Barry J. Shelp, William J. Sutton, and Edward J. Flaherty
cultivars of subirrigated greenhouse-grown chrysanthemum
improves phosphorus-use efficiency in two contrasting

Strategic timing and rate of phosphorus fertilization
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Abstract: Greenhouse floriculture operations pose significant environmental risk due to extensive inputs of fertilizer, especially nitrogen and phosphorus (P). Recent evidence shows that the use efficiency for nitrogen or sulphur is markedly improved in subirrigated potted chrysanthemums (Chrysanthemum morifolium Ramat.) by supplying a moderate level of the nutrient during vegetative growth, and removing the entire nutrient suite at the onset of reproductive growth, without adverse effects on plant quality. Here, two split-plot experiments were conducted with subirrigated, potted, disbudded chrysanthemums grown in a peat:perlite mixture under greenhouse conditions (high- or low-ambient light) with inorganic orthophosphate (Pι) treatment (2.6 mmol L\(^{-1}\) Pι supplied during the vegetative and reproductive stages, and 2.6, 1.95, or 1.3 mmol L\(^{-1}\) Pι supplied during the vegetative stage only) as the main plot and cultivar (‘Olympia’ and ‘Covington’) as the subplot. Market quality plants with sufficient tissue P were produced even when Pι delivery was reduced by approximately 75% over the crop cycle, compared with industry standards. The primary mechanism for sustaining plant growth with decreasing Pι delivery was improved acquisition or uptake efficiency, although some changes in internal P-utilization efficiency were evident, including the remobilization of both organic P and Pι during inflorescence development. Differences in biomass yields, tissue P concentrations, content-based P-use efficiency (PUEC = mg shoot dry mass/mg shoot P content) with constant Pι acquisition, and uptake- versus remobilization-based P supply for inflorescence growth established that ‘Olympia’ has a greater P-utilization efficiency than ‘Covington’. This modified subirrigation practice could contribute significantly to low-input production of floricultural crops.

Key words: chrysanthemum, greenhouse floriculture, phosphorus-acquisition efficiency, phosphorus management, phosphorus-utilization efficiency, remobilization.

Résumé : La floriculture de serre pose des risques importants pour l’environnement en raison du grand volume d’engrais utilisés, principalement des engrais azotés et phosphorés (P). Des résultats récents indiquent que lorsqu’il est nécessaire de réduire l’apport d’engrais pour des chrysanthèmes (Chrysanthemum morifolium Ramat.) en pot irrigués de façon souterraine avec l’apport d’une quantité modérée d’oligoéléments durant la croissance végétative, puis le retrait complet de ces derniers quand s’amorce la période de reproduction, sans que la qualité de la plante s’en ressente. Les auteurs ont procédé à deux expériences à dispositif en tiroir sur des chrysanthèmes en pot éboutonnés, irrigués de façon souterraine et cultivés dans un mélange de tourbe et de perlite. Les plants ont été cultivés en serre (sous un fort ou faible éclairage ambiant). Le paramètre principal consistait en un amendement d’orthophosphate inorganique (Pι — « inorganic orthophosphate ») (2,6 mmol de Pι par litre pendant les stades végétatif et reproductif, ou 2,6, 1,95 ou 1,3 mmol de Pι par litre uniquement pendant le stade végétatif), le cultivar (‘Olympia’ ou ‘Covington’) constituant le paramètre secondaire. Les chercheurs ont obtenu des plants de qualité marchande dont les tissus contenaient suffisamment de P, même après réduction d’environ 75% de l’apport de Pι pendant le cycle complet, comparativement aux pratiques en usage dans l’industrie. Le principal mécanisme qui permet à la plante de croître en dépit d’une baisse de la quantité de Pι fournie est une meilleure absorption ou assimilation de cet élément, bien qu’on ait observé quelques changements au niveau de l’assimilation du P interne par la plante, notamment la remobilisation du P organique et du Pι pendant le développement des inflorescences. Les variations au niveau du rendement en biomasse, de la concentration de
P dans les tissus, de l’assimilation du P en fonction du contenu [PUEC — « content-based P-use efficiency » = mg de matière sèche des pousses/mg de P dans les racines] avec l’acquisition constante de P, et l’absorption plutôt que la remobilisation du P pour la croissance de l’inflorescence indiquent que la variété ‘Olympia’ assimile mieux le P que ‘Covington’. Cette méthode d’irrigation souterraine modifiée pourrait sensiblement concourir à la production de fleurs avec un plus faible apport d’engrais. [Traduit par la Rédaction]

Mots-clés : chrysanthème, floriculture de serre, efficacité de l’acquisition du P, gestion du P, assimilation du P, remobilisation.

Introduction

Greenhouse floriculture operations pose significant environmental risk due to extensive inputs of fertilizer, especially in the form of nitrogen (N) and phosphorus (P) (Ontario Ministry of the Environment 2012). Subirrigation was developed to reduce nutrient and water usage (MacDonald et al. 2013; Ferrarezi et al. 2015), but its utility could be further enhanced by optimization of nutrient delivery (Hansen and Lynch 1998; Zheng et al. 2004; Heins and Yelanch 2013). Recent research with chrysanthemum combined a moderate level of N or sulphur (S) during vegetative growth with the elimination of the entire suite of nutrients during reproductive growth (MacDonald et al. 2014; Shelp et al. 2017; Sutton et al. 2019). The delivery of N and S, respectively, was reduced by approximately 75% and 87.5% over the crop cycle compared with industry standards, without adverse effects on plant and flower quality. With S, the primary mechanism for maintaining plant growth was improved acquisition (or uptake) efficiency, whereas with N improved utilization (including remobilization) efficiency during reproductive growth, as well as acquisition efficiency over the crop cycle, was important.

In the present paper, the modified nutrient delivery practice was extended to the study of P. The regulation of P homeostasis in plants is complex (Gu et al. 2016; Młodzińska and Zboińska 2016; López-Arredondo et al. 2017; Wang et al. 2018; Chang et al. 2019). High-affinity inorganic orthophosphate (P_i) transporters (PTs) belong to the PHOSPHATE TRANSPORTER 1 (PHT1) gene family; they are plasma membrane–located and associated with free P_i uptake at the root–soil interface when external P_i is limited, as well as root-to-shoot transfer and remobilization. Low-affinity PTs belong to the PHT2, PHT3, and PHT4 gene families, are associated with organellar membranes, and are believed to participate in P_i distribution within the plant. The PHOSPHATE 1 transporter facilitates P_i efflux from cells into the root xylem, and the tonoplast transporters, VPI1 (also called PHT5:1) and VPE1/2, respectively, mediate influx into and efflux from vacuoles (Młodzińska and Zboińska 2016; López-Arredondo et al. 2017; Wang et al. 2018; Xu et al. 2019). In this context, P_i can be liberated from organic sources in vacuoles and mature/senescent plant parts, respectively, for buffering cytosolic contents and recycling to actively growing organs and tissues (Plaxton and Tran 2011; Veneklaas et al. 2012). Transcription factors known as PHOSPHATE RESPONSE regulate a large subset of P_i-starvation-responsive genes, including those belonging to the PHT1 gene family (López-Arredondo 2017; Huang et al. 2018; Wang et al. 2018).

Phosphorus, like N, is readily translocated in both xylem and phloem streams, and low levels of P within the plant do not immediately lead to deficiency symptoms (White 2012; Młodzińska and Zboińska 2016). To date, most work on P remobilization from vegetative (petiole, stem, and leaf) tissue has focused on the grain filling period in field crops (Veneklaas et al. 2012) and on leaves exhibiting clear signs of senescence (Himelblau and Amasino 2001; Maillard et al. 2015), rather than early inflorescence growth. The partitioning of total P (P_t) between P_i and organic phosphate esters (P_o) may vary among plant parts, but tissue-P_t concentration generally reflects the P_i supply (Veneklaas et al. 2012). It is unclear however, if the tissue levels of P_i, P_o, and P_i influence their remobilization.

In productive agricultural systems, P_i fertilizer is typically added on an annual basis to avoid soil depletion in the long term, and the availability of P_i for crop development declines over the growing season and fluctuates with the weather conditions (Veneklaas et al. 2012). In contrast, under greenhouse floriculture conditions the P_i supply can be strictly managed throughout the growth cycle. Zheng et al. (2004, 2010) have shown that subirrigated, potted gerbera and miniature rose can be grown to the commercial stage with a P_i supply of 1.1–1.2 mmol L^{-1}. For our studies, we chose a popular year-round greenhouse floriculture crop in Canada: potted chrysanthemum (Chrysanthemum morifolium Ramat). Thus, the modified subirrigation practice was used to manipulate the P_i supply to two chrysanthemum cultivars grown under both summer (high-ambient light) and winter (low-ambient light) greenhouse conditions (Experiments 1 and 2, respectively) typical of southern Ontario. The morphological characteristics and accumulation and partitioning of P within plant strata were monitored to assess the acquisition and internal utilization of P_i over the crop cycle, and the remobilization of P_i during inflorescence development.

Materials and Methods

Plant growth conditions

Chrysanthemum (cultivars ‘Olympia’ and ‘Covington’) cuttings were produced in a commercial setting and then rooted in Jiffy Plugs (Model CF Hort Plug,
70000088) filled with peat moss (Shelp et al. 2017). The rooted cuttings were transferred to the University of Guelph (Guelph, ON, Canada) and individually placed in 10 cm plastic pots containing a peat moss and perlite mixture (50:50 by volume, initial pH of 5.4–6.2) as described previously (Shelp et al. 2017); a saturated medium extract of the peat alone had a P level of 0.02 mmol L\(^{-1}\) (Berger, Boisbriand, QC, Canada). The potted cuttings were distributed across the 16 troughs (445 cm \(\times\) 11 cm \(\times\) 4.7 cm, Farm Tek HydroCycle 6” Pro NFT Series, Dyerville, IA, USA) composing a computer-controlled, ebb-and-flow subirrigation and blackout system, which was located in a naturally-lit greenhouse (Sutton et al. 2019). Nutrient solution was supplied every 2 d until inflorescence emergence, and nutrient solution or deionized water was supplied every 1 or 2 d as necessary thereafter until the flowers were fully expanded. The nutrient solution (completely replenished about once per week) or deionized water was held in adjacent 50 L tanks and pumped twice into the troughs (one tank supplied the identical treatment troughs from two blocks) at 1100 h for 2.5 min, allowing the troughs to fill to an approximate depth of 2 cm, and then to drain over a 20 min period into the 50 L tank.

Experiment 1 was conducted under high-ambient light (March–April 2016), and the plants were immediately exposed to flower-inducing short days; the 10–14 h light-dark cycle was imposed 1 h before sundown (Sutton et al. 2019). Experiment 2 was conducted under low-ambient light (October–December 2016), and 2 wk of long days were imposed prior to the short days. Supplemental lighting (Solar Max Spectrum LED, BML Horticulture, Austin, TX, USA; 20–50 \(\mu\)mol m\(^{-2}\) s\(^{-1}\) photosynthetic photon flux density at pot level) was provided in the low-ambient light experiment to compensate for shading that could result from the overhead frame supporting the blackout blinds used to impose the short-day cycle.

Experimental design

A balanced split-plot experiment was conducted with four Pi treatment regimens in otherwise complete nutrient solutions (MacDonald et al. 2014). The standard industry practice control consisted of 2.6 mmol L\(^{-1}\) Pi supplied during both vegetative and reproductive stages, a period of approximately 9–10 wk after flower-inducing short days were initiated (denoted as 2.6VR). The experimental regimens consisted of 2.6, 1.95 and 1.3 mmol L\(^{-1}\) Pi supplied during the vegetative stage, followed by deionized water during the reproductive stage (denoted as 2.6V, 1.95V, and 1.3V, respectively), so that plants received approximately 50%, 37.5%, and 25%, respectively, of the Pi received over the crop cycle with the industry control treatment. A negative control treatment was not included. The Pi treatments were arranged as the main plot and the two cultivars (‘Olympia’ and ‘Covington’) as the subplot (one cultivar was randomly placed in one half of the treatment trough, and the other cultivar was placed in the adjacent half). The main plots were arranged as a randomized complete block design of four blocks over two benches with no border rows. There were 21 plants (disbudded) per treatment replicate, and 10 plants per treatment replicate were harvested when inflorescences emerged (approximately 4 and 4.5 wk after the beginning of short days for the high- and low-ambient light experiments, respectively) or were fully expanded (approximately 4–4.5 wk later) for evaluation of visual symptoms and plant characteristics. Thereafter, individual plants were divided into the bottom 12 leaves (i.e., lower), lower stem and petioles, the remaining leaves (i.e., upper), upper stem and petioles, and inflorescence for determination of fresh mass, and then dried at 70 °C for 3 d and ground into a fine powder. The 10 plants in each treatment replicate were randomly divided into groups of two, which were combined for dry mass (DM) yield and P analyses (i.e., five biological replicates per treatment replicate).

P analysis

Analysis of Pi was conducted by the Agriculture and Food Laboratory at the University of Guelph. The tissue samples were microwave acid–digested and diluted to an appropriate volume with nanopure water before measurement by an inductively coupled plasma-mass spectrometry method developed and validated in-house (based on USEPA Method 6020; United States Environmental Protection Agency 2014). Analysis of Pi in nanopure-water extracts of the dried plant tissues was conducted according to USEPA Method 365.1 (United States Environmental Protection Agency 1993) using an Astoria-2 flow cell analyzer (Astoria-Pacific International, Clackamas, OR, USA). Organic phosphate was calculated as the difference between Pi and Pt.

Statistical analysis and indices of P-use efficiency

All statistical analyses were conducted using SAS version 9.4 with the PROC GLIMMIX method at \(\alpha = 0.05\) level (SAS Institute Inc. 2013). Normality and homogeneity of variance were confirmed before further statistical analyses were performed. Transformation of the dependent variable was conducted as necessary, and the resulting outcomes back-transformed for presentation. Variance was separated into fixed effects (Pi treatment, cultivar, and harvest), random effects (block), and all relevant interactions within and between the fixed and random effects. Repeated measures analysis was used whenever samples were compared across the two harvest points using a compound symmetry covariance structure. Analyses of variance were performed \((N = 4\) treatment replicates\) and when effects were significant, the means were compared with each other using Tukey’s Honest Significant Difference test using the slice function. Means within a treatment and component strata were compared across harvest points using...
contrast and estimate statements. Linear regression analyses were performed to compare tissue P at the beginning of the reproductive period with net changes in P content over the reproductive period for each biological replicate (\(N = 80\)). Efron’s Pseudo \(R^2\) was calculated as the square of the correlation coefficient between observed values and values predicted by the equation for the line of best fit.

Dry mass and P content of the various strata, as well as P level in the nutrient solution, were used to calculate supply-based Pi-use efficiency (\(\text{PUES} = \text{mg shoot DM/mg Pi input}\)) and Pi-uptake efficiency (\(\text{PUpES} = \text{mg shoot P content/mg Pi input}\)), as well as content-based P-use efficiency (\(\text{PUEC} = \text{mg shoot DM/mg shoot P content}\)), P harvest index (\(\text{PHI} = \text{mg inflorescence P content/mg shoot P content}\)), and P utilization efficiency (\(\text{PUE} = \text{mg inflorescence DM/mg shoot P content}\)) (Good et al. 2004). The P usage index \([\text{PUI} = \text{mg shoot DM} \times (\text{shoot DM/mg shoot P content})]\) accounts for the absolute increase in biomass (Siddiqi and Glass 1981). Inputs of nutrient solution and Pi into each pot were estimated from the change in volume of nutrient solution in the supply tank over 1 wk intervals as a function of the number of pots currently being supplied in the two troughs.

### Results

#### Morphological characteristics

Across the two ambient light conditions and the four Pi regimens, the fresh and DM yields of the whole shoot, the shoot length, and the inflorescence diameter of ‘Covington’ at final harvest were approximately 75%–78%, 70%–82% and 83%–93% of the corresponding values for ‘Olympia’ (\(P \leq 0.05\)) (Table 1, Supplementary Fig. S1). Notably, there was no impact of Pi treatment

| Table 1. Impact of inorganic orthophosphate (Pi) treatment on the final morphological characteristics of two chrysanthemum cultivars grown under high- (Experiment 1) and low-ambient (Experiment 2) light conditions. |
|---|---|---|---|---|
| Cultivar/Pi treatmenta | Shoot mass (g FM plant\(^{-1}\)) | Shoot length (cm plant\(^{-1}\)) | Inflorescence diameter (cm plant\(^{-1}\)) | Shoot mass (g DM plant\(^{-1}\)) |
| **High-ambient light** | | | | |
| ‘Olympia’ | | | | |
| 2.6VR | 31.91 | 35.00 | 10.14 | 4.98 |
| 2.6V | 34.44 | 35.10 | 10.50 | 4.79 |
| 1.95V | 33.73 | 33.57 | 10.81 | 4.95 |
| 1.3V | 35.57 | 32.55 | 10.59 | 5.34 |
| ‘Covington’ | | | | |
| 2.6VR | 26.38 | 27.50 | 9.71 | 3.30 |
| 2.6V | 28.65 | 28.75 | 9.82 | 3.25 |
| 1.95V | 28.87 | 27.34 | 10.04 | 3.30 |
| 1.3V | 30.85 | 26.61 | 10.12 | 3.56 |
| **Low-ambient light** | | | | |
| ‘Olympia’ | | | | |
| 2.6VR | 34.74 | 56.73 | 10.11 | 5.04 |
| 2.6V | 37.09 | 58.02 | 10.48 | 5.17 |
| 1.95V | 40.88 | 58.43 | 10.74 | 5.65 |
| 1.3V | 40.48 | 57.89 | 10.80 | 5.28 |
| ‘Covington’ | | | | |
| 2.6VR | 24.81 | 40.11 | 8.44 | 3.23 |
| 2.6V | 27.11 | 41.27 | 8.87 | 3.23 |
| 1.95V | 28.57 | 40.57 | 9.03 | 3.55 |
| 1.3V | 28.49 | 41.23 | 8.81 | 3.35 |

**Note:** None of the means were significantly different (\(P \leq 0.05\)). FM, fresh mass; DM, dry mass. Data represent the mean of four treatment replicates; shoot FM, shoot length, and inflorescence diameter are derived from 10 biological replicates, whereas DM is derived from five biological replicates.

a2.6VR received 2.6 mmol L\(^{-1}\) Pi during both vegetative and reproductive stages, whereas 2.6V, 1.95V, and 1.3V, respectively, received 2.6, 1.95, and 1.3 mmol L\(^{-1}\) Pi during the vegetative stage only.

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<sup>a</sup>Supplementary data are available with the article through the journal Web site at [http://nrcresearchpress.com/doi/suppl/10.1139/cjps-2019-0173](http://nrcresearchpress.com/doi/suppl/10.1139/cjps-2019-0173).
Fig. 1. Impact of inorganic orthophosphate (Pi) treatment on the dry mass (DM) yields over the reproductive period of whole plants or their strata in two chrysanthemum cultivars under high-ambient light conditions. 2.6VR received 2.6 mmol L\(^{-1}\) Pi during both vegetative and reproductive stages, whereas 2.6V, 1.95V, and 1.3V, respectively, received 2.6, 1.95, and 1.3 mmol L\(^{-1}\) Pi during the vegetative stage only. The top and bottom rows represent Experiments 1 and 2, respectively; panels a and c represent ‘Olympia’, and panels b and d represent ‘Covington’. The initial and final harvests are compared within each treatment; *indicates a significant difference (\(P \leq 0.05\)). Each bar represents from bottom to top: lower leaves, ▪; upper leaves, ◇; lower stem and petioles, ◆; upper stem and petioles, ▣; inflorescence, ●. Data represent the mean of four treatment replicates, each consisting of five biological replicates derived from two plants.

on these morphological characteristics or the DM yields of the plant strata with either cultivar. Development of the inflorescence was consistently accompanied by DM accumulation in all plant strata of ‘Olympia’, except the lower leaves from the 2.6VR, 2.6V, and 1.95V treatments. In contrast, inflorescence development in ‘Covington’ was accompanied by DM accumulation in lower leaves and lower stem plus petioles only, and for only plants grown under high-ambient light conditions; these results were unaffected by Pi treatment (Fig. 1). There were no visual symptoms of P stress on the vegetative and reproductive parts of either cultivar at final harvest (Fig. 2). Thus, none of the Pi regimens employed here limited growth over the crop cycle, compared with the industry standard, regardless of the ambient light conditions.

**P-use efficiency and accumulation**

Various indices of PUE were calculated to assess mechanisms that may be involved in sustaining the growth of chrysanthemums as Pi delivery was reduced by up to 75% over the crop cycle. The supply-based indices, PUES and PUpES, showed the most dramatic increases over the delivery range, regardless of experiment and cultivar (Figs. 3 and 4). The other indices were less affected by the reductions, but some increases were evident at 1.95V and (or) 1.3V. Of particular interest were the increases in PUEC, PUI, PHI and PUE in ‘Olympia’ plants grown under high-ambient light conditions. Across all treatments and ambient light conditions, ‘Olympia’ showed significantly greater values for PUI, PUES and PUpES than ‘Covington’ (\(P \leq 0.05\)); under high-ambient light conditions ‘Olympia’ also showed a greater value for PUEC (\(P \leq 0.05\)). Overall, these findings indicate that the primary mechanism responsible for the growth of chrysanthemums, regardless of the ambient light conditions, was improved acquisition efficiency, although this was accompanied by changes in utilization efficiency with the more severe reductions in Pi delivery. Furthermore, they suggest that ‘Olympia’ utilized both external and internal Pi more efficiently than ‘Covington’.

Information for calculation of the various indices for PUE reported above is provided in the Supplementary data\(^1\). Across the two experiments, the amounts of Pi delivered to each pot over the crop cycle were estimated to be 29.1–179.4 mg and 64.8–192.6 mg, a 3- to 6-fold range in inputs (Supplementary Tables S1 and S2\(^1\)). The mean tissue-Pi levels had a narrower range than that for the Pi inputs: the lower leaves, upper leaves, lower stem and petioles, upper stem and petioles, and inflorescence were 0.43%–0.95% DM, 0.43%–0.89% DM, 0.26%–0.31% DM, 0.37%–0.45% DM, and 0.37%–0.48% DM.
Fig. 2. Impact of inorganic orthophosphate (Pi) treatment on the morphological characteristics of two subirrigated chrysanthemum cultivars under high-ambient light conditions (Experiment 1). 2.6VR received 2.6 mmol L\(^{-1}\) Pi over both vegetative and reproductive stages, whereas 2.6V, 1.95V, and 1.3V, respectively, received 2.6, 1.95, and 1.3 mmol L\(^{-1}\) Pi over the vegetative stage only. Plants were arranged in a split-plot experimental design and grown until the inflorescences were fully expanded. Panels a and b represent ‘Olympia’ and ‘Covington’, respectively. Two plants each are arranged from right to left with decreasing Pi supply over the life cycle.

in ‘Olympia’, and 0.70%–1.00% DM, 0.85%–1.20% DM, 0.25%–0.29% DM, 0.35%–0.50% DM, and 0.40%–0.42% DM in ‘Covington’ (Supplementary Tables S3–S5\(^1\)). The mean accumulation of Pi in the shoots of ‘Olympia’ and ‘Covington’ was 20.3–30.1 and 18.5–22.1 mg P plant\(^{-1}\), respectively (Supplementary Tables S6–S8\(^1\)). Even though the roots were not examined in this study, these data suggest that ‘Covington’ was 75%–78% as efficient as ‘Olympia’ in acquiring Pi at the corresponding Pi regimen. With high-ambient light conditions, ‘Olympia’ showed a slight decreasing trend in Pi with decreasing Pi delivery, although the difference between the 2.6VR and 1.3V treatments was only 13% (Supplementary Table S6\(^1\)). ‘Olympia’ and ‘Covington’ acquired 18%–37% and 13%–25%, respectively, of the whole shoot Pi at final harvest during the period of inflorescence development (Supplementary Tables S9 and S10\(^1\)), suggesting that remobilization in ‘Covington’ is a more important source of P for inflorescence development than in ‘Olympia’.

**P remobilization and partitioning**

Linear correlation analysis of the individual biological replicates was used to assess the impact of tissue-Pi status on the net changes in P contents of each stratum during inflorescence development. The fractionation of P [Pi, Po, and (or) Pt] was determined for plants grown under high-ambient light conditions. Organic phosphate was clearly a more important constituent of Pi than Pi in all strata (Fig. 4), and the widest range in changes in net P content, regardless of the P fraction, was in the upper leaves, followed by lower leaves, for both cultivars (Figs. 5 and 6). The concentration of Pi was lower in the leaves of ‘Olympia’, especially the upper leaves, than in the corresponding leaves of ‘Covington’. Net changes in Pi and Po contents in lower leaves of ‘Olympia’ were not correlated with tissue Pt, whereas net changes in Pi contents in lower leaves of ‘Covington’ were moderately correlated with tissue Pt (Fig. 5, Supplementary Table S11\(^1\)). Notably, the net changes in Po contents in upper leaves of ‘Olympia’ were moderately correlated with tissue Pi, in a positive manner, whereas in ‘Covington’ they were moderately correlated in a negative manner. In contrast, the net changes in both Pi and Pt contents of the lower or upper stem and petioles in both cultivars were moderately to strongly correlated with tissue Pt in a negative manner. In approximately half of these cases, the significant correlations between net changes in Po or Pi contents and tissue Pt were associated with significant correlations between net changes in Pi contents and tissue Pt. While the fractionation of P was not determined for plants grown under low-ambient light conditions, evidence was obtained for moderate to strong negative correlations between net changes in Pi contents and tissue Pt in the lower stem and petioles and the upper stem and petioles of ‘Olympia’, and in the upper leaves and upper stem and petioles of ‘Covington’ (Fig. 6, Supplementary Table S12\(^1\)). Overall, the maximal losses of Pi from leaves, especially the upper leaves, exceeded those from stem and petioles. These findings indicate that the net losses from leaves of ‘Covington’ were generally associated with relatively high-P status, but there was some evidence of net loss in ‘Olympia’ leaves with relatively low-P status. In contrast, net losses from the stem and petioles were always associated with relatively high-P status. Furthermore, the findings establish that remobilized Po and to a lesser extent Pi are more important sources of P for inflorescence development in ‘Covington’ than in ‘Olympia’, likely due to the higher P status at inflorescence emergence.

The highlights described above were derived from data provided in the Supplementary data\(^1\). Across the two ambient light conditions, the mean tissue-Pi levels in the lower leaves, upper leaves, lower stem and petioles, and upper stem and petioles at inflorescence emergence were 0.53%–0.67% DM, 0.69%–0.77% DM, 0.33%–0.35% DM, and 0.50%–0.51% DM in ‘Olympia’, and 0.72%–0.88% DM, 1.24%–1.33% DM, 0.27%–0.29% DM, and 0.49%–0.52% DM in ‘Covington’ (Supplementary Tables S3–S5\(^1\)). For high-ambient light plants in particular, 24%–26%, 15%–19%, 23%–26%, and 26%–28% of the Pi was present as Pi in the lower leaves, upper leaves, lower stem and petioles, and upper stem and petioles of the
two cultivars at initial harvest, whereas 22%–27%, 23%–27%, 23%–26%, and 25%–27% was present as Pi in the corresponding strata, and 19%–22% as Pi in the inflorescence at final harvest (Supplementary Tables S6 and S71). Across all ambient light conditions and cultivars the net losses of P from all strata could account for 2%–44% of the Pt accumulated by the inflorescence (Supplementary Tables S9 and S10), suggesting that direct acquisition of Pi, rather than remobilization from shoot organs and tissues, was the most important P source for inflorescence development in both cultivars.

Discussion

High quality chrysanthemums with sufficient tissue

P were produced over a wide range of P, delivery

Subirrigation enabled easy removal of the entire suite of nutrients from the water supply at the onset of reproductive growth and reduction of the P, supply from 2.6 to 1.3 mmol L$^{-1}$ during vegetative growth. While plants from the two cultivars, 'Olympia' and 'Covington', differed substantially in biomass accumulation at final harvest, there was no external evidence that P, availability ever became limiting during the crop cycle. This indicates that the P delivered over the crop cycle could be reduced by at least 75%, compared with industry standards, without adversely affecting plant and inflorescence quality. Similar results have been found with N and S using this delivery strategy (MacDonald et al. 2014; Shelp et al. 2017; Sutton et al. 2019). Previous research has shown that plants with some fully-expanded inflorescences are successfully produced with 1.125 mmol L$^{-1}$ Pi in subirrigated potted miniature rose (Zheng et al. 2010), 1.2 mmol L$^{-1}$ Pi and then 0.6 mmol L$^{-1}$ Pi in the final stage of production for subirrigated potted gerbera (Zheng et al. 2004), and 0.25 mmol L$^{-1}$ Pi (in combination with starter fertilizer) in fertigated potted poinsettia (Ku and Hershey 1997). In our study, market-quality subirrigated, potted chrysanthemums were produced with 1.3 mmol L$^{-1}$ Pi delivered over the vegetative growth phase only, and the corresponding tissue P, of the lower and upper leaves of chrysanthemums at inflorescence emergence ranged from 0.53%–1.36% DM,
which compares favourably with the sufficiency range in a diagnostic leaf (0.23%–1.15% DM) (Ontario Ministry of Agriculture, Food and Rural Affairs 2014; Hill Laboratories 2019). Thus, the recommended industry supply of 1–2.6 mmol L$^{-1}$ Pi in the nutrient solution over the crop cycle (Sonneveld and Kreij 1987; Green Leaf Plants 2015) can probably be reduced for many popular ornamental crops, regardless of the irrigation method.

Phosphorus homeostasis is mediated via multiple mechanisms

One important feature of PHT1s is their inducibility by low Pi concentrations (López-Arredondo et al. 2017; Wang et al. 2018). For example, Arabidopsis thaliana (L.) Heynh., PHT1;1, and PHT1;4 contribute to Pi uptake at both high (0.5 mmol L$^{-1}$) and low (2–5 μmol L$^{-1}$) external Pi, with similar affinity ($K_m = \sim 10$ μmol L$^{-1}$) (Misson et al. 2004; Shin et al. 2004). Interestingly, AtPHT1 expression is barely detectable with 1.25 mmol L$^{-1}$ external Pi, but it increases substantively in a linear fashion as availability decreases to 25–50 μmol L$^{-1}$; further decreases in Pi have little effect (Karthikeyan et al. 2002). CmPHT1;2 expression is evident in roots, stems, and leaves, and is highest in the stems of vegetative plants grown under a high-Pi condition (300 μmol L$^{-1}$) (Liu et al. 2018). However, CmPHT1;2 is induced approximately five times more in roots in the absence of Pi, yet not at all in the stem and leaves. The Pi-uptake rate of plants in the presence of 300 μmol L$^{-1}$ Pi is approximately twice that with 15 μmol L$^{-1}$ Pi (low-Pi condition). While the tissue-Pi levels are similar under the high- and low-Pi conditions in both roots and shoots, the tissue-Pi levels are approximately 60%–150% greater with high Pi than low Pi. Furthermore, the DM yields of the roots, but not the shoots, are reduced with low Pi, so that the root-to-shoot ratio decreases, contrary to the response typically reported for P deficiency (Marschner 1995). These findings suggest that root growth and P partitioning within the chrysanthemum plant can be modified by the availability of external Pi, even when Pi acquisition is unaffected.

In the present study, the delivery of Pi to chrysanthemum over the crop cycle was modified approximately...
4-fold. Regardless of the ambient light conditions and the timing and level of P<sub>i</sub> fertilization, the DM yields and P<sub>i</sub> contents of plants for both cultivars were reasonably steady, resulting in corresponding improvements in the efficiency of P acquisition with decreasing P delivery. Thus, it can be suggested that all the reductions in P<sub>i</sub> delivery were sufficient to induce the transcription of high-affinity PTs located in the root system of chrysanthemums, although the precise signalling mechanism(s) by which this would occur is uncertain (López-Arredondo et al. 2017; Wang et al. 2018). In some cases, the more severe reductions in P delivery resulted in improvements in the efficiency of internal P utilization, due at least in part to changes in P usage or remobilization from the

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Fig. 5. Relationship between changes in phosphorus (P) contents in various strata of two chrysanthemum cultivars over inflorescence development and the corresponding tissue P at the beginning of inflorescence development under high-ambient light conditions (Experiment 1). Panels a, c, e, and g represent ‘Olympia’, panels b, d, f, and h represent ‘Covington’. The figure combines biological replicates, derived from two plants each, from all four treatments: 2.6 mmol L<sup>-1</sup> inorganic orthophosphate (P<sub>i</sub>) during both vegetative and reproductive stages; and 2.6, 1.95, or 1.3 mmol L<sup>-1</sup> P<sub>i</sub> during the vegetative stage only. Each datum represents a single biological replicate; blue squares, red triangle, s and green circles represent total P, organic P, and inorganic P, respectively. The replicates were pooled for regression analysis (N = 80 biological replicates); the line of best fit is shown in black. *indicates a significant relationship (see Supplementary Table S11 for outputs of the regression analysis). DM, dry mass.
Fig. 6. Relationship between changes in total phosphorus (P) content in various strata of two chrysanthemum cultivars over inflorescence development and the corresponding tissue P at the beginning of inflorescence development under low-ambient light conditions (Experiment 2). Panels a and b represent ‘Olympia’ and ‘Covington’, respectively. The figure combines biological replicates, derived from two plants each, from all four treatments: 2.6 mmol L\(^{-1}\) inorganic orthophosphate (Pi) during both vegetative and reproductive stages; and 2.6, 1.95, or 1.3 mmol L\(^{-1}\) Pi during the vegetative stage only. Each datum represents a single biological replicate; gray circles, blue circles, red squares, and green squares represent lower leaves, upper leaves, lower stem plus petioles, and upper stem plus petioles, respectively. The replicates were pooled for regression analysis (\(N = 80\) biological replicates); the line of best fit is shown in black. ‘indicates a significant relationship (see Supplementary Table S12 for outputs of the regression analysis). DM, dry mass.

Various plant organs and (or) tissues, even though the concentrations of tissue P\(_i\) never decreased below the sufficiency range (see discussion above). Notably, the levels of tissue P\(_i\) over the period of inflorescence development ranged widely among the various plant strata (0.25%–1.36% DM), but this was accompanied by minor differences in partitioning of P between P\(_o\) and P\(_i\). Furthermore, P\(_o\) levels always dominated those for P\(_i\) and the relative partitioning between the two fractions did not change with the range in P\(_i\) delivery employed here. Other studies have reported that the P\(_o\) pool dominates at low concentrations of tissue P\(_i\) (≤ 0.5% DM) in photosynthetic tissues of many grain crops, but as tissue P\(_i\) increases, the P\(_i\) pool becomes increasingly important (Veneklaas et al. 2012). In our study, the extent of P remobilization from source organs was generally correlated with their P status, although some cultivar-dependent exceptions were evident with low tissue P\(_i\).

Differences in biomass yields, tissue-P\(_i\) concentrations, and PUEC suggest that ‘Olympia’ uses internal P more efficiently than ‘Covington’ (Rose and Wisuwa 2012). Notably, the accumulation of P\(_i\) was reasonably similar in ‘Olympia’ and ‘Covington’ across all P\(_i\) delivery regimens after both vegetative and reproductive stages, but especially so with 1.3V and 1.95V, respectively. Together, these findings establish that ‘Olympia’ had greater internal utilization efficiency than ‘Covington’ over the entire crop cycle, which is associated, at least in part, with a greater uptake-based P supply for inflorescence development. A remobilization-based P supply for inflorescence development was apparently more important in ‘Covington’ than in ‘Olympia’. Thus, we conclude that the two contrasting chrysanthemum cultivars utilized internal P differently. Whilst the P-acquisition efficiency in both cultivars was very sensitive to the changes in availability of non-limiting P\(_i\), the PUE was less sensitive. Furthermore, the PUtE for ‘Covington’ appeared to be less sensitive to the changes than ‘Olympia’. A recent study reported that the wheat cultivar ‘Chinese 80-55’ maintains higher P\(_i\) concentrations in all organs upon P\(_i\) withdrawal, as well as higher P\(_i\) acquisition in the presence of P\(_i\) than the cultivar ‘Machete’ (Aziz et al. 2014). The authors concluded that ‘Chinese 80-55’ has a higher PUtE, which is most likely achieved through greater P remobilization. Consequently, we propose that the enhanced storage and remobilization of previously acquired P would make ‘Covington’ chrysanthemum more tolerant than ‘Olympia’ to conditions of P\(_i\) starvation, despite its lower internal PUtE.

**Application to floricultural industry**

Closed subirrigation systems, which recirculate water and nutrients, are gaining popularity as environmentally friendly strategies for managing the nutrition of popular potted ornamental plants (MacDonald et al. 2013; Ferrarezi et al. 2015). Here, we have continued to establish that a modified subirrigation practice, based on removal of nutrients at the onset of reproductive growth and reduced fertilizer supply during vegetative growth, improves the nutrient use efficiency of chrysanthemums, regardless of the ambient light conditions. This delivery strategy was developed with an understanding of three plant processes: (i) nutrient uptake by roots during reproductive growth is less than during vegetative growth; (ii) reproductive growth relies to a large extent on nutrients acquired and stored during vegetative growth; and, (iii) high-affinity nutrient uptake by roots is induced during vegetative growth on low to moderate nutrient levels (Marschner 1995; Sutton et al. 2019). To date, we have reduced the delivery of N, S, and P to chrysanthemums grown with subirrigation in a peat:perlite mixture by approximately 75%–87.5%, while

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producing plants of similar yield and quality (MacDonald et al. 2014; Shelp et al. 2017; Sutton et al. 2019). Optimization of the delivery for the remaining macronutrients, as well as micronutrients, and validation of the complete optimized nutrient regimen in a commercial setting could enhance the adoption of subirrigation by the floricultural industry and lay the foundation for improved low-input production.

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