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STRATEGIC TIMING AND RATE OF PHOSPHORUS FERTILIZATION IMPROVES PHOSPHORUS USE EFFICIENCY IN TWO CONTRASTING CULTIVARS OF SUBIRRIGATED GREENHOUSE-GROWN CHRYSANTHEMUM

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ABSTRACT

Greenhouse floriculture operations pose significant environmental risk due to extensive inputs of fertilizer, especially nitrogen and phosphorus. 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 (P_i) treatment (2.6 mmol L^{-1} P_i supplied during the vegetative and reproductive stages, and 2.6, 1.95 or 1.3 mmol L^{-1} P_i supplied during the vegetative stage only) as the main plot and cultivar ('Olympia' and 'Covington') as the sub-plot. Market quality plants with sufficient tissue-P were produced, even when P_i delivery was reduced by approximately 75% over the crop cycle, compared to industry standards. The primary mechanism for sustaining plant growth with decreasing P_i 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 P_i during inflorescence development. Differences in biomass yields, tissue-P concentrations, content-based P use efficiency (PUE_C = mg shoot DM/mg shoot P content) with constant P_i 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.

Keywords: chrysanthemum, greenhouse floriculture, P acquisition efficiency, P management, P utilization efficiency, remobilization

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 Yelanich 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 to 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 2016; Młodzińska and Zboińska 2016; López-Arredondo et al. 2017; Wang et al. 2018; Chang et al. 2019). High-

affinity P_i transporters (PT) belong to the *PHOSPHATE TRANSPORTER 1* (*PHT1)* gene family, are plasma membrane-located and associated with free inorganic orthophosphate (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 organelle membranes, and believed to participate in P_i distribution within the plant. The PHOSPHATE 1 (PHO1) transporter facilitates P_i efflux from cells into the root xylem, and the tonoplast transporters, VPT1 (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/senescing plant parts, respectively, for buffering cytosolic contents and recycling to actively growing organs and tissues (Plaxton and Trans 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; Wang et al. 2018; Huang 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/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_i concentration generally reflects the P_i supply (Veneklaas et al.

2012). It is unclear however, if the tissue levels of P_t, 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 in order to assess the acquisition and internal utilization of P_i over the crop cycle, and the remobilization of P 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 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 x 11 x 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 ~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 1100hr 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 h–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; 20-50 μmol 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 P_i treatment regimens in otherwise complete nutrient solutions (MacDonald et al. 2014). The standard industry practice control consisted of 2.6 mmol L^{-1} P_i 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} P_i 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 P_i received over the crop cycle with the industry control treatment. A negative control treatment was not included. The P_i treatments were arranged as the main plot and the two cultivars ('Olympia' and 'Covington) as the sub-plot (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 weeks 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 + petioles, the remaining leaves (i.e., upper), upper stem + petioles, and inflorescence for determination of fresh mass, and then dried at 70 °C for 3 days 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 P_t 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 P_i 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, Oregon, 97015-0830, USA). P_o was calculated as the difference between P_t and P_i.

Statistical Analysis and Indices of P Use Efficiency

All statistical analyses were conducted using SAS 9.4 with the PROC GLIMMIX method at $\alpha = 0.05$ level (SAS Institute Inc., Cary, NC 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 (P_i 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 (ANOVA) were performed (N =4 treatment replicates) and when effects were significant, the means were compared to each other using Tukey's Honest Significant Difference (HSD) 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 P_i use efficiency (PUE_S = mg shoot DM /mg P_i input) and P_i uptake efficiency ($PUpE_S$ = mg shoot P content/mg P_i input), as well as content-based P use efficiency (PUE_C = mg shoot DM/mg shoot P_t content), P harvest index (PHI = mg inflorescence P content/mg shoot P_t content), and P utilization efficiency (PUtE = mg inflorescence DM/mg shoot P_t content) (Good et al. 2004). The P usage index (PUI = mg shoot DM x (shoot DM/mg shoot P_t content) accounts for the absolute increase in biomass (Siddiqi and Glass 1981). Inputs of nutrient solution and P_i 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 P_i 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 P_i treatment 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 P_i 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 P_i regimens employed here limited growth over the crop cycle, compared to 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 P_i delivery was reduced by up to 75% over the crop cycle. The supply-based indices, PUE_S and $PUpE_S$, 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 PUE_C, PUI, PHI and PUtE

in 'Olympia' plants grown under high-ambient light conditions. Across all treatments and ambient light conditions, 'Olympia' showed significantly greater values for PUI, PUE_S and $PUpE_S$ than 'Covington' ($P \leq 0.05$); under high-ambient light conditions 'Olympia' also showed a greater value for PUE_C ($P \leq 0.05$). 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 P_i delivery. Furthermore, they suggest that 'Olympia' utilized both external and internal P_i more efficiently than 'Covington'.

Information for calculation of the various indices for PUE reported above is provided in the Supplementary Files. Across the two experiments, the amounts of P_i 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 S1and Table S2). The mean tissue-P_t levels had a narrower range than that for the P_i inputs: the lower leaves, upper leaves, lower stem + petioles, upper stem + 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 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). The mean accumulation of P_t 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). Even though the roots were not examined in this study, these data suggest that 'Covington' was 75-78% as efficient as 'Olympia' in acquiring P_i at the corresponding P regimen. With high-ambient light conditions, 'Olympia' showed a slight decreasing trend in P_t with decreasing P_i delivery, although

the difference between the 2.6VR and 1.3V treatments was only 13% (Supplementary Table S6). 'Olympia' and 'Covington' acquired 18-37% and 13-25% , respectively, of the whole shoot-P at final harvest during the period of inflorescence development (Supplementary Tables S9-S10), 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-P_t status on the net changes in P contents of each stratum during inflorescence development. The fractionation of P (P_i, P_o and/or P_t) was determined for plants grown under high-ambient light conditions. Organic phosphate was clearly a more important constituent of P_t than P_i 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 P_t was lower in the leaves of 'Olympia', especially the upper leaves, than in the corresponding leaves of 'Covington'. Net changes in P_i and P_o contents in lower leaves of 'Olympia' were not correlated with tissue-P_t, whereas net changes in P_i contents in lower leaves of 'Covington' were moderately correlated with tissue-P_t (Fig. 5, Supplementary Table S11). Notably, the net changes in P_o contents in upper leaves of 'Olympia' were moderately correlated with tissue-P_t in a positive manner, whereas in 'Covington', they were moderately correlated in a negative manner. In contrast, the net changes in both P_o and P_i contents of the lower or upper stem + petioles in both cultivars were moderately to strongly correlated with tissue-P_t in a negative manner. In approximately half of these

cases, the significant correlations between net changes in P_o or P_i contents and tissue P_t were associated with significant correlations between net changes in P_t contents and tissue-P_t. 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 P_t contents and tissue-P_t in the lower stem + petioles and the upper stem + petioles of 'Olympia' and in the upper leaves and upper stem + petioles of 'Covington' (Fig. 6, Supplementary Table S12). Overall, the maximal losses of P_t from leaves, especially the upper leaves, exceeded those from stem + 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 + petioles were always associated with relatively high-P status. Furthermore, the findings establish that remobilized P_o and to a lesser extent P_i are more important sources of P for inflorescence development in 'Covington' than in 'Olympia', likely due to the higher P_t status at inflorescence emergence.

The highlights described above were derived from data provided in the Supplementary Files. Across the two ambient light conditions, the mean tissue-P_t levels in the lower leaves, upper leaves, lower stem + petioles and upper stem + 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). For high-ambient light plants in particular, 24-26%, 15-19%, 23-26%, and 26-28% of the P_t was present as P_i in the lower leaves, upper leaves, lower stem + petioles, upper stem + petioles of the

two cultivars at initial harvest, whereas 22-27%, 23-27%, 23-26%, and 25-27% was present as P_i in the corresponding strata, and 19-22% as P_i in the inflorescence at final harvest (Supplementary Tables S6-S7). Across all ambient light conditions and cultivars, the net losses of P from all strata could account for 2-44% of the total-P accumulated by the inflorescence (Supplementary Tables S9 and S10), suggesting that direct acquisition of P_i, 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_i 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_i 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_i 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 to 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} P_i in subirrigated potted miniature rose (Zheng et al. 2010), 1.2 mmol L^{-1} P_i and then 0.6 mmol L^{-1} P_i in the final stage of production for subirrigated

potted gerbera (Zheng et al. 2004), and 0.25 mmol L^{-1} P_i (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} P_i delivered over the vegetative growth phase only, and the corresponding tissue-P_t 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} P_i 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 P_i concentrations (López-Arredondo et al. 2017; Wang et al. 2018). For example, *Arabidopsis thaliana* PHT1;1 and PHT1;4 contribute to P_i uptake at both high (0.5 mmol L^{-1}) and low (2-5 µmol L^{-1}) external P_i with similar affinity (K_m = ~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 P_i, but it increases substantively in a linear fashion as availability decreases to 25-50 µmol L^{-1}; further decreases in P_i have little effect (Karthikeyan et al. 2002). *CmPHT1;2* expression is evident in roots, stems and leaves, and highest in the stems of vegetative plants grown under a high-P_i condition (300 µmol L^{-1}) (Liu et al. 2018). However, it is induced approximately five times in roots in the absence of P_i, but not at all in the stem

and leaves. The P_i uptake rate of plants in the presence of 300 μmol L^{-1} P_i is approximately twice that with 15 μmol L^{-1} P_i (low-P_i condition). While the tissue-P_t levels are similar under the high- and low-P_i conditions in both roots and shoots, the tissue-P_i levels are approximately 60-150% greater with high-Pi than low-P_i. Furthermore, the dry mass yields of the roots, but not the shoot, are reduced with low-P_i, 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 P_i, even when P_i acquisition is unaffected.

In the present study, the delivery of P_i to chrysanthemum over the crop cycle was modified by approximately 4-fold. Regardless of the ambient light conditions and the timing and level of P_i fertilization, the DM yields and P_t 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_i 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, but not all, 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 various plant organs/tissues, even though the concentrations of tissue-P_t never decreased below the sufficiency range (see discussion above). Notably, the levels of tissue-P_t 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_t (≤ 0.5% DM) in photosynthetic tissues of many grain crops, but as tissue-P_t 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_t.

Differences in biomass yields, tissue-P_t concentrations, and content-based P use efficiency suggest that 'Olympia' uses internal P_t more efficiently than 'Covington' (Rose and Wisuwa 2012). Notably, the accumulation of P_t 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 P utilization efficiency was less sensitive. Furthermore, the P utilization efficiency 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 P utilization efficiency, 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 P utilization efficiency.

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 producing plants of similar yield and quality (MacDonald et al. 2014; Shelp et al. 2017; Sutton et al. 2019; this paper). Optimization of the delivery for the

remaining macronutrients, as well as micronutrients, then 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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FIGURE LEGENDS

Fig. 1. Impact of P_i treatment on the dry mass 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} P_i 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} P_i 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 + petioles, ■; upper stem + petioles, ■; inflorescence, ■. Data represent the mean of four treatment replicates, each consisting of five biological replicates derived from two plants.

Fig. 2. Impact of P_i 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} P_i 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} P_i 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 P_i supply over the life cycle.

Fig. 3. Impact of P_i treatment on indices of P use efficiency in plants of two chrysanthemum cultivars under high-ambient light conditions (Experiment 1). 2.6VR received 2.6 mmol L^{-1} P_i 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} P_i during the vegetative stage only. Panels a, c, e, g, i and k represent 'Olympia', and panels b, d, f, h, j and l represent 'Covington'. Top panels: supply-based P use efficiency (PUE_S) = mg shoot DM/mg P_i input; middle panels: content-based P use efficiency (PUE_C) = mg shoot DM/mg shoot P; bottom panels: supply-based P_i uptake efficiency ($PUpE_S$) = mg shoot P /mg P_i input. P usage index (PUI) = mg shoot DM x (mg shoot DM/mg shoot P) ($x10^{-6}$); P harvest index (PHI) = mg inflorescence P/mg shoot P; P utilization efficiency (PUtE) = mg inflorescence DM/mg shoot P. The data represent the mean of four treatment replicates, each consisting of five biological replicates derived from two plants; bars sharing the same lowercased letters within cultivars and indices are not significantly different ($P \leq 0.05$).

Fig. 4. Impact of P_i treatment on indices of P use efficiency in plants of two chrysanthemum cultivars under low-ambient light conditions (Experiment 2). 2.6VR received 2.6 mmol L^{-1} P_i 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} P_i during the vegetative stage only. Panels a, c, e, g, i and k represent 'Olympia', and panels b, d, f, h, j and l represent 'Covington'. Top panels: supply-based P_i use efficiency (PUE_S) = mg shoot DM/mg P_i input; middle panels: content-based P use efficiency (PUE_C) = mg shoot DM/mg shoot P; bottom panels: supply-based P_i uptake efficiency ($PUpE_S$) = mg shoot P

/mg P_i input. P usage index (PUI) = mg shoot DM x (mg shoot DM/mg shoot P) ($x10^{-6}$); P harvest index (PHI) = mg inflorescence P/mg shoot P; P utilization efficiency (PUtE) = mg inflorescence DM/mg shoot P. The data represent the mean of four treatment replicates, each consisting of five biological replicates derived from two plants; bars sharing the same lowercased letters within cultivars and indices are not significantly different ($P \leq 0.05$).

Fig. 5. Relationship between changes in 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^{-1} P_i during both vegetative and reproductive stages; and 2.6, 1.95 or 1.3 mmol L^{-1} P_i during the vegetative stage only. Each datum represents a single biological replicate; blue squares, red triangles 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).

Fig. 6. Relationship between changes in total-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} P_i during both vegetative and reproductive stages; and 2.6, 1.95 or 1.3 mmol L^{-1} P_i 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).

Table 1. Impact of P_i treatment on the final morphological characteristics of two chrysanthemum cultivars grown under high- (Experiment 1) and low-ambient (Experiment 2) light conditions.

Cultivar/ P_i treatment[a]	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*a*	35.00*a*	10.14*a*	4.98*a*
2.6V	34.44*a*	35.10*a*	10.50*a*	4.79*a*
1.95V	33.73*a*	33.57*a*	10.81*a*	4.95*a*
1.3V	35.57*a*	32.55*a*	10.59*a*	5.34*a*
'Covington'				
2.6VR	26.38*a*	27.50*a*	9.71*a*	3.30*a*
2.6V	28.65*a*	28.75*a*	9.82*a*	3.25*a*
1.95V	28.87*a*	27.34*a*	10.04*a*	3.30*a*
1.3V	30.85*a*	26.61*a*	10.12*a*	3.56*a*
		Low-ambient light		
'Olympia'				
2.6VR	34.74*a*	56.73*a*	10.11*a*	5.04*a*
2.6V	37.09*a*	58.02*a*	10.48*a*	5.17*a*
1.95V	40.88*a*	58.43*a*	10.74*a*	5.65*a*
1.3V	40.48*a*	57.89*a*	10.80*a*	5.28*a*
'Covington'				
2.6VR	24.81*a*	40.11*a*	8.44*a*	3.23*a*
2.6V	27.11*a*	41.27*a*	8.87*a*	3.23*a*
1.95V	28.57*a*	40.57*a*	9.03*a*	3.55*a*
1.3V	28.49*a*	41.23*a*	8.81*a*	3.35*a*

Note: Means sharing a lowercase italic letter within columns and cultivars are not 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.

[a]2.6VR received 2.6 mmol L^{-1} P_i 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} P_i during the vegetative stage only.

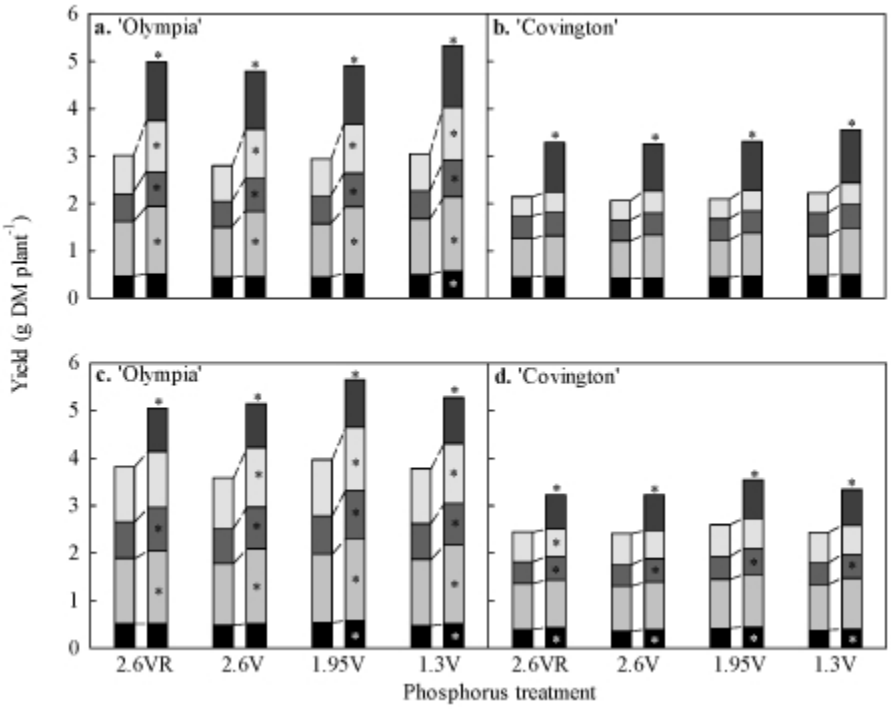
a. 'Olympia'
b. 'Covington'
c. 'Olympia'
d. 'Covington'
Yield (g DM plant-1)
0
1
2
3
4
5
6
2.6VR
2.6V
1.95V
1.3V
Phosphorus treatment

Fig. 1.

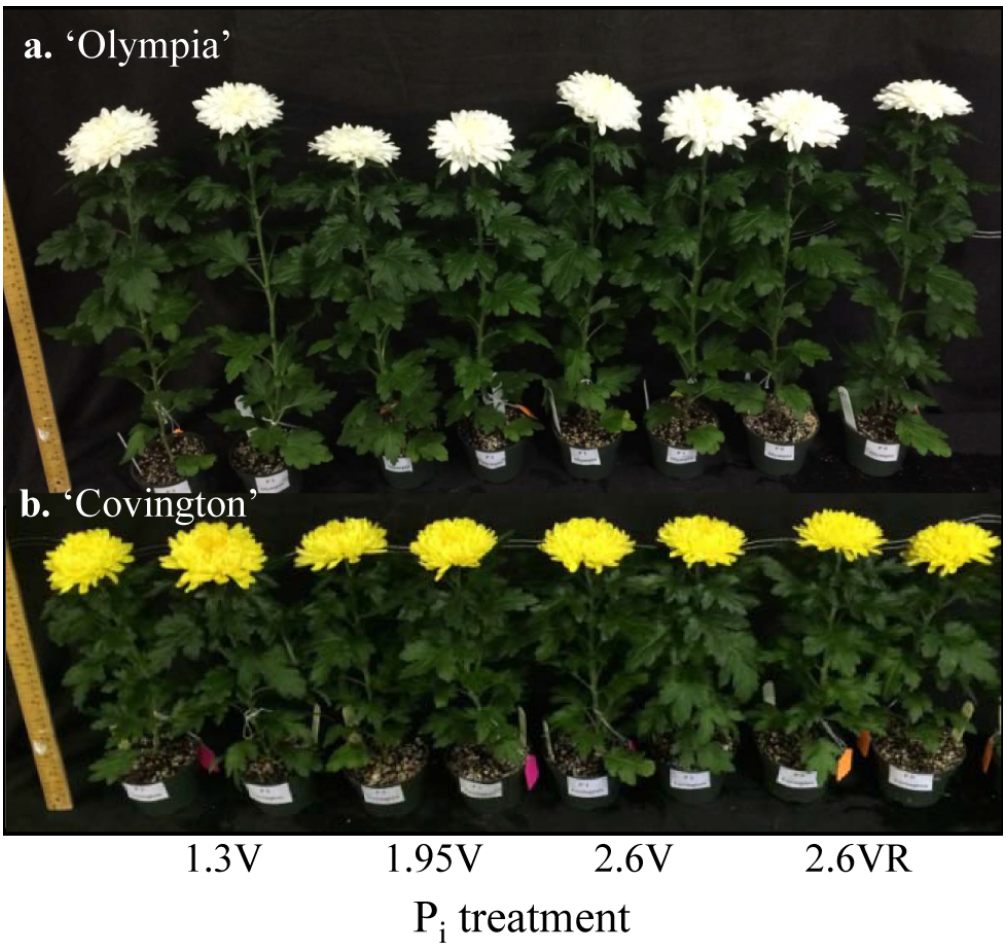

Fig. 2.

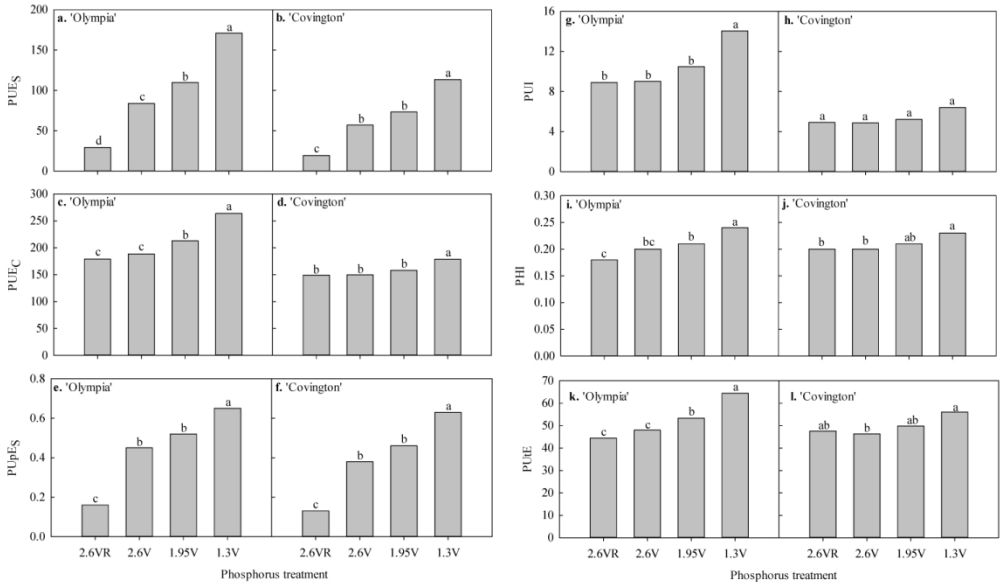
a. 'Olympia'
b. 'Covington'
g. 'Olympia'
h. 'Covington'
c. 'Olympia'
d. 'Covington'
i. 'Olympia'
j. 'Covington'
e. 'Olympia'
f. 'Covington'
k. 'Olympia'
l. 'Covington'
PUE$_S$
PUE$_C$
PUpE$_S$
PUI
PHI
PUtE
2.6VR
2.6V
1.95V
1.3V
Phosphorus treatment

Fig. 3.

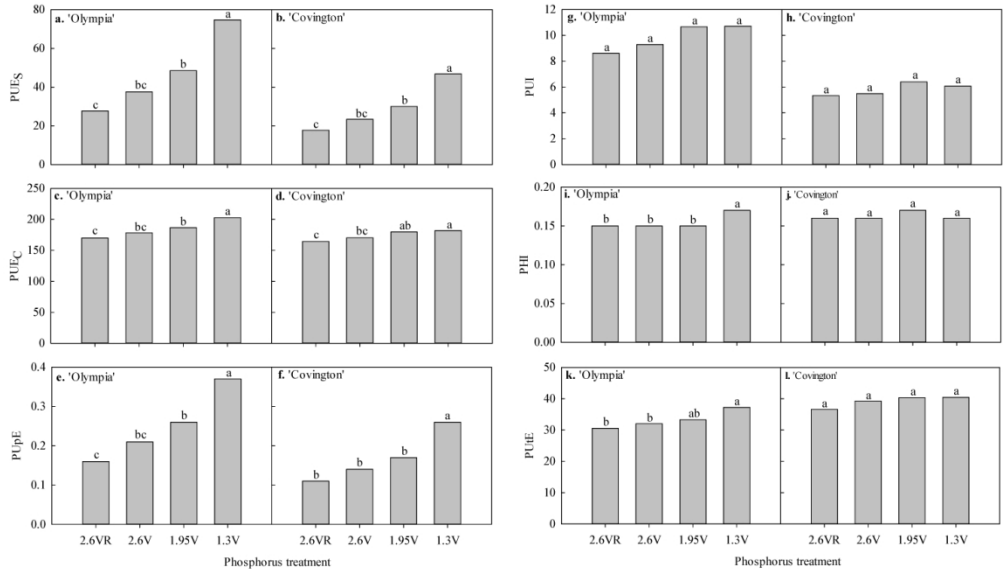
a. 'Olympia'
b. 'Covington'
g. 'Olympia'
h. 'Covington'
c. 'Olympia'
d. 'Covington'
i. 'Olympia'
j. 'Covington'
e. 'Olympia'
f. 'Covington'
k. 'Olympia'
l. 'Covington'
PUE$_S$
PUE$_C$
PUpE
PUI
PHI
PUtE
2.6VR
2.6V
1.95V
1.3V
Phosphorus treatment

Fig. 4.

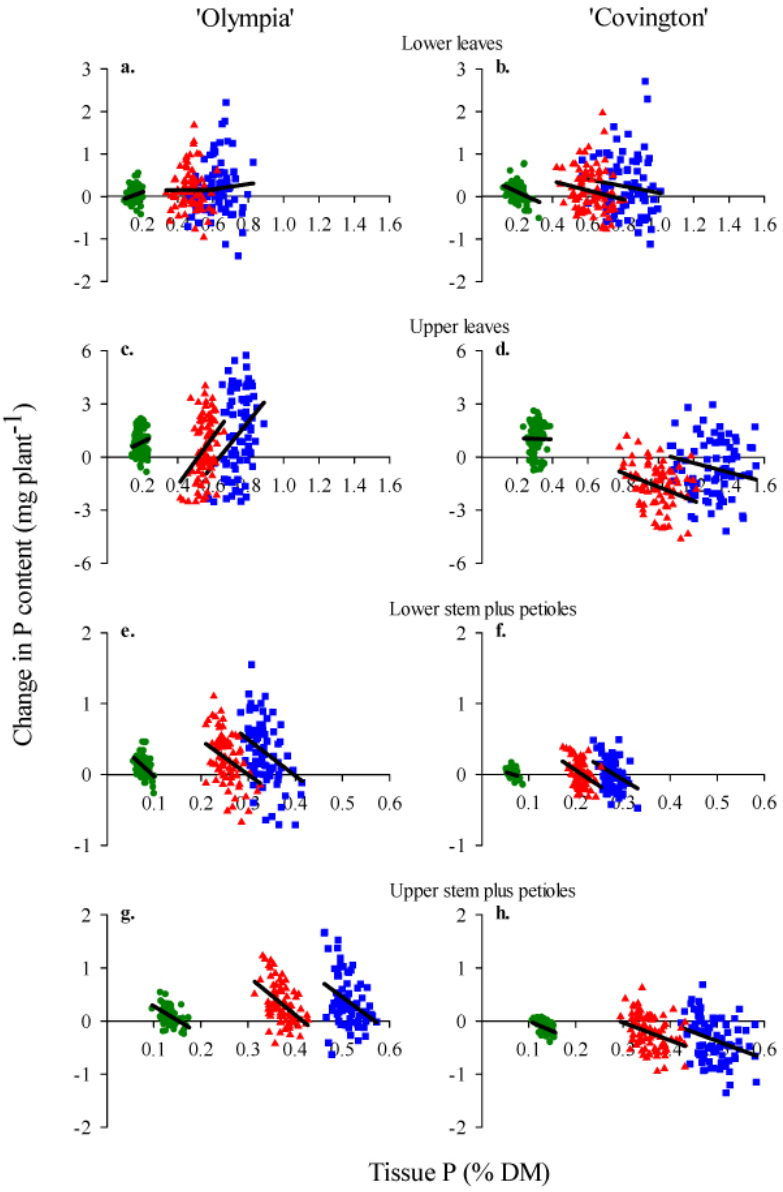
'Olympia'
'Covington'
Lower leaves
Upper leaves
Lower stem plus petioles
Upper stem plus petioles
a.
b.
c.
d.
e.
f.
g.
h.
Change in P content (mg plant-1)
Tissue P (% DM)

Fig. 5.

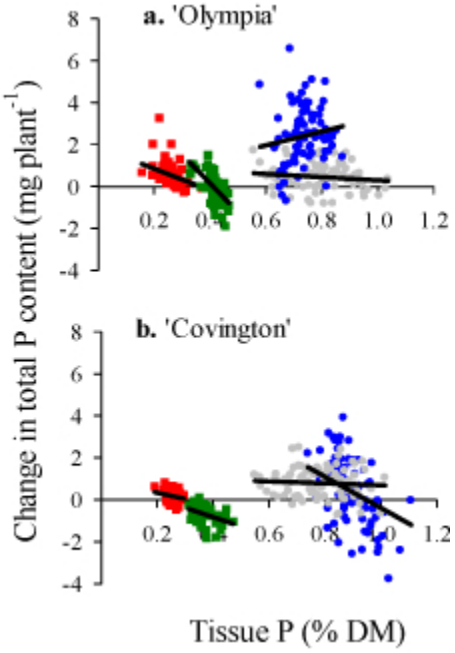
a. 'Olympia'
b. 'Covington'
Change in total P content (mg plant-1)
Tissue P (% DM)
8
6
4
2
0
-2
-4
0.2
0.6
0.8
1.0
1.2

Fig. 6.