mg $L^{-1}$ for all three taxa. Phosphorus concentration below 2.5 mg $L^{-1}$ reduced leaf size and resulted in greater partitioning of biomass and P to root growth. Analysis of responses of photosynthesis to intercellular carbon dioxide concentration (*A/Ci* curves) indicated a continuous increase in photosynthetic parameters to increasing P concentration. Rate of rubisco for carboxylation ($V_{cmax}$), RuBP regeneration rate (*J*), and the rate of triose phosphate use (*TPU*) limited photosynthesis in P-deficient plants for all three taxa. Light-harvesting efficiency (Fv'/Fm') for all three taxa was less sensitive to P addition than photosynthetic biochemistry or plant growth. The optimal P concentrations identified in this study are lower than common recommendations and less than the amounts provided by typical commercial fertilizers. Thus, for these three taxa, application of P above 4 mg $L^{-1}$ in combination with excess irrigation resulting in leachate could have negative environmental consequences without improving crop growth or physiology.

## 1. Introduction

Horticulture is a large and economically significant industry, both in the U.S. and around the world. In the U.S., the wholesale value of horticultural crops was worth $13.8 billion in 2014 (United States Department of Agriculture National Agricultural Statistics Service, 2016). Containerized nursery production is a major sector of horticulture and requires frequent, usually daily, irrigation and continuous additions of mineral nutrients when crops are actively growing. For container-grown ornamentals, growers seek to optimize irrigation by applying enough water to meet losses due to evapotranspiration while leaching out deleterious accumulated soluble salts. A commonly cited best management practice (BMP) is to irrigate to the level that results in 10–20 % leaching of applied water (leaching fraction) (Bilderback et al., 2013). However, even this recommendation may lead to over-irrigation (Pershey et al., 2015). Nonetheless, in practice, application of water often exceeds the BMP, and a large portion of irrigation water may be lost as agrichemical laden irrigation return flow (Danelon et al., 2010; Warsaw et al., 2009). Container nursery crops in the U.S. are typically grown in soil-less media composed primarily of softwood bark. Phosphorus leaching is higher in soil-less media (pine bark, sphagnum peat, vermiculite or sand) in comparison to mineral soil (Broschat, 1995) due to low P adsorption capacity of soil-less media and preferential flow through porous substrates (Fields et al., 2014; Owen et al., 2008). Thus, 30–60 % of applied P is commonly leached when using bark-based substrates (Newman, 2014). This leachate and resultant irrigation return flow can pollute surface and groundwater systems (United States Environmental Protection Agency, 2016, 2005).

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Water quality concerns associated with nursery and greenhouse irrigation return flow, such as eutrophication and algal blooms, are primarily related to nitrogen (N) and P present in runoff water (Conley et al., 2009; Fulcher et al., 2016; Paerl, 2009). In 2014, P runoff was the primary cause of harmful algal blooms (HABs) in Lake Erie that left more than half a million people without drinking water (Michalak et al., 2013; Watson et al., 2016). Lowering P fertilization is an option for nursery growers to protect water resources, but lowering P below the sufficiency threshold will reduce plant growth and quality, potentially resulting in decreased profits for producers. For container-grown ornamentals, 5–10 mg $L^{-1}$ P in the applied solution is often considered the target level for optimum plant growth (Kim and Li, 2016; Owen et al., 2008; Shreckhise et al., 2018, 2019b; Zhang et al., 2004) but many liquid and controlled-release fertilizers commonly used in the landscape nursery trade provide 15–50 mg $L^{-1}$ P when applied at labeled rates (Broschat, 1995; Soti et al., 2015). Phosphorus requirements of plants vary by taxa, and recent studies suggest the possibility of reducing P additions below 10 mg $L^{-1}$ without compromising plant growth and quality (Shreckhise et al., 2018, 2019b). For example, *Hydrangea paniculata* 'Limelight' and *Rhododendron* sp. 'Karen' grown in pine bark substrate achieved maximum shoot dry weight at 4.7 mg $L^{-1,}$ and 2.9 mg $L^{-1}$ P in applied solutions, respectively (Shreckhise et al., 2018). For *Lantana camara* 'New Gold' grown in a mixture of perlite and vermiculite, P concentration of $\leq$ 10 mg $L^{-1}$ in the applied solution was sufficient for optimum growth at the reproductive stage of the plant (Kim and Li, 2016). In contrast, *Impatiens hawkeri* 'Paradise Violet', and *Catharanthus roseus* 'Pacifica Red' grown in a soil-less substrate had maximum growth and shoot dry weight at 31 mg $L^{-1}$ and 23 mg $L^{-1}$ P application (Whitcher et al., 2005).

The impacts of P availability on growth reflect the integration of its effect on physiological processes, particularly photosynthetic biochemistry. Phosphorus is an essential plant nutrient that is present in plants in various membranes, nucleic acids, and energy compounds (Armstrong, 1999). The effects of P deficiency on growth may be due to both source and sink limitations of photosynthesis (Pessarakli, 2005). Phosphorus is required for numerous physiological processes, including light reactions and the Calvin-Benson cycle of photosynthesis (Poorter et al., 2010; Brooks, 1986). Phosphorus deficiency reduces photosynthesis by limiting RuBP regeneration (Fredeen et al., 1990), inhibiting ATP synthesis (Carstensen et al., 2018), inhibiting enzymes such as phosphoglycerate kinase and nicotinamide adenine dinucleotide phosphate dependent glyceraldehyde-3-phosphate dehydrogenase (NADP-GAPDH) required in the Calvin-Benson cycle (Rao and Terry, 1989), and reducing stomatal conductance (Martins et al., 2015). Phosphorus deficiency also reduces chlorophyll fluorescence (Nowak and Stroka, 2001) by lowering the efficiency of Photosystem II (PSII) and damages the photosynthetic apparatus by increasing the production of free radicals (Xu et al., 2007). Physiological impacts in response to P are predominantly determined by the severity of P deficiency and the duration of P starvation (Terry and Ulrich, 1973; Xu et al., 2007).

Photosynthesis in light-saturated conditions can be rubisco limited, RuBP limited, or triose phosphate use (*TPU*) limited, and a well-constructed carbon dioxide response (*A/Ci*) curve can be used to determine the extent and nature of these limitations (Farquhar et al., 1980; Sharkey, 2016). Improving our understanding of the interrelationships between P effects on photosynthetic biochemistry and plant productivity may provide additional insights into optimizing P fertilization to maximize growth while minimizing adverse environmental impacts. To date, holistic studies optimizing P fertilization for maximum physiological and morphological performance of plants while reducing effluent P are still lacking. Therefore, the goal of this study was to investigate the feasibility of reducing P fertilization without reducing crop growth or quality and estimating the amount of effluent P at various P application rates. Our specific objectives were to (1) determine the effect of P on photosynthesis (*A/Ci* curves and light-adapted fluorescence) and morphological responses (total dry weight, root-shoot ratio, leaf number, and leaf size) in three different ornamental plant taxa, (2) identify the type of photosynthetic limitation that may result from P deficiency, and (3) categorize P partitioning to plant growth and P in the leachate.

## 2. Materials and methods

### 2.1. Plant material and nursery culture

On May 6, 2016, *Hydrangea quercifolia* 'Queen of Hearts' (oakleaf hydrangea), *Cornus obliqua* 'Powell Gardens' Red Rover® (silky dogwood) and *Physocarpus opulifolius* 'Seward' Summer Wine® (ninebark) liners from 10-cm diameter plug cells were potted into #3 (11.36 L) containers using an unamended mixture of aged *Pinus resinosa*(red pine) bark (85 % by volume) and Sphagnum peat moss (15 % by volume) (Renewed Earth LLC, Otsego, MI, USA). Plants were grown outdoors under typical nursery practices for the region at the Michigan State University Horticulture Teaching and Research Center near East Lansing, MI, USA. During the growing season, all plants were irrigated daily with 19 mm of water provided by an overhead sprinkler irrigation system and top-dressed with controlled-release fertilizer (19:1.75:6.65; N:P:K) with micronutrients (5–6 months release rate, Harrell's LLC, Lakeland, FL, USA). On October 28, 2016, all taxa were relocated to an unheated hoop-house covered with 0.15 mm poly film to receive partial chilling. At this time, each container was carefully checked for residual fertilizer prills, which were removed if found. Plants remained in the unheated hoop house for 40 days, then they were moved to a walk-in cooler (6° C) for five weeks to complete their chilling requirement. A period of five weeks at 6° C was identified in a preliminary research trial as sufficient to meet the chilling requirement for all taxa in the study (data not shown).

### 2.2. Experimental set-up

For the current study, plants were brought into a temperature-controlled research greenhouse at Michigan State University on January 11, 2017 and observed until bud-break. The temperature in the greenhouse was set to 22 °C for 18 h (6:00 to 24:00) and 20 °C for the remaining 6 h. Supplemental lighting (approx. 500 μmol $m^{-2}$·$s_{-}^{1}$) was provided by high-pressure sodium lamps when the photosynthetic photon flux density was lower than 440 μmol $m^{-2}$·$s_{-}^{1}$ to provide a 16-h photoperiod.

### 2.3. Phosphorus treatments

Application of P treatments started on January 26, 2017, after the emergence of leaves on the study plants. Plants were hand-watered with a mineral nutrient solution with nominal concentrations of 0, 1, 2, 4, 6, or 8 mg $L^{-1}$ P derived from dibasic potassium phosphate ($K_2HPO_4$; Sigma-Aldrich, St. Louis, Missouri, USA), 100 mg $L^{-1}$ N {urea [$(NH_2)_2CO$], Alpha chemicals, USA}, 60 mg $L^{-1}$ potassium [K from potassium chloride (KCl), Sigma-Aldrich, St. Louis, Missouri, USA] and 80 mg $L^{-1}$ micronutrients that contained 4.8 mg $L^{-1}$ calcium (Ca), 2.4 mg $L^{-1}$ magnesium (Mg), 9.6 mg $L^{-1}$ sulfur (S), 0.08 mg $L^{-1}$ boron (B), 1 mg $L^{-1}$copper (Cu), 13 mg $L^{-1}$ iron (Fe), 2 mg $L^{-1}$ manganese (Mn), 0.04 mg $L^{-1}$ molybdenum (Mo), and 0.8 mg $L^{-1}$ zinc (Zn) (supplemental table, 1-Micromax® micronutrients, ICL Fertilizers, Dublin, Ohio, USA) using a 1000 mL beaker. The amount of K from $K_2HPO_4$ was included in calculating the amount of K in the nutrient solution. Mineral salts were precisely weighed and completely dissolved in irrigation water just before each application to supply and ensure availability of mineral nutrients at the desired concentrations. Plants of all three taxa were arranged in a completely randomized design with six replicates per each P rate. Plastic saucers were placed under each container and we collected and measured the amount of leachate from each plant after each fertigation event. The fertigation frequency and volume applied

**Table 1**
Nominal versus actual concentration of total phosphorus (P) in the nutrient solution. S.E represents standard error. Actual P represents total P determined post digestion of unfiltered sample via colorimetry using a flow injection analysis system.

| Total P concentration (mg/L) | |
|---|---|
| Nominal | Actual ± S.E. |
| 0 | 0.0 ± 0.01 |
| 1 | 0.7 ± 0.19 |
| 2 | 1.3 ± 0.39 |
| 4 | 2.5 ± 0.58 |
| 6 | 3.7 ± 0.60 |
| 8 | 6.5 ± 0.86 |

was periodically adjusted to account for changes in plant water use during the study, targeting a 15–20 % leaching fraction (leached volume/fertigation volume× 100). During early growth (February to March 2017), fertigation was less frequent (weekly or two times a week). Beginning April 2017, plants started growing vigorously, therefore, fertigation frequency was increased to once every other day to every day. Fertigation was carried out for almost six months, until July 10, 2017. Plants were fertigated a total of 45 times during the study period. Pour-through pH and electrical conductivity (EC) of the leachate were measured four times (March 22, May 6, June 6, and July 10, 2017) during the study (LeBude and Bilderback, 2009). Pour-through pH values were the same for all six treatments within taxa ($p = 0.24$) and did not change during the time of the study ($p = 0.1$). The average pH value (± standard error) of the mean for all three taxa was 6.49 ± 0.02. Unlike pH, average EC for all three taxa gradually increased over the course of the study. The average EC (dS/m) value (± standard error) of the mean of *P. opulifolius, H. quercifolia, and C. obliqua* increased ($p < 0.05$) from 0.45 ± 0.01, 0.41 ± 0.02 and 0.45 ± 0.01 at the start of study to 1.00 ± 0.02, 0.83 ± 0.03 and 0.69 ± 0.05 at the end of study, respectively.

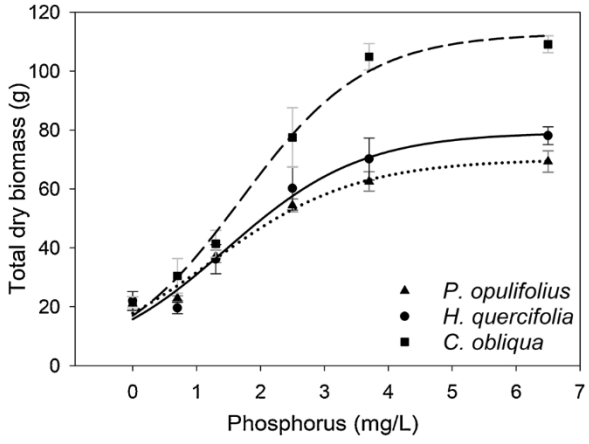


**Fig. 1.** Total dry biomass (TDB) of *Physocarpus opulifolius* 'Seward', *Hydrangea quercifolia* 'Queen of hearts', and *Cornus obliqua*'Powell Gardens' in response to increasing application of phosphorus (P) as fertigation. Non-linear regression curves (logistic growth curves) are plotted for TDB at various concentrations of P. Standard errors of the means are denoted as vertical lines on the curves.

### 2.4. Applied phosphorus

Phosphorus concentration of the nutrient solutions was determined via flow injection analysis (QuikChem 8500 series 2 with Total Nitrogen and Total Phosphorus manifolds) after a persulfate digestion (Lachet QuikChem method 10−115-01−4-B). Samples for P analysis were collected after every sixth fertigation event, resulting in seven collection dates for 45 fertigation events (~16 % sample rate). Samples were collected on March 13, April 11, May 12, May 29, June 13, June 30, and July 6 of 2017. Actual P concentrations were consistently lower than the nominal rates (Table 1). The concentration of actual P applied was on average 32 % less than targeted. The decrease in actual P versus nominal P could be a result of sorption of P with Ca, Fe, and Mn when making fertigation solutions. Similarly, Shreckhise et al. (2020) reported decreased pore-water P due to interactions with Micromax within a pine bark based substrate. For the remainder of this paper, we refer to actual P concentrations.

### 2.5. Plant growth and morphology

At the end of the study all leaves were detached from the stems of each plant, counted, and leaf area determined using a leaf area meter (LI-3100, LI−COR Inc., Lincoln, NE, USA). Leaf size ($cm^2$ $leaf^{-1}$) was calculated as total plant leaf area/number of leaves per plant. Stems were cut at the substrate surface. Container substrate was removed from roots by allowing the root ball, roots, and the surrounding substrate to air-dry before shaking the root ball and gently separating roots from the dried substrate. Leaves, stems, and roots were each dried in an oven at 45 °C for three days and then weighed to determine dry mass. Root and shoot dry weights were summed to calculate total dry biomass (TDB), and the root-to-shoot ratio was calculated by dividing root mass with shoot mass.

### 2.6. Phosphorus partitioning

An individual P budget was estimated for each P rate via a calculated mass balance using each plant replicate: $P_{applied} = P_{leached} + P_{uptake} + P_{substrate}$.

Where:

$P_{applied}$ = total phosphorus applied during the experiment via fertigation

$P_{leached}$ = total phosphorus leached from each container

$P_{uptake}$ = phosphorus assimilated by each plant during the experiment

**Table 2**
Root and shoot dry weight (g) of *Physocarpus opulifolius* 'Seward', *Hydrangea quercifolia* 'Queen of hearts', and *Cornus obliqua* 'Powell Gardens' in response to six applied phosphorus concentrations. Mean separations were carried out using Fisher Least Significant Difference (LSD) post hoc test at p-value < 0.05. Means within a taxon that are followed by same letters are not significantly different at given p-value.

| P ($mg{\cdot}L^{-1}$) | *P. opulifolius* | | *H. quercifolia* | | *C. obliqua* | |
|---|---|---|---|---|---|---|
| | Root | Shoot | Root | Shoot | Root | Shoot |
| 0 | 10.09c | 10.94d | 7.75c | 14.22d | 8.70e | 12.80d |
| 0.7 | 10.09c | 12.78d | 6.70c | 12.86d | 11.21de | 19.28d |
| 1.3 | 12.70c | 24.39c | 10.70c | 25.44c | 13.26cd | 28.10d |
| 2.5 | 17.59b | 36.83b | 15.01b | 45.17b | 21.49bc | 55.98c |
| 3.7 | 18.22ab | 44.30a | 17.31ab | 52.84ab | 30.87a | 74.06b |
| 6.5 | 21.88a | 47.42a | 19.39a | 58.68a | 25.9ab | 83.19a |
| *p*-value | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 | <0.0001 |

$P_{substrate}$ = estimated total phosphorus stored in the substrate at the end of the study

$P_{applied}$ was calculated as total volume of fertigation solution applied (in L) × mean P concentration of fertigation solution from seven periodic samples (mg/L)

$P_{leached}$ was estimated as the total volume of leachate from each container (in L) × mean P concentration of leachate from seven periodic samples (mg/L)

$P_{uptake}$ was estimated as the P content of each plant at the end of the study [calculated as sum of the biomass of each plant component (leaves, stems, roots) × the P concentration for respective components] minus the P content of the plant at the beginning of the experiment

$P_{substrate}$ was estimated the difference between the amount of P applied during the study and the amount of P taken up by plants or leached from containers

For $P_{leached}$, Phosphorus concentration in the leachate was determined seven times during the course of study on same dates as the nutrient solutions using flow injection analysis (section 2.4).

Samples for tissue P concentration were sent to a commercial plant laboratory (Waters Agricultural Laboratories, Camilla, GA, USA) for analysis where they were thoroughly washed with distilled water, blotted dry, and underwent open vessel -wet digestion with nitric acid and hydrogen peroxide before analysis with inductively coupled plasma optical emission spectrometry (ICP-OES) (Cunniff and Association of Official Analytical Chemists International, 1995). Six plants per taxa were sampled at the beginning of the trial to determine the initial plant P content before treatments began (supplemental Table 2). Mean initial plant P contents (P concn. × weight = P content) were subtracted from final P content to calculate total plant P uptake. Tissue P concentration in leaves and stems were determined on six replicates per P rate per taxa and P content in roots was determined on three replicates per P rate per taxa. We attempted to determine initial P concentration of the pine bark-peat moss substrate using same method as tissue P, however the concentrations were too low (<0.01 %) to be reliably measured. Pine bark has minimal available P (Wilkerson, 1981; Yeager and Wright, 1982). Although sphagnum peat moss can contain significant amount of P (Pakarinen and Tolonen, 1977), available P was minimal (0.01 %, by weight), hence we assumed it to be zero.

### 2.7. Physiological measurements

A portable photosynthesis system (LI-6400 XT, LI−COR, Inc., Lincoln, NE, USA) equipped with a fluorescence chamber head (LI-6400−40, LI−COR, Inc., Lincoln, NE, USA) was used to measure photosynthesis and light-adapted fluorescence (Fv'/Fm') for all three taxa. All physiological measurements were conducted on five replicates of each taxon receiving each P rate.

#### 2.7.1. A/Ci curves

A/Ci curves were obtained by plotting photosynthesis rate at intercellular carbon dioxide concentrations starting at 400 ppm, then incrementally decreasing to 0 ppm and then increasing to 800 ppm (i.e. 400, 300, 200, 100, 50, 0, 300, 400, 600 and 800 ppm) at the rate of approximately 2.5 min per concentration (Singh et al., 2013).

Measurements were conducted on a section of a fully expanded healthy leaf enclosed in the fluorescence chamber head at either the 3$^{rd}$ or 4$^{th}$ node from the top for *P. opulifolius* and *C. obliqua* and at the 1st or 2nd node for *H. quercifolia*. Photosynthetically active radiation (PAR) in the chamber was set to 1500 μmol $m^{-2}{\cdot}s^{-1}$. The block temperature on the chamber head was set to 25 °C and the reference $CO_2$ was 400 ppm. Photosynthesis values at various internal carbon dioxide concentrations (*Ci*) were used to generate *A/Ci* curves using the non-linear rectangular hyperbola model developed by Archontoulis and Miguez (2015),

$$y = \frac{a * x * Yasym}{\{Yasym + a * x\} - Rd}$$

Where y = photosynthesis, x = Intercellular $CO_2$ concentration, Yasym = Asymptotic value of Y, a = initial slope of curve at low x levels (<200 ppm), and Rd = day respiration.

Data from *A/Ci* curves were used to estimate the maximum velocity of rubisco for carboxylation ($V_{cmax}$), rate of photosynthetic electron transfer for RuBP regeneration (*J*), and *TPU* using calculations and the hyperbola model provided by Sharkey (2016). Curves used to generate values for $V_{cmax}$, *J,* and *TPU* first solved for the minimum sum of squares. Visual confirmation for the best fit was conducted for all the curves to detect any errors during curve fitting. Where the calculator estimated unrealistic values of day respiration (Rd) or mesophyll conductance (gm), the values of these variables were constrained to < 6 μmol $m^{-2}{\cdot}s^{-1}$ and < 3 μmol $m^{-2}{\cdot}s^{-1}$ $Pa^{-1}$, respectively, to ensure calculated values were not outside normal ranges.

#### 2.7.2. Chlorophyll fluorescence

Light-adapted chlorophyll fluorescence (*Fv'/Fm'*) was used to determine the rate of electron transport or the efficiency of PSII (Murchie and Lawson, 2013), therefore, a section of a healthy leaf of each plant was enclosed in the fluorescence chamber head with 1500 μmol $m^{-2}{\cdot}s^{-1}$ of PAR, 400 ppm $CO_2$, and 40–60 % humidity at 25 °C. After approximately 5 min of acclimatization, *Fv'/Fm'* was measured (LI-6400XT, LI−COR Inc., Lincoln, NE, USA).

### 2.8. Statistical analysis

All data were analyzed separately for each taxon using SAS software (SAS Institute Inc., Cary, NC, USA). A logistic growth curve was used to determine optimum P fertilization for TDB (Archontoulis and Miguez, 2015; Shreckhise et al., 2018),

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

Where, y = TDB, c = asymptote of the curve, a = growth rate and b = inflection point of maximum growth rate.

The equation for the logistic growth curve was differentiated to find the point of asymptotic deceleration for TDB (Mischan et al., 2011) using the fourth-order derivative, adapting the process of Shreckhise et al. (2018). For all other comparisons, data were analyzed using one-way ANOVA. Post-hoc mean comparisons were made using Fisher's least significance difference (LSD) at p-value of < 0.05. Correlations among

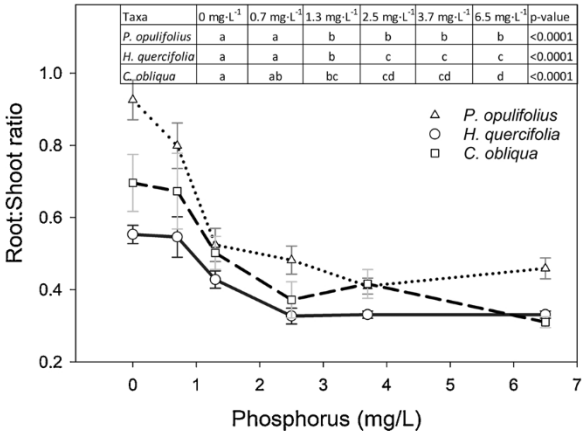


**Fig. 2.** Root-to-Shoot (R:S) ratio of *Physocarpus opulifolius* 'Seward', *Hydrangea quercifolia* 'Queen of hearts', and *Cornus obliqua* 'Powell Gardens' in response to six phosphorus concentrations applied via fertigation. Mean separations were carried out using Fisher Least Significant Difference (LSD) post-hoc test at p-value < 0.05 and presented as an inset table. Means within a taxon indicated by the same letter are not different at given p-value. Standard errors are denoted as vertical lines on the curves.

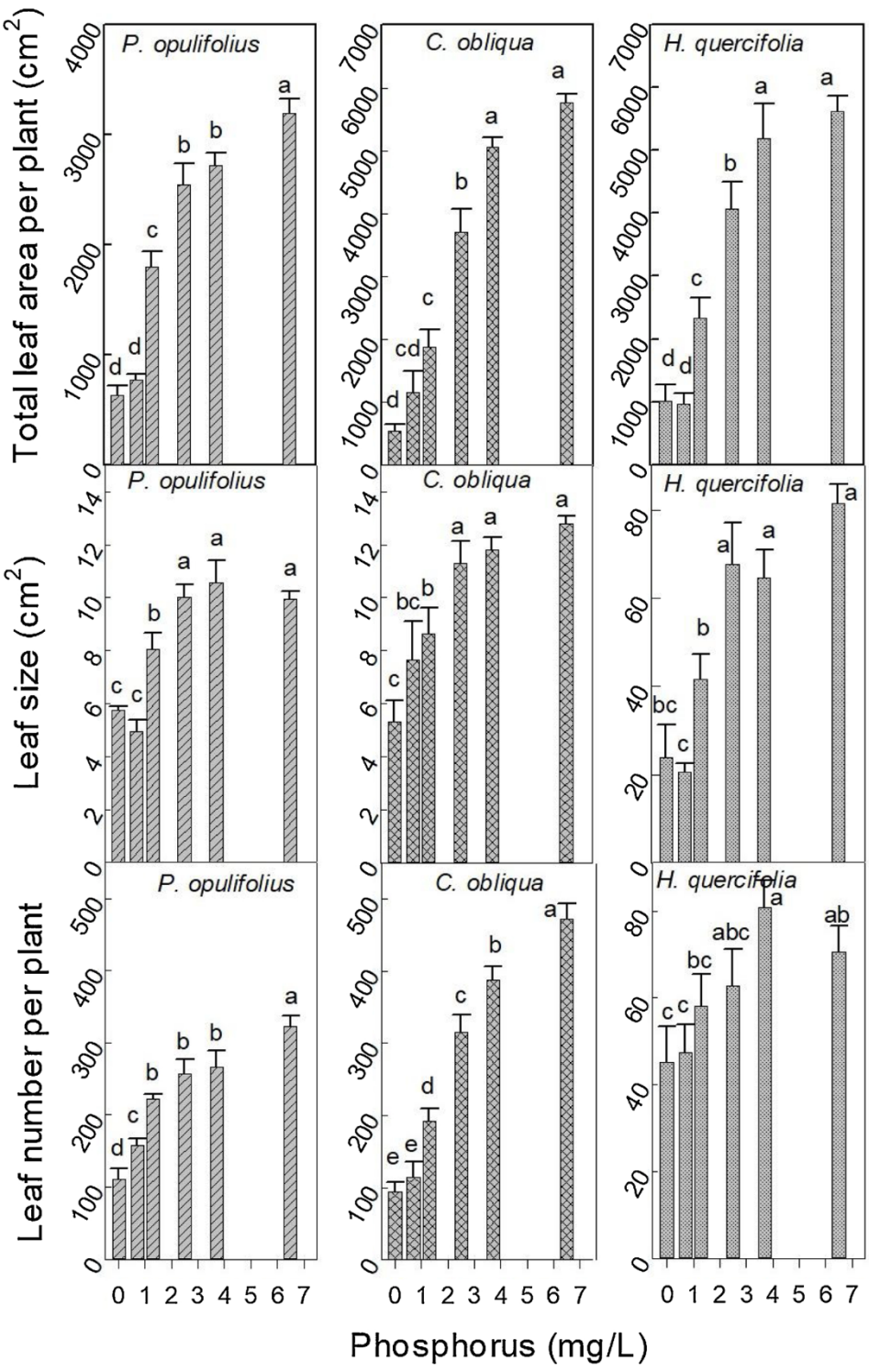


**Fig. 3.** Leaf number per plant, leaf size, and total leaf area per plant for *Physocarpus opulifolius* 'Seward', *Cornus obliqua* 'Powell Gardens', and *Hydrangea quercifolia* 'Queen of hearts' in response to applied phosphorus as fertigation. Standard error of the means are denoted by vertical 'T' lines. Mean separations for each taxon were carried out using Least Significant Difference (LSD) post-hoc test at p value $< 0.05$. Means within a taxon followed by same letters are not significantly different at $p < 0.05$.

morpho-physiological variables were analyzed using the Pearson correlation coefficient.

## 3. Results

### 3.1. Total dry biomass in response to P concentration

Plant growth response to P concentration varied by taxa (Fig. 1). Total dry biomass was lowest for plants receiving no P fertilizer in the solution. Calculated optimum P for TDB was achieved for *P. opulifolius*, *H. quercifolia*, and *C. obliqua* when applying 3.8, 3.9, and 4.0 mg $L^{-1}$ P, respectively (Fig. 1).

### 3.2. Root and shoot dry weight and root-to-shoot ratio in response to P concentration

Increasing P concentration increased root and shoot growth for all three taxa. Roots had maximum dry weight when 3.7 mg $L^{-1}$ P was applied, regardless of taxa. Maximum shoot dry weight for *P. opulifolius* and *H. quercifolia* was achieved when 3.7 mg $L^{-1}$ P was applied and at

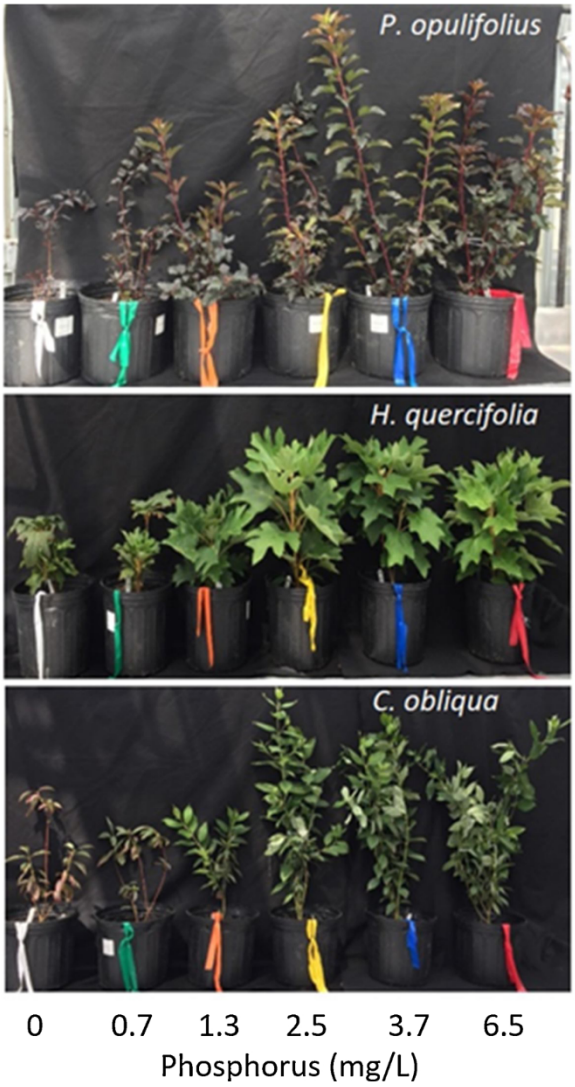


**Fig. 4.** Representative plants of *Physocarpus opulifolius* 'Seward', *Hydrangea quercifolia* 'Queen of hearts', and *Cornus obliqua* 'Powell Gardens', after receiving 0 to 6.5 mg $L^{-1}$ phosphorus for six months in the greenhouse.

6.5 mg $L^{-1}$ P for *C. obliqua* (Table 2).

Root-to-shoot ratio for *P. opulifolius* decreased from 0.93 to 0.52 when the applied P concentration was increased from 0 mg $L^{1-}$ to 1.3 mg $L^{-1}$. Further increases in applied P concentration did not decrease root-to-shoot ratio (Fig. 2). Root-to-shoot ratio of *H. quercifolia* and *C. obliqua* decreased from 0.55 to 0.33 and 0.7 to 0.37, respectively, when the applied P concentration was increased from 0 mg $L^{-1}$ to 2.5 mg $L^{-1}$; further increase in P concentration did not decrease root-to-shoot ratio (Fig. 2).

### 3.3. Leaf characteristics in response to P concentration

Leaf number, leaf size, and total leaf area per plant increased with increasing P concentration (Fig. 3). *P. opulifolius* and *C. obliqua* had maximum leaf number at 6.5 mg $L^{-1}$ P in the solution, while *H. quercifolia* had maximum leaf number at 2.5 mg $L^{-1}$ P. Applied P below 2.5 mg $L^{-1}$ reduced leaf size, while application above 2.5 mg $L^{-1}$ P did not affect leaf size across taxa (Fig. 3). Total leaf area per plant for *C. obliqua* and *H. quercifolia* reached a maximum when fertigated with 3.8 mg $L^{-1}$ P, while total leaf area for *P. opulifolius* was greatest at 6.5 mg $L^{-1}$ applied P. Visual symptoms of P deficiency, i.e., shorter internodes, purpling of leaves, and smaller leaf sizes, were observed in all three taxa when fertigated with 0 and 0.7 mg $L^{-1}$ P (Fig. 4).

### 3.4. Partitioning of applied phosphorus

To assess the fate of P applied, we compared the total amount of P in each fraction (P in leaves, stems, roots, and leachate). Non-substrate P (P in leaf, stem, root, and leachate) was higher than the total P applied for 0 mg $L^{-1}$ P treatment for all three taxa (Table 3). For all plants receiving 0.7 mg $L^{-1}$ P or more, the non-substrate P was lower than the total P applied. Leaves accounted for the largest fraction of P taken up by plants, except for *P. opulifolius* at application rate of 0 mg $L^{-1}$ P (Table 3).

For all three taxa, increasing the applied P concentration increased the total amount of P output as leachate and P in the substrate (Table 3). Increasing applied P from 0 to 1.3 mg $L^{-1}$ increased P allocation to leaves, but when fertigating with greater than 2.5 mg $L^{-1}$ P, P allocation to leaves decreased (Table 3). Increasing P concentration increased P partition to leachate. For example, increasing P concentration in *P. opulifolius* from 0.7 mg $L^{-1}$ to 6.5 mg $L^{-1}$ resulted in an increase of the leachate P fraction from 10 % to 17 %, respectively. Total P leached increased from 18 mg to 35 mg for *P. opulifolius*, from 19 mg to 30 mg for *H. quercifolia,* and from 20 mg to 34 mg for *C. obliqua* when P concentration was increased from 3.5 mg $L^{-1}$ to 6.5 mg $L^{-1}$ (Table 3).

### 3.5. Photosynthetic response to phosphorus concentration

*A/Ci* curves were modeled with a non-linear model of a rectangular hyperbola (R-squared $>$ 0.96 for all three taxa). For all three taxa, increasing concentration of applied P increased net photosynthesis. Increases in photosynthesis associated with P were consistently greater at higher values of *Ci* ($>$ 300 ppm) (Fig. 5).

For *P. opulifolius*, $V_{cmax}$ was lowest when fertigated with $\leq$ 0.7 mg $L^{-1}$ P and reached a plateau at 1.3 mg $L^{-1}$ P (Fig. 6A). For *H. quercifolia*, $V_{cmax}$ was lowest at $\leq$ 1.3 mg $L^{-1}$ of applied P and highest at $\geq$ 2.5 mg $L^{-1}$ P. *C. obliqua* fertigated at 0 mg $L^{-1}$ P had the lowest $V_{cmax}$ while $\geq$ 2.5 mg $L^{-1}$ P did not show a significant difference in $V_{cmax}$ (Fig. 6A). Photosynthetic limitation for *J* was also evident at lower P concentrations. *P. opulifolius* and *H. quercifolia* had lowest *J* at application of $\leq$ 0.7 mg $L^{-1}$ P, and the lowest *J* for *C. obliqua* was at 0 mg $L^{-1}$ P. *P. opulifolius* and *C. obliqua* had maximum *J* when fertigated with $\geq$ 1.3 mg $L^{-1}$ P, while *H. quercifolia* had maximum *J* at $\geq$ 2.5 mg $L^{-1}$ P (Fig. 6B). Applied P concentration also affected photosynthesis limitation because of *TPU* but was less sensitive compared to $V_{cmax}$ and *J*. For all three taxa, *TPU* was lowest when P concentrations were $\leq$ 0.7 mg $L^{-1}$ and highest when $\geq$ 1.3 mg $L^{-1}$ P was applied. Therefore, increasing applied P from 1.3–6.5 mg $L^{-1}$ did not increase *TPU* (Fig. 6C).

Light-adapted fluorescence (*Fv'/Fm'*) reached maximum levels at relatively low P concentrations for all three taxa (Fig. 7). Increasing applied P from 0 to 0.7 mg $L^{-1}$ increased Fv'/Fm' to a maximum for *H. quercifolia*, similarly, increasing applied P from 0 to 1.3 mg $L^{-1}$ maximized fluorescence for *P. opulifolius and C. obliqua*.

### 3.6. Correlation among morpho-physiological variables

For all three taxa, *TDB* correlated ($p < 0.05$) with P percentage in leaf ($r = 0.87$ *P. opulifolius*; $r = 0.44$ *H. quercifolia*; $r = 0.65$ *C. obliqua*) and average leaf size ($r = 0.85$ *P. opulifolius*; $r = 0.91$ *H. quercifolia*; $r = 0.81$ *C. obliqua*) (Table 4). Total dry biomass also correlated well ($r \geq 0.56$) with parameters related to photosynthetic biochemistry such as $V_{cmax}$, *J,* and *TPU* for all taxa (Table 4). Correlation order of TDB with those physiological parameters for all three taxa were in the order $V_{cmax} > J >$ *TPU*. Total dry biomass also correlated ($r \geq 0.45$) with *Fv'/Fm'* for all three taxa but was weaker compared to photosynthetic biochemistry (Table 4). Foliar P concentration was correlated with parameters related to photosynthetic biochemistry ($V_{cmax}$, *J, TPU,* and *Fv'/Fm'*) only for *P. opulifolius* and *C. obliqua*. Root-to-shoot ratio was negatively

**Table 3**
Partitioning of phosphorus (P) to leachate, leaf, stem, and root, and calculated amount of P stored in the substrate at harvest for *P. opulifolius* 'Seward', *H. quercifolia* 'Queen of hearts', and *C. obliqua* 'Powell Gardens'. Partition was based on total P applied. Mean separation were carried out using Fisher Least Significant Difference (LSD) post-hoc test at p-value <0.05. Means that are followed by same letters are not significantly different given p value.

| P ($mg{\cdot}L^{-1}$) | Total-P input[#] (mg) | | Total P in leachate (mg) | Total P in leaf (mg) | Total P in stem (mg) | Total P in root (mg) | Total P in substrate (mg) | Percent of P in leachate | Percent of P in leaf | Percent of P in stem | Percent of P in root | Percent of P in substrate |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | *P. opulifolius 'Seward'* | | | | | | | | | |
| 0 | 0.6 | = | 1.4d | 3.8d | 1.3c | 6.5b | −12.5* | – | – | – | – | – |
| 0.7 | 22.5 | = | 2.3cd | 5.5d | 1.7c | 4.5b | 8.5b | 10.3 | 24.6 | 7.7 | 19.8 | 37.9 |
| 1.3 | 40.3 | = | 3.6c | 12.7c | 5.1c | 7.5b | 11.4b | 9.0 | 31.5 | 12.6 | 18.7 | 28.4 |
| 2.5 | 79.0 | = | 11.7b | 24.2b | 11.6b | 17.5a | 14.0b | 14.9 | 30.7 | 14.7 | 22.2 | 17.8 |
| 3.7 | 119.0 | = | 18.4b | 30.9a | 18.8a | 26.2a | 25.0b | 15.5 | 25.9 | 15.8 | 22.0 | 21.0 |
| 6.5 | 204.2 | = | 35.2a | 36.7a | 24.1a | 29.4a | 79.0a | 17.3 | 18.0 | 11.8 | 14.4 | 38.7 |
| *p*-value | | | <0.0001 | <0.0001 | <0.0001 | <0.0005 | <0.0001 | | | | | |
| | | | *H. quercifolia 'Queen of hearts'* | | | | | | | | | |
| 0 | 0.5 | = | 1.7f | 5.4d | 2.4bc | 5.3bc | −14.4* | – | – | – | – | – |
| 0.7 | 21.0 | = | 2.3e | 8.5d | 1.4c | 3.2c | 5.6c | 11.0 | 40.5 | 6.5 | 15.3 | 26.9 |
| 1.3 | 37.6 | = | 3.4d | 18.2cd | 2.6bc | 6.2bc | 7.3c | 9.0 | 48.4 | 7.1 | 16.4 | 19.4 |
| 2.5 | 73.7 | = | 5.7c | 30.7bc | 5.8ab | 10.3ab | 21.4bc | 7.7 | 41.5 | 8.0 | 14.0 | 29.0 |
| 3.7 | 111.1 | = | 18.8b | 37.1ab | 6.3ab | 10.4ab | 38.6b | 16.9 | 33.4 | 5.7 | 9.4 | 34.8 |
| 6.5 | 192.7 | = | 29.8a | 48.0a | 7.0a | 16.2a | 89.7a | 15.5 | 26.0 | 3.7 | 8.5 | 46.6 |
| *p*-value | | | <0.0001 | <0.0005 | <0.05 | <0.01 | <0.0001 | | | | | |
| | | | *C. obliqua 'Powell Gardens'* | | | | | | | | | |
| 0 | 0.6 | = | 2.7d | 4.9d | 2.5d | 0.7d | −10.2* | – | – | – | – | – |
| 0.7 | 21.9 | = | 2.7d | 8.6cd | 3.5cd | 1.2d | 5.9c | 12.2 | 39.2 | 16.2 | 5.4 | 27.2 |
| 1.3 | 39.2 | = | 3.4d | 17.8c | 5.8bcd | 3.0cd | 9.3c | 8.6 | 45.4 | 14.8 | 7.6 | 23.8 |
| 2.5 | 76.7 | = | 9.6c | 33.8b | 6.5bc | 7.1c | 19.7b | 12.5 | 44.1 | 8.5 | 9.3 | 25.8 |
| 3.7 | 115.6 | = | 20.0b | 41.0b | 8.1b | 21.6a | 24.9b | 17.4 | 35.5 | 7.1 | 18.7 | 21.6 |
| 6.5 | 198.4 | = | 34.1a | 58.5a | 13.3a | 16.2b | 76.4a | 17.2 | 29.5 | 6.7 | 8.2 | 38.5 |
| *p*-value | | | <0.0001 | <0.0001 | <0.0005 | <0.0005 | <0.0001 | | | | | |

* The value was not considered in ANOVA or post-hoc mean separation test because it is negative value.
# Total P input includes total amount phosphorus applied during the study via fertigation.

correlated with *TDB* for all taxa (r = -0.73 *P. opulifolius*; r = -0.73 *H. quercifolia*; r = -0.62 *C. obliqua*) and with P concentration in leaf for *P. opulifolius* (r=-0.78) and *C. obliqua* (r = -0.66) (Table 4).

## 4. Discussion

Plant productivity is the integrated result of leaf surface area accretion, net photosynthetic activity, and allocation of photosynthate to plant organs. In this study, P concentration in the applied solution affected all aspects of plant productivity.

### 4.1. Morphological response to phosphorus concentration

For all three taxa, P required for maximum TDB accumulation varied but was less than approximately 4 mg $L^{-1}$. This is consistent with recent observations of growth in woody ornamentals such as *Hydrangea paniculata* 'Limelight' (hydrangea), *Ilex crenata* 'Helleri' (holly), and *Rhododendron* 'Karen' (azalea) where growth was maximized at 2.9–4.7 mg $L^{-1}$ of applied P (Shreckhise et al., 2018). Leaf size for all three taxa followed a similar trend as TDB. Leaf size increased with increasing applied P up to 2.5 mg $L^{-1}$ with no further increase at higher P concentrations. Phosphorus fertigated at 6.5 mg $L^{-1}$ for *P. opulifolius* and 3.7 mg $L^{-1}$ for *C. obliqua* and *H. quercifolia* produced maximum total leaf area per plant. Hence, P fertilization is required for leaf expansion and growth. An increase in fertilizer P concentration was reported to increase leaf area in *Phaseolus vulgaris* (common bean), *Helianthus annuus* (sunflower) and *Trifolium repens* (white clover) (Høgh-Jensen et al., 2002; Lynch et al., 1991; Rodríguez et al., 1998). Root-to-shoot ratio was maximum with fertigation of 0.7 mg $L^{-1}$ P across taxa. Minimum root-to-shoot ratio was observed when *P. opulifolius* was fertigated at 1.3 mg $L^{-1}$ P, or 2.5 mg $L^{-1}$ P for *H. quercifolia* and *C. obliqua*. Thus, these results reveal the effect of P on carbon allocation. Phosphorus was utilized more for root growth at critically low application rates of P (≤ 1.3 mg $L^{-1}$) when possibly limiting and needed to expend resources to acquire P via the root, whereas when P was more readily available, there was a shift of resources to maximize shoot growth (≥ 2.5 mg $L^{-1}$). Allocation of resources such as P for shoot growth subsequently increased plant net photosynthesis and Fv'/Fm'. Root-to-shoot ratio had a negative correlation to TDB and foliar P. Therefore, decreasing root-to-shoot ratio was primarily because of an increase in shoot growth.

### 4.2. Fate of applied phosphorus

Combined P in leachate and the plant tissue was greater than total P applied for the treatment receiving 0 mg $L^{-1}$ P. This could be a result of uptake from initial substrate release and subsequent labile P adsorbed or precipitated in the substrate (Shreckhise et al., 2020). Ristvey et al. (2004) observed a similar response in container-grown *Rhododendron* 'Karen' (azalea) grown with P addition as low as 0 $mg{\cdot}week^{-1}$. The total amount of P in leaves and stems increased with increasing applied P (to 3.7 mg $L^{-1}$ for *H. quercifolia* and *P. opulifolius* and to 6.5 mg $L^{-1}$ for *C. obliqua*), but P application above approximately 4 mg $L^{-1}$ did not increase TDB or any physiological parameter in any of the taxa. Therefore, the increase in tissue P content when P is applied above 4.0 mg $L^{-1}$ indicates luxury consumption (i.e., absorption and storage of P beyond the current plant requirement), which also has been observed in a wide range of plant species including container-grown plants (Ristvey et al., 2004) and forest trees (Lawrence, 2001). Increasing the concentration of P applied via fertigation increased P loss in leachate and the total amount of P in the substrate. Similar observations were made in other studies where increasing P application increased P loss from the system (Ristvey et al., 2004; Shreckhise et al., 2019a, 2018). Therefore, increasing P beyond the optimum requirement would have no benefit on growth and physiological processes but could increase P leaching. Hence, we do not recommend the application of P over the optimum requirement of approximately 4.0 mg $L^{-1}$ for all three taxa. We cannot

accurately predict how our findings of optimum P requirement in liquid fertilization will directly relate to temperature-based release of controlled release fertilizers with or without micronutrients, but can be used as a proxy for fertilization when optimizing pore-water P via monitoring using the pour-through method.

### 4.3. Physiological performance in response to phosphorus concentration

To fix one molecule of $CO_2$ in the Calvin-Benson cycle, three molecules of ortho-phosphate ($PO_4$) are required (Walker and Robinson, 1978), therefore, P deficiency can limit photosynthesis. In our study, increasing P concentration, up to a point, increased net photosynthesis for a wide range of intercellular $CO_2$ concentrations (0–600 ppm) in all three taxa. Similar increases in net assimilation with increasing applied P was observed in *Helianthus annuus* 'Asmer'(sunflower), *Zea mays* 'Eta'(maize) (Jacob and Lawlor, 1991) and *Pinus* sp. (pine) seedlings (Loustau et al., 1999). For *H. quercifolia* and *C. obliqua*, applied P < 2.5 mg $L^{-1}$ reduced $V_{cmax}$. For those two taxa, photosynthesis at lower P fertigation rates (< 2.5 mg $L^{-1}$) was reduced partly because of the limited supply of rubisco enzyme. For *P. opulifolius*, rubisco restricted photosynthesis only at < 1.3 mg $L^{-1}$ of applied P. The rate of RuBP regeneration (*J*) may also limit photosynthesis in P deficient plants. For *P. opulifolius* and *C. obliqua*, photosynthesis was limited by *J* at the application rate of of < 1.3 mg $L^{-1}$ P. For *H. quercifolia J* limited

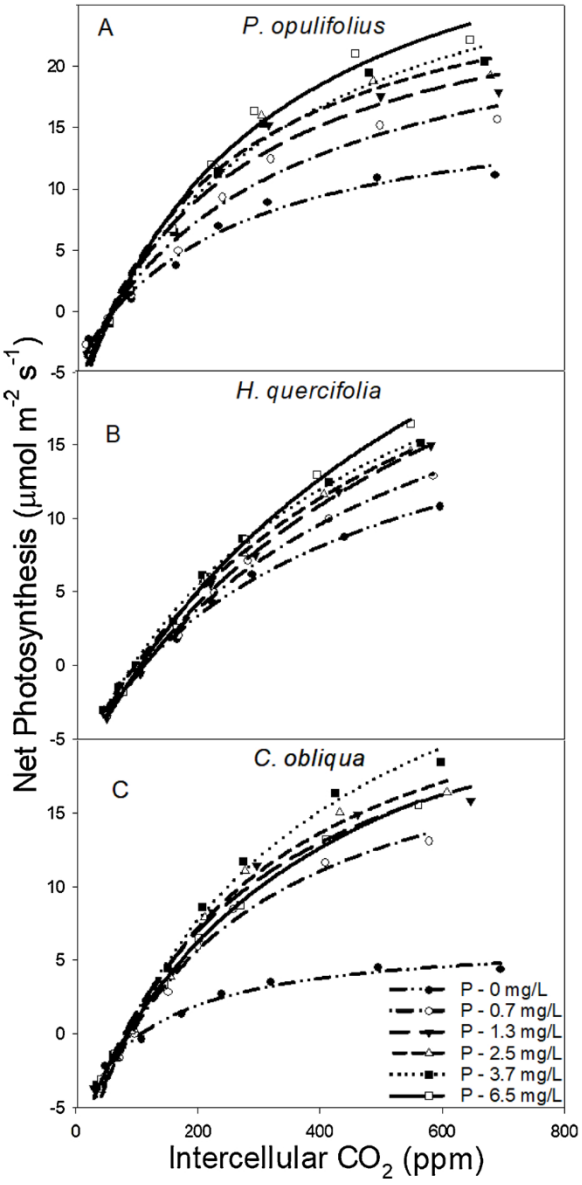


**Fig. 5.** Response of photosynthesis to increasing internal carbon dioxide concentration (A/Ci Curve) for *Physocarpus opulifolius* 'Seward', *Hydrangea quercifolia* 'Queen of hearts', and *Cornus obliqua* 'Powell Gardens' for each of the 6 applied P concentrations. Curves were generated as the mean of five replicates. All the curves followed non-linear model of rectangular hyperbola. R-squared values for all models for all three taxa were above 0.96.

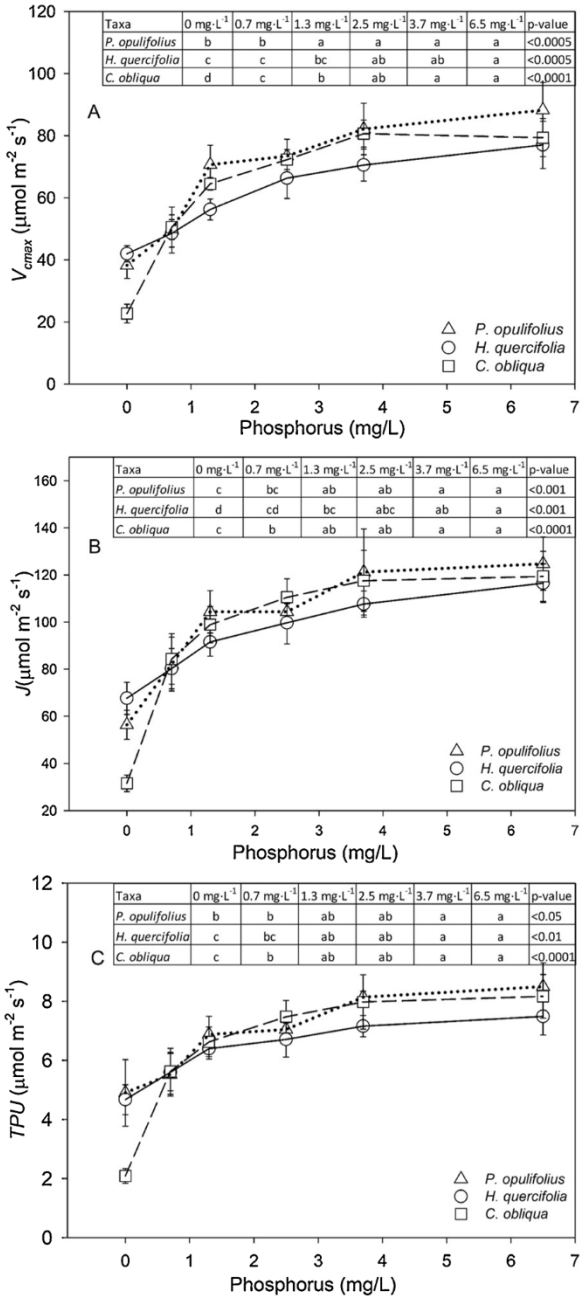


**Fig. 6.** Maximum velocity of rubisco for carboxylation ($V_{cmax}$) (A); rate of photosynthetic electron transport for RuBP regeneration (J) (B), and triose phosphate use (TPU) (C) of *P. opulifolius* 'Seward', *H. quercifolia* 'Queen of hearts', and *C. obliqua* 'Powell Gardens' in response to varied application of phosphorus (P) concentration. Values of A/Ci Curves were analyzed based on equations provide by Sharkey (2016) to generate $V_{cmax}$, J and TPU for each replicate. Fisher Least Significant Difference (LSD) was used to compare means among P fertilization levels at p-value < 0.05 and presented as inset table. Means within a taxon indicated by the same letter are not different at given p-value. Standard errors are denoted as vertical lines on the curves.

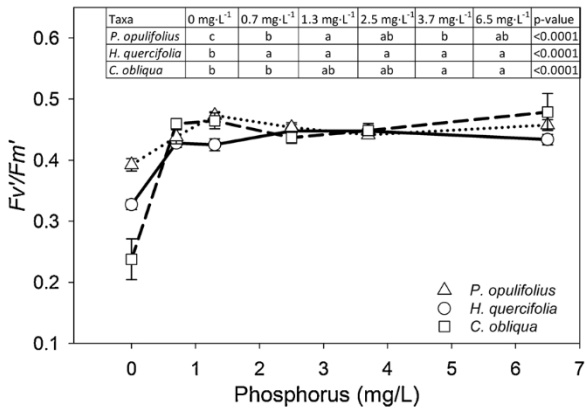


**Fig. 7.** Light-adapted fluorescence (Fv'/Fm') response to increasing phosphorus (P) concentration for *P. opulifolius* 'Seward', *H. quercifolia*'Queen of hearts', and *C. obliqua* 'Powell Gardens'. Fisher Least Significant Difference (LSD) was used to compare means among P fertilization levels at p-value < 0.05 and presented in as inset table. Means within a taxon indicated by the same letter are not different at given p-value. Standard errors are denoted as vertical lines on the curves.

photosynthesis at an application of < 2.5 mg $L^{-1}$ P. At higher rates of photosynthesis, export of carbon compounds from Calvin-Benson cycle slows down, causing down-regulation of photosynthesis (Yang et al., 2016), also referred to as *TPU* limited photosynthesis. In our study, application of 1.3 mg $L^{-1}$ P was sufficient to overcome the limitation caused by *TPU* for all three taxa. Therefore, photosynthesis limitations associated with $V_{cmax}$ and *J* were more sensitive compared to the limitation related to *TPU*. This sensitivity is also further evidenced by the correlation analysis of TDB with $V_{cmax}$, *J*, and *TPU*. Phosphorus deficiency has also been observed to reduce $V_{cmax}$ and *J* for several other taxa (Lin et al., 2009; Loustau et al., 1999; Singh et al., 2013).

In contrast to the parameters of the Calvin-Benson cycle, light utilization by plants of all three taxa was less affected by P concentration. For all three taxa, plants that received no P had lower *Fv'/Fm'* compared to plants that received P. Applying 0.7 mg $L^{-1}$ P for *H. quercifolia* and *C. obliqua* and 1.3 mg $L^{-1}$ P for *P. opulifolius* was sufficient to maximize *Fv'/Fm'*. Other studies have observed no reduction in chlorophyll content and light-harvesting capacity at low rates of P application (Brooks, 1986; Campbell and Sage, 2006). In our study, light-harvesting capacity was reduced when no P was supplied, but a low rate of P was sufficient for optimum functioning of photosystem II.

## 5. Conclusion

For all three taxa, TDB was most sensitive to applied P concentration; thus, a higher concentration of P in fertigation solution was required for optimizing TDB compared to other morphological and physiological parameters. Analysis of *A/Ci* curves indicated a broader response to applied P concentration compared to *Fv'/Fm'*. This suggests that overall photosynthetic response to P is driven more by photosynthetic biochemistry rather than light harvesting reactions. When compared among all three taxa, $V_{cmax}$ (rubisco limited) was the main reason for reduction in photosynthesis followed by *J* (RuBP regeneration) then by *TPU*.

Overall, biomass growth was optimized when fertigating with approximately 4.0 mg $L^{-1}$ P for all three taxa, which is much lower than P rates of water-soluble fertilizers or P release rate of controlled-release fertilizers that are commonly available and used in the nursery industry. Therefore, nursery growers may be able to reduce P fertilization without reducing crop growth. Even a slight reduction in P rates over a long period can substantially reduce total P in the leachate. For example, if P concentration were lowered from 6.5 mg $L^{-1}$ to 3.8 mg $L^{-1}$, leachate P concentration would be reduced by 59–91 % depending on taxa grown. Reducing P in irrigation return flow can ultimately lower growers' environmental footprint without affecting physiological or morphological processes across many ornamental taxa.

**Table 4**
Pearson's correlation coefficient for *Physocarpus opulifolius* 'Seward', *Hydrangea quercifolia* 'Queen of hearts', and *Cornus obliqua* 'Powell Gardens'. TDB is total dry biomass, R/S ratio is root to shoot ratio, Leaf size is (total leaf area per plant/ leaf number per plant), P% in leaf is phosphorus percent in leaf by weight, $V_{cmax}$ is maximum velocity of rubisco for carboxylation, J is the rate of photosynthetic electron transport for RuBP regeneration, TPU is triose phosphate use, and Fv'/Fm' is light-adapted fluorescence.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Pearson's correlation coefficient for *P. opulifolius*** | | | | | | | |
| | R/S ratio | Leaf size | P% in leaf | $V_{cmax}$ | *J* | *TPU* | *Fv'/Fm'* |
| TDB | −0.73*** | 0.85*** | 0.87*** | 0.69*** | 0.65*** | 0.56** | 0.45* |
| R/S ratio | | −0.74*** | −0.78*** | −0.60*** | −0.50** | −0.44* | −0.55*** |
| Leaf size | | | 0.75*** | 0.62*** | 0.58** | 0.51** | 0.42* |
| P% in leaf | | | | 0.77*** | 0.63*** | 0.55** | 0.48** |
| $V_{cmax}$ | | | | | 0.92*** | 0.81*** | 0.47* |
| *J* | | | | | | 0.99*** | 0.46* |
| *TPU* | | | | | | | 0.34$^{NS}$ |
| **Pearson's correlation coefficient for *H. quercifolia*** | | | | | | | |
| | R/S ratio | Leaf size | P% in leaf | $V_{cmax}$ | *J* | *TPU* | *Fv'/Fm'* |
| TDB | −0.73*** | 0.91*** | 0.44* | 0.77*** | 0.75*** | 0.68*** | 0.51** |
| R/S ratio | | −0.68*** | −0.23$^{NS}$ | −0.60*** | −0.56** | −0.52** | −0.52** |
| Leaf size | | | 0.44* | 0.70*** | 0.68*** | 0.61*** | 0.46** |
| P% in leaf | | | | 0.31$^{NS}$ | 0.30$^{NS}$ | 0.24$^{NS}$ | 0.04$^{NS}$ |
| $V_{cmax}$ | | | | | 0.92*** | 0.89*** | 0.44* |
| *J* | | | | | | 0.98*** | 0.47* |
| *TPU* | | | | | | | 0.47* |
| **Pearson's correlation coefficient for *C. obliqua*** | | | | | | | |
| | R/S ratio | Leaf size | P% in leaf | $V_{cmax}$ | *J* | *TPU* | *Fv'/Fm'* |
| TDB | −0.62*** | 0.81*** | 0.65*** | 0.78*** | 0.7*** | 0.69*** | 0.51** |
| R/S ratio | | −0.75*** | −0.66*** | −0.6** | −0.59** | −0.58** | −0.53** |
| Leaf size | | | 0.53** | 0.7*** | 0.61** | 0.60** | 0.61** |
| P% in leaf | | | | 0.65*** | 0.56** | 0.57** | 0.43* |
| $V_{cmax}$ | | | | | 0.91*** | 0.91*** | 0.70*** |
| *J* | | | | | | 0.99*** | 0.68*** |
| *TPU* | | | | | | | 0.66*** |

*** p-value of ≤ 0.0005; ** p-value of ≤ 0.005; and * p-value of ≤ 0.05, NS p-value > 0.05.

## Contributions

Shital Poudyal and Bert Cregg: Conceptualization and Visualization, Shital Poudyal, Thomas D. Sharkey, James S. Owen and Bert Cregg: Methodology, Shital Poudyal: Software, Shital Poudyal: Investigation, Bert Cregg, Thomas R. Fernandez, Thomas D Sharkey and James S. Owen: Resources, Shital Poudyal and Bert Cregg: Original draft preparation, Shital Poudyal and Bert Cregg: Funding acquisition, Shital Poudyal, Bert Cregg, Thomas R. Fernandez, Thomas D Sharkey and James S. Owen: Reviewing and editing, Bert Cregg, Thomas R. Fernandez, Thomas D Sharkey and James S. Owen: Supervision.

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## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.scienta.2020.109719.

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