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Response to Phosphorus Availability during Vegetative and Reproductive Growth of Chrysanthemum: I. Whole-plant Carbon Dioxide Exchange

Conny W. Hansen[1] and Jonathan Lynch
Department of Horticulture, The Pennsylvania State University, 103 Tyson Building, University Park, PA 16802

Carl-Otto Ottosen
Department of Ornamentals, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, 5792 Aarslev, Denmark

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ABSTRACT. **Whole-plant CO_2 exchange and root–shoot interactions during transition from vegetative to reproductive growth of 'Coral Charm' chrysanthemum (*Dendranthema ×grandiflorum* Ramat.) were investigated over a range of P concentrations considered to be deficient (1 µM), adequate (100 µM), or high (5 mM). Transition from vegetative to reproductive growth resulted in reduced photosynthate production, root respiration, biomass accumulation, and starch accumulation in leaves. Root respiration was low in high-P plants regardless of growth stage. Reduced root respiration may indicate changes in source–sink relationships during the transition from vegetative to reproductive growth, making roots less competitive sinks than developing flowers. Plant responses to P deficiency included decreased CO_2 assimilation and shoot biomass accumulation but increased root respiration, root:shoot ratio, specific leaf mass (SLM), and starch accumulation in leaves. Reduced root respiration activity in high-P plants was presumably due to differences in root architecture resulting in proportionately fewer root apices in high P. Daily CO_2 assimilation, shoot biomass, SLM, and root:shoot ratio were similar in plants grown with adequate-P and high-P availability, although plant P accumulation increased with P availability. Our results suggest that the excessive P fertilization often used in ornamental production systems is detrimental to root activity.**

Roots are strong sinks for photoassimilates during vegetative growth (Amthor, 1989). Transition from vegetative to reproductive growth may lead to decreasing root metabolism (Amthor, 1989; Kalleracka and Milburn, 1985; Hood et al., 1993), making the root a less competitive sink than developing flowers and fruit. However, only a few studies have described if and when such a change in root activity occurs. Hood et al. (1993) found that P uptake (measured by tissue P content) in snapdragons decreased during flower bud initiation and increased during the stage of visible buds to anthesis to reach a level similar to P uptake during vegetative growth. Smaller fluctuations were found in chrysanthemum (Boodley and Meyer, 1965), although P content in leaves increased after the visible bud stage and until anthesis.

Several studies have shown that low-P availability increases the root to shoot biomass ratio (e.g., Gutschick and Kay, 1995; Rao et al., 1993). Preferential biomass allocation to roots may represent a strategy to increase P acquisition in natural soils, in which P mobility is strongly limited.

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[1]To whom reprint requests should be addressed at Dept. of Ornamentals, Danish Institute of Agricultural Sciences, Kirstinebjergvej 10, 5792 Aarslev, Denmark; e-mail cwh@afp.sp.dk.

We hypothesized that the high levels of P fertilizer used in conventional horticultural production may reduce root growth, which may in turn increase plant sensitivity to postproduction stress. If so, reduced P fertilization might improve plant quality while simultaneously reducing undesirable environmental impacts from fertilizer effluents. The effects of P nutrition on root activity might be particularly important in floral crops, where reproductive growth creates additional competition for photoassimilates. The overall objective of this study was to test this hypothesis by analysis of whole-plant CO_2 exchange, biomass, and P dynamics in chrysanthemum as affected by P supply, with particular attention to the transition from vegetative to reproductive growth. This paper only includes the gas-exchange results, whereas biomass and P dynamics results are discussed in Hansen and Lynch (1997, 1998).

Materials and Methods

PLANT CULTURE. Cuttings of 'Coral Charm' chrysanthemum (*Dendranthema ×grandiflorum*) (Zander, 1993) were carefully selected for vigor and uniformity and propagated in a nutrient solution at 25% of the concentration of the P-free solution used during the experiment. In the nutrient solution used for propagation, P concentration was 1 µM. Stock plants and cuttings were kept under long-day conditions (18-h day and 6-h night) to ensure vegetative growth.

Four-week-old rooted cuttings were transplanted into a nonrecirculating, aerated hydroponic system containing 66 tanks (68 L each) and assigned randomly to three P treatments, two daylength treatments, and three blocks. Each tank contained six plants and was covered with foil to eliminate solar heating of the root zone. The stem was placed in a slit in a circular Styrofoam

sheet, which was fastened in an 11-cm-wide × 40-cm-deep plastic tube submerged in the tank to avoid tangling of roots from different plants.

Phosphorus treatment. Three P treatments were used: 1, 100, and 5 mM. These were considered to be low P, adequate P, and high P, respectively, and denoted as P1, P2, and P3. To maintain low and stable P concentrations, a solid-phase alumina-P buffer (Al_2O_3-P) technique as described in Lynch et al. (1990) was used in the two lower P treatments. Bags containing alumina-P buffer were placed directly in a P-free nutrient solution, so that the total amount of P taken up by the plants experiencing low-P and adequate-P conditions was provided from the alumina-P buffer. The P concentration in the high-P treatment was not substantially depleted and so was unbuffered; i.e., 5 mM KH_2PO_4 was added directly to the nutrient solution. The composition of the P-free nutrient solution was (in µM): 420 K_2SO_4, 1720 KNO_3, 1350 $Ca(NO_3)_2$, 320 $MgSO_4$, 830 NH_4NO_3, 500 $Mg(NO_3)_2$, 110 NaCl, 40 Fe-EDTA, 5 $MnSO_4$, 2 $ZnSO_4$, 0.6 $CuSO_4$, 8 H_3BO_3, and 0.008 $NH_4Mo_7O_{24}$.

Solution samples were collected periodically, and P content was analyzed as described by Murphy and Riley (1962). When the desorption P concentration began to decrease, the depleted buffer bag was replaced with a new one providing the desired P concentration. Solution electrical conductivity (EC) and pH were monitored three times a week and pH was adjusted with either 1.0 M KOH or 0.5 M HCl if necessary to maintain a pH of 5.5 to 6.0. The nutrient solution was replaced 4 weeks after transplanting (WAT), and an EC of 1.5 to 2.0 $mS\cdot cm^{-1}$ was maintained throughout the experiment.

Alumina-P buffer. Ten kilograms of alumina (Alcoa, grade F1, 28-48 mesh) was treated with 10 L of 0.3 M HCl. The solution was stirred and replaced every 2 h for 24 h, then rinsed with deionized water every 2 h for 12 h. Subsequently, pH was adjusted with 1.0 M KOH from pH 3 to equilibrium pH 5. Alumina was loaded with P by exposing the acid-treated alumina to 10-mM and 1-M KH_2PO_4 solutions, for P-deficient and adequate-P treatments, respectively, in the ratio of 10 kg alumina to 10 L solution. The P absorption solutions were replaced every 3 h for 72 h, then rinsed with deionized water every 1 h for 6 h to reduce the amount of excess P on the outer surface of the alumina particles.

Buffer bags made of double layers of nylon stockings and containing 500 g alumina-P buffer were placed in the aerated nutrient solution on the bottom of the hydroponic tanks. Each plant had separate aeration in the bottom of the plant tube to ensure an even distribution of P from the buffer bags.

Photoperiod treatment. Flower induction was controlled by modifying the natural photoperiod. All plants were covered with dark cloth after a 9-h (0800 to 1700 HR) photoperiod in naturally lit greenhouse conditions in State College, Pa. (lat. 41° N). Night-break lighting for 3.5 h (from midnight to 0330 HR) was given to half of the plants to maintain vegetative growth for comparison with reproductive plants. Incandescent light (1 $\mu mol\cdot m^{-2}\cdot s^{-1}$) used to prolong the photoperiod was installed underneath the dark cloth. The reason for covering all the plants from both daylength treatments was to ensure similar temperature and photosynthetic photon flux density (PPFD) in all treatments.

Growth conditions. Environmental data were monitored with 1-min intervals and averaged over 10 min during April through July 1994. Average day/night air temperature during the growth period was 22.1/20.1 °C. Minimum and maximum day/night air temperature was 18.5/18 °C and 28.8/22.5 °C, respectively. Only minor variations in solution temperature were seen diurnally and during the growth period. Average day/night solution temperature during the growth period was 21.6/20.6 °C. Average daytime relative humidity (RH) was 24%, and average nighttime RH was 67%.

During the CO_2-exchange measurements, plants were placed in 5.7-L PVC root cylinders in a nutrient solution similar to the solution for each P treatment in the hydroponic tanks. Rubber stoppers covered with parafilm sealed the base of the stem. Average solution temperature during measurements was 22.5 ± 2.9 °C.

PPFD varied considerably during the photoperiod; it was typically ≈1000 $\mu mol\cdot m^{-2}\cdot s^{-1}$ during midafternoon CO_2 exchange measurements and 600 to 800 $\mu mol\cdot m^{-2}\cdot s^{-1}$ during midmorning measurements. Cloudy days 5 WAT resulted in low average midmorning PPFD (228 $\mu mol\cdot m^{-2}\cdot s^{-1}$) and midafternoon PPFD of 604 $\mu mol\cdot m^{-2}\cdot s^{-1}$.

Data collection. Whole-plant CO_2 exchange was measured under greenhouse conditions to estimate actual rather than potential CO_2 exchange. The diurnal pattern of whole-plant CO_2 exchange was determined by measuring leaf and root CO_2 exchange at the following three times in a diurnal period: midmorning (2 h after the beginning of the light period), midafternoon (6 h after the beginning of the light period), and evening (6 h after beginning of the dark period). This represented one plant per treatment per day repeated three times a week at an interval of 2 d. Plants from different replications had the same physiological age. Measurements started 3 weeks after the plants were transplanted into the hydroponic tanks to provide a sufficient period for adapting to the new growth conditions and continued until anthesis. Net photosynthesis (P_N) and dark respiration from the whole aboveground biomass were determined with a portable photosynthesis system (LI-6200; LI-COR Inc., Lincoln, Nebr.) operated as a closed system. One of two cuvettes (27 or 36 L) was used depending on plant size. Photosynthesis and dark respiration were measured over a 20 or 30-s period, depending on cuvette size used, to avoid increases in air temperature and RH in excess of 1 °C and 2%, respectively. Carbon dioxide exchange data from the photosynthesis system were analyzed by linear regression to evaluate coefficients of variation and discarding outliers if r^2 was low. Low r^2 was typically due to an initial lag period (1 to 2 s) of CO_2 depletion inside the cuvette. After discarding outliers, $r^2 > 0.9$ was obtained. The linear correlation coefficient for dark respiration from shoots varied among P treatments from $r^2 > 0.8$ in P2 and P3 plants to $r^2 < 0.5$ in P1 plants.

Respiration from the entire, intact root system was determined using a Micro-carbon dioxide electrode (MI-720; Microelectrodes, Inc., Bedford, Mass.). The CO_2 electrode was connected to a pH meter (model 720A; Orion Research Inc., Boston). Calibration was performed by bubbling gas with known CO_2 concentrations through nutrient solutions similar to those in the P1 treatment, gaining a steady-state mV value in ≈30 min. The following formula from the manual was used to convert the percentage of CO_2 to solubility (S) (in $mol\cdot L^{-1}$):

$$S = (a/22.414) \times ((760 - p)/760) \times (r\%/100)$$

where a = absorption coefficient of gas at temperature; p = vapor pressure in mm Hg of water at temperature; r% = actual reading in percent CO_2.

Aeration of the solution was stopped during measurements, and the CO_2 increments observed from 5 to 10 min after the aeration was stopped were used to estimate root respiration. During root respiration measurements, the pH of the solution in the cylinders was kept at 4.5 to 5.0 to avoid the chemical binding of CO_2 as carbonate or bicarbonate. A considerable part of the root respiration data was discarded from the analyses due to technical difficul-

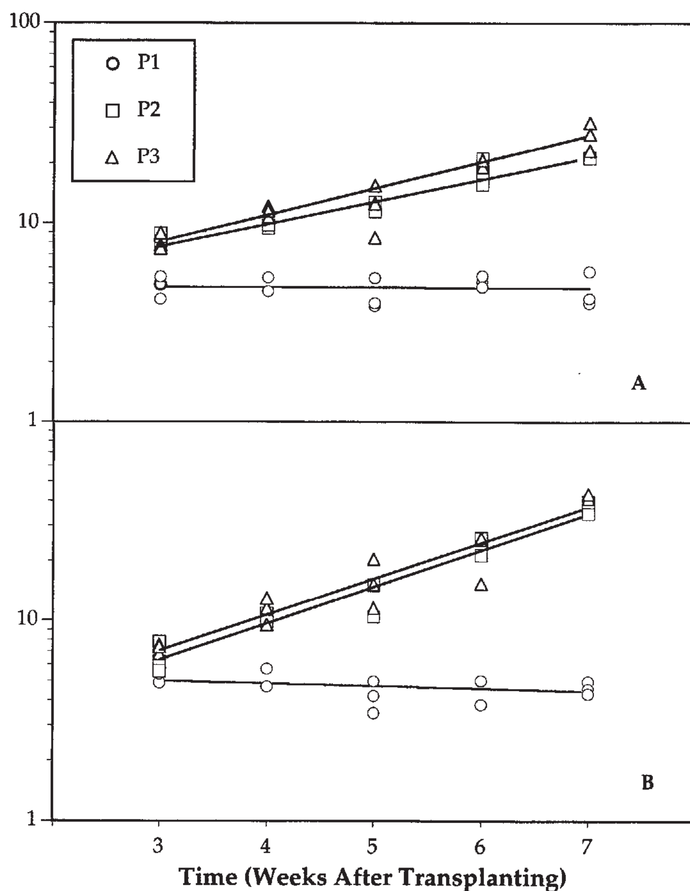

Fig. 1. Growth response over time for reproductive (**A**) and vegetative (**B**) plants expressed as the total plant dry mass. Individual replicate plants in a treatment are identified with a circle for P1 (1 μM P); square for P2 (100 μM P), and triangle for P3 (5 mM P).

ties in measuring root respiration of plants with a relatively small root mass or very low root respiration activity compared with the size of measuring cylinder.

A destructive harvest followed the whole-plant gas exchange measurements. Leaf area was estimated from 10 randomly selected leaf punches. The dry mass of the punches was used to calculate the area per unit dry mass of punches, from which total plant leaf area was estimated. Biomass was determined after plants were dried for 3 d at 70 ± 1 °C. Total N content of leaves was analyzed as described by Bremner (1965). At 11 WAT, young fully expanded leaves and roots were sampled at the end of a 15-h dark period for starch analyses. Starch content was assayed enzymatically as described by Åman and Hesselman (1984).

DATA ANALYSIS. A numerical method using the trapezoid rule as described by Lynch and Rodriguez (1994) was used to integrate instantaneous midmorning and midafternoon P_N and dark respiration from the entire shoot into daily leaf CO_2 assimilation. A similar numerical method was used to estimate integrated diurnal root respiration (DRR). Daily leaf CO_2 assimilation and DRR were calculated for each individual replicate plant and then averaged within each treatment group. Numerical differentiation of leaf area was performed to obtain values for leaf area development over time. Differentiation was based on least squares fitting of polynomials by a three-point formula (Erickson, 1976).

Daily C budgets were estimated on a whole-plant basis by indexing the values of daytime CO_2 assimilation, dark respiration from shoot, nighttime root respiration, and daytime root respiration with daytime CO_2 assimilation as 100. Daily C gain was defined as daytime CO_2 assimilation not lost to respiration.

Results were analyzed statistically in SAS (SAS Institute, Cary, N.C.) as a randomized complete-block design. The following data were logarithmically transformed allowing data to be normally distributed before analysis of variance by SAS's general linear models (GLM) procedure: daily CO_2 assimilation, total dry mass, leaf area, and leaf N content. The statistical models were reduced by stepwise discarding of nonsignificant variables ($p > 0.05$). Least square means (LSM) and standard errors of means are presented in the figures. Root respiration data are presented as mean values instead of LSM values in Table 1 due to missing values.

Results

TRANSITION TO REPRODUCTIVE GROWTH. Transition from vegetative to reproductive growth resulted in reduced total biomass accumulation (Fig. 1), and root biomass accumulation tended to decline in reproductive plants (Table 1). Significantly less leaf area in reproductive plants occurred from 5 WAT (Fig. 2A). Differences in the rate of leaf area development occurred at 3 WAT (Fig. 3); it took 2 weeks for the effect of the altered rate to manifest itself as altered leaf area. A similar decrease occurred in daily CO_2 assimilation (Fig. 2B). Although the highest leaf N content was seen in reproductive plants (Fig. 4), total leaf N declined slightly over time regardless of P availability and growth stage (data not shown).

Considerable variation in instantaneous values of whole-shoot CO_2 assimilation among replicate plants was interpreted as a result of measuring CO_2 assimilation at variable PPFD under greenhouse conditions. Instantaneous values of midmorning CO_2 assimilation were significantly ($p < 0.01$) higher than midafternoon CO_2 assimilation, even though PPFD typically was 200 to 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$ higher during midafternoon measurements (data not shown).

Vegetative plants showed a higher DRR (when expressed on a root dry matter basis and on a whole-plant basis) than reproductive plants when grown with deficient-P and adequate-P availability

Table 1. Diurnal root respiration incorporates day and night root respiration giving an estimate of the root respiration activity for a 24-h period. Root respiration is expressed here on a root dry matter and on a whole-plant basis. Each value is the mean of 5 to 10 plants (standard error in parenthesis).

	Diurnal root respiration (CO_2)					
	Specific root dry mass ($mmol \cdot g^{-1} \cdot d^{-1}$)		Total plant ($mmol \cdot d^{-1}$)		Root dry mass (g)	
P treatment	Vegetative	Reproductive	Vegetative	Reproductive	Vegetative	Reproductive
P1	43.0 (3.7)	20.6 (2.0)	42.5 (7.6)	38.9 (5.3)	1.4 (0.1)	1.3 (0.1)
P2	35.4 (11.0)	14.9 (7.1)	45.1 (8.2)	25.6 (8.4)	2.3 (0.1)	2.2 (0.1)
P3	16.2 (2.2)	17.6 (10.3)	35.9 (5.8)	18.2 (7.2)	2.4 (0.1)	2.2 (0.1)

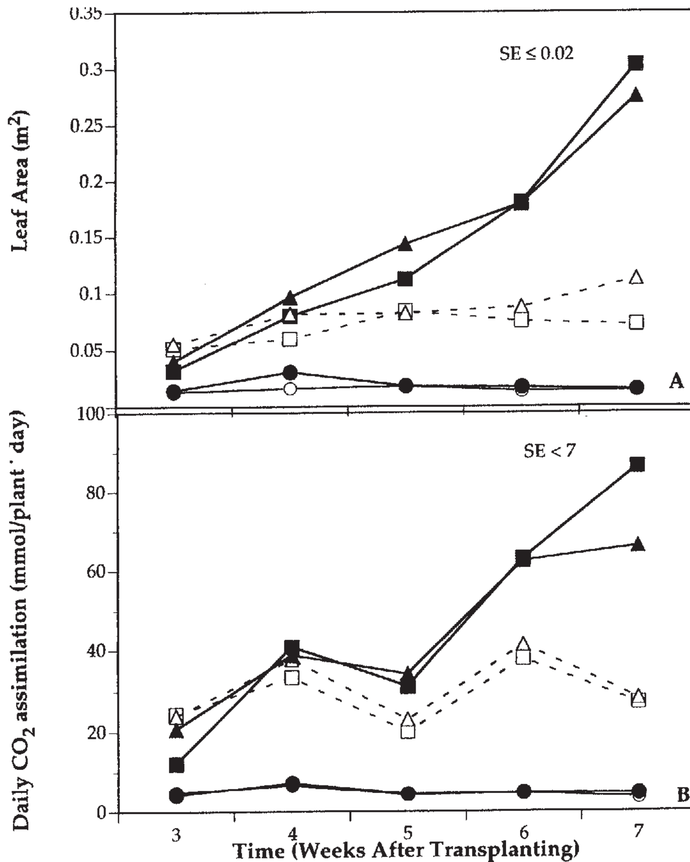

Fig. 2. Leaf area (**A**) and Daily P_N (**B**) estimated from whole-shoot CO_2 exchange including daily net photosynthesis and dark respiration from shoots giving an estimate of net P_N for a 24-h period. Circle = low P; square = adequate P; triangle = high P. Open symbols for reproductive plants; closed symbols for vegetative plants. Each point is the mean of three replicate plants.

and likewise in high-P plants when expressed on a whole-plant basis (Table 1).

Although reproductive plants tended to have a slightly higher shoot C gain, the decline in CO_2 assimilation coinciding with a decline in root respiration resulted in no substantial differences in shoot C-gain between vegetative and reproductive plants. Daily C budgets estimated from whole-plant CO_2 exchange measured 6 weeks after transplanting are shown in Fig. 5.

Substantially less starch accumulated in leaves of reproductive compared with vegetative plants, but there was no significant difference ($p < 0.5$) in specific leaf mass (SLM) (Table 2).

Effects of p availability. Plants grown with low P exhibited small dark-green leaves, wilting of basal leaves, and a reddish coloration on the basal stem, whereas plants grown with adequate P and high P showed no visual symptoms of nutrient deficiency. Plants grown with the two highest P concentrations did not differ significantly in total biomass accumulation (Fig. 1), leaf area (Fig. 2A), and total leaf N content (expressed on a leaf area basis in Fig. 4). This resulted in similar daily CO_2 assimilation of plants grown with adequate P and high P (Fig. 2B).

Root respiration capacity (DRR per unit root dry mass) was negatively correlated with P availability (Table 1) for vegetative plants. The difference in DRR was less pronounced when expressed on a whole-plant basis where the highest root respiration was seen in plants grown with adequate P. Excluding data from two time points having a high frequency of missing values resulted in F and p values as shown in Table 2. The root respiration capacity declined slightly over time except for 4 WAT, when a generally low root respiration was seen (data not shown).

Nighttime root respiration affected the daily C budget more than daytime root respiration, and dark respiration from shoots only insignificantly influenced the daily C budgets (Fig. 5). A high root respiration activity (Table 1) coinciding with a relatively low rate of photosynthesis in P-deficient plants (Fig. 2B) resulted in an overall daily C loss (Fig. 5) and premature senescence of basal leaves, whereas the net shoot C gain was similar for plants grown with adequate P and high P.

Phosphorus-deficiency responses included reduced total biomass accumulation (Fig. 1), a low leaf area (Fig. 2A) resulting in low daily CO_2 assimilation (Fig. 2B), and a high root:shoot ratio (Hansen and Lynch, 1997, 1998), reflecting that root growth was favored over shoot growth. Total leaf N content, expressed on a leaf area basis, was significantly higher in P-deficient plants compared with adequate-P and high-P plants (Fig. 4).

SLM increased up to 38% in P-deficient plants compared with nondeficient plants (Fig. 6), and the highest amount of starch was present in leaves of vegetative P-deficient plants (180 $mg \cdot g^{-1}$), which was 6% higher than in adequate-P plants, and 4% more than in high-P plants. Considerably less starch accumulated in leaves of reproductive plants (3 to 99 $mg \cdot g^{-1}$) and, conversely to vegetative plants, starch content increased with P availability. Very low levels of starch were found in roots compared with leaves: from 0.4 in P-deficient plants to 1.9 $mg \cdot g^{-1}$ root dry mass in adequate-P and high-P plants, regardless of growth stage.

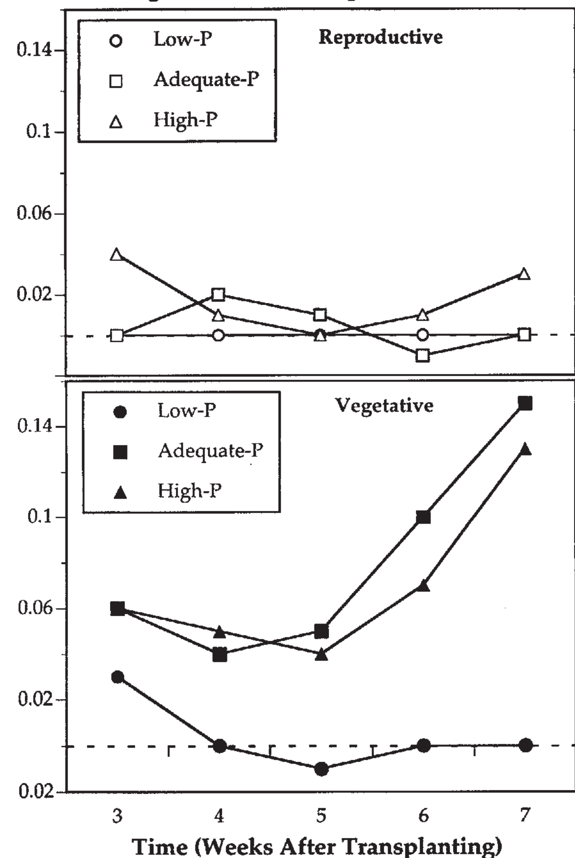

Fig. 3. Rate of leaf area development in vegetative and reproductive plants as influenced by P availability. Numerical differentiation of leaf area data was performed to obtain values for the rate of change in leaf area over time. Each point represents the differentiated value of the mean of three replications.

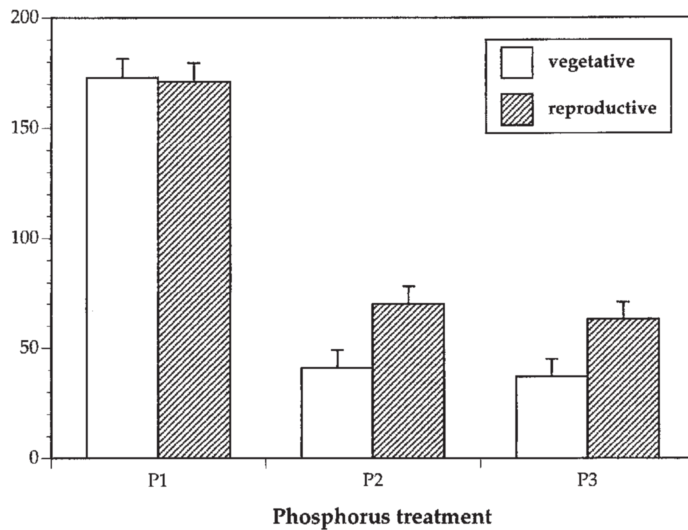

Fig. 4. Total leaf N content of vegetative and reproductive plants at three P treatments. Each point is the mean of 15 plants (3 replications × 5 weeks after transplanting) and error bars represent SEs of the mean.

Discussion

Leaf biomass accumulation, leaf area, and daily CO_2 assimilation were quite similar in vegetative and reproductive plants until 5 WAT, coinciding with the time of visible buds occurring in plants grown with short photoperiod regardless of P availability. Differences in the rate of change in leaf area over time already occurred from 3 WAT (Fig. 3), which may indicate that plants grown with short photoperiod were floral induced 3 weeks after treatment initiation. The significant decline in these parameters found in reproductive compared with vegetative plants after 5 WAT reflect that some changes in the source–sink relationships took place during transition from vegetative to reproductive growth, with the developing flower buds being major sinks. The reduced root respiration and root biomass accumulation in reproductive compared with vegetative plants likewise indicate changes in the metabolic activity in roots as a consequence of transition to reproductive growth, making the root a less competitive sink than developing flowers. This is supported by a general decline in the component production rate (CPR) of roots and leaves but increasing CPR_{stem} (including developing flowers) in reproductive compared with vegetative plants grown with the two highest P concentrations (Hansen and Lynch, 1997, 1998), showing a higher commitment of the whole plant to the production of developing flowers than to roots and leaves. Further, the decline in starch accumulation in leaves of reproductive compared with vegetative plants may indicate remobilization of stored photoassimilates from leaves to developing flowers reflecting increased sink demand by developing flowers.

Most leaf N is located in the proteins of the Calvin cycle and thylakoids; therefore, photosynthetic capacity is strongly related to the N content of leaves (Evans, 1989). When P was not deficient, vegetative plants had significantly less leaf N content than reproductive plants although photosynthesis was significantly higher. This may indicate a lower photosynthetic capacity per unit of N in reproductive compared with vegetative plants. However, significantly higher leaf area, leaf dry matter content, and RGR (Hansen and Lynch, 1997, 1998) in vegetative compared with reproductive plants suggest that the decline in leaf N may be explained by the N content being diluted in more tissue. The quantity of ribulose bisphosphate carboxylase (Rubisco) increases as young leaves develop until they are fully expanded, and if young leaves had a lower N content per unit leaf area than older fully expanded leaves, this may account for the difference between vegetative and reproductive plants. The low leaf area in P-deficient plants accounts for the high N content when expressed on a leaf area basis considering that the total leaf N (in units of percent dry mass) was lower in P-deficient plants (2.7% ± 0.05%) than in nondeficient plants (4.7% ± 0.05%).

The large general decrease in daily CO_2 assimilation measured in plants from all treatments at 5 WAT was due to the low PPFD in this period.

PPFD levels giving maximal photosynthetic rates are for most species considerably higher on a whole-plant (canopy) basis than for single leaves (Jones, 1992) due to internal shading. PPFD in our study is considered to be below light saturation for canopy photosynthesis in this species. We interpret the decrease in midafternoon

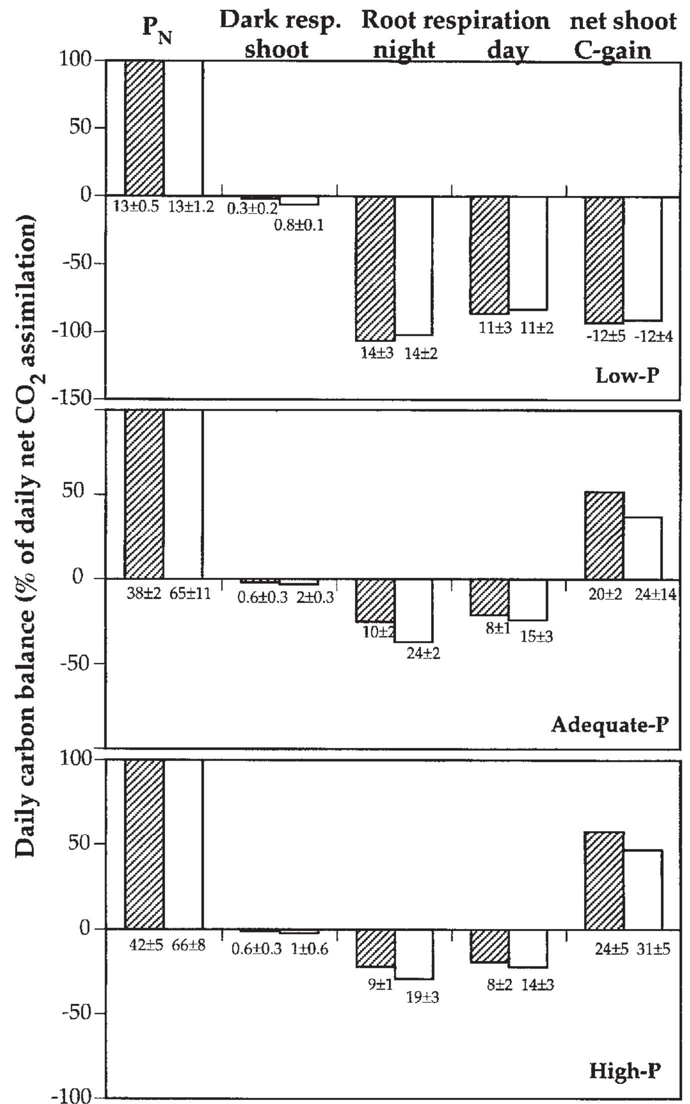

Fig. 5. Daily C budget estimated on a whole-plant basis as values of photosynthesis, dark respiration from shoot, night time, and daytime root respiration, indexed with photosynthesis as 100. All data shown in this figure refer to CO_2 exchange measurements obtained 6 weeks after transplanting. The number at each column is the mean value of two to three plants (in mmol/plant/d) followed by standard deviation.

Table 2. Summary of F values from the analyses of variance (GLM) followed by the p values in parenthesis. F values are from successive models reduced by stepwise discarding of NS variables.

Parameter	Root respiration	CO_2 assimilation	Total dry mass	Root dry mass	Leaf area	SLM	Leaf N content	*df*
P treatment (P)	3.2^{NS,*z}	311***	511***	62***	557***	126***	131***	2
Daylength (DL)	2.1^{NSy}	10**	7**	3^{NS}	35***	<1^{NS}	26***	1
WAT	7.1**	10***	96***	<1^{NS}	32***	4**	21***	4
P × DL	0.7^{NS}	1^{NS}	2^{NS}	139***	7**	1^{NS}	11***	2
P × WAT	4.1*	3**	28***	8***	7***	4***	5***	8
DL × WAT	0.2^{NS}	5***	4**	8***	12***	2^{NS}	7***	4
P × DL × WAT	---^{x}	1^{NS}	2*	3*	3**	1^{NS}	2^{NS}	8

^{z}Value is 4.77 (<0.05) when excluding P1 from the analysis.
^{y}Value is 4.77 (<0.04) when excluding P3 from the analysis
^{x}Not included in the analysis.
^{NS,*,**,***}Nonsignificant or significant at $p = 0.05$, 0.01, or 0.001, respectively.

compared with midmorning CO_2 assimilation as a midday depression of P_N. The midday depression in our study may be explained by stomatal closure during peak evaporative demand midday as a response to increasing light and temperature under very low RH (down to 10% RH in the middle of the day), a strategy to avoid severe water-stress conditions. Lynch and Rodriguez (1994) compared integrated values for daily P_N from three data points using the trapezoid rule with values obtained from integrating over 13 to 15 data points using Newton's method (Burden et al., 1978). They found the trapezoid method to be an acceptable alternative to more frequent diurnal measurements for estimating daily P_N. Considering the midday depression found in our study and similar time points for measuring instantaneous P_N during the diurnal period, we find it reasonable to estimate Daily P_N using the trapezoid rule after the formula described by Lynch and Rodriguez (1994).

Quite similar biomass accumulation, leaf area, leaf N content, CO_2 assimilation, and SLM in plants grown with adequate P and high P indicated that there were no quantitative benefits from using high external P concentrations.

Root respiration was found to be positively correlated with root growth rate for a number of species (Van der Werf et al., 1989; Poorter et al. 1991). In our study there were no significant differences in root growth rates of plants grown with adequate- and high-P availability (Hansen and Lynch, 1997, 1998), so growth rate cannot explain the reduced root respiration activity in high-P plants. The difference in root respiration could also be due to differences in the respiratory costs for synthesis of root biomass or the cost of nutrient acquisition. Since the biggest cost to roots in terms of ion uptake is usually from N, plants with high rates of N uptake per unit root mass may have higher respiration rates (Eissenstat, 1992). We observed the lowest total N content per unit root mass in high-P plants (1.55 $g·g^{-1}$) compared with 1.74 $g·g^{-1}$ in adequate-P and 1.90 $g·g^{-1}$ in low-P plants. Changes in root architecture in response to P availability (Nielsen et al., 1994) may explain differences in root respiration. Analysis of the number of basal and higher-order roots in scanned images of root subsamples showed that plants adapted to high-P conditions had 37% fewer higher-order roots per unit root length than adequate-P plants, indicating differences in root topology (Hansen and Lynch, 1997, 1998). Since respiration is highest in the meristematic zone (Nielsen et al., 1994), a reduced number of growing points in high-P plants could account for the low root respiration activity. In spite of the reduced root respiration in high-P plants, P uptake expressed as the total P tissue content increased with P availability (Hansen and Lynch, 1997, 1998). Phosphorus-deficient plants had a more herringbone root system topology than nondeficient plants (Hansen and Lynch, 1997, 1998). It has been proposed that a herringbone root system is more expensive to construct and maintain (Fitter and Stickland 1991; Nielsen et al., 1994), which may explain the high root respiration in P-deficient plants in our study. In hydroponically grown plants, roots are not exposed to the same physical resistances (a type of stress) that they would experience in the soil or in an artificial substrate in a container. Although such physical resistance may influence root architecture, low-P plants of different species have been shown to have a herringbone topology in sand culture systems (Fitter and Stickland, 1991). We would therefore not expect the root respiration results to be very different if plants were grown in a different substrate.

SRL declined in low-P plants compared with nondeficient plants, suggesting that P-deficient plants had a lower ratio of fine to thick roots, in contrast to observations of Snapp and Lynch (1996) in common bean. Fine roots usually have higher respiration per gram root dry mass than thick roots, so differences in root topology may account for the high root respiration in P-deficient plants. Reduced lateral branching of roots in P-deficient plants is probably a strategy to enhance root elongation and thereby the exploitation efficiency.

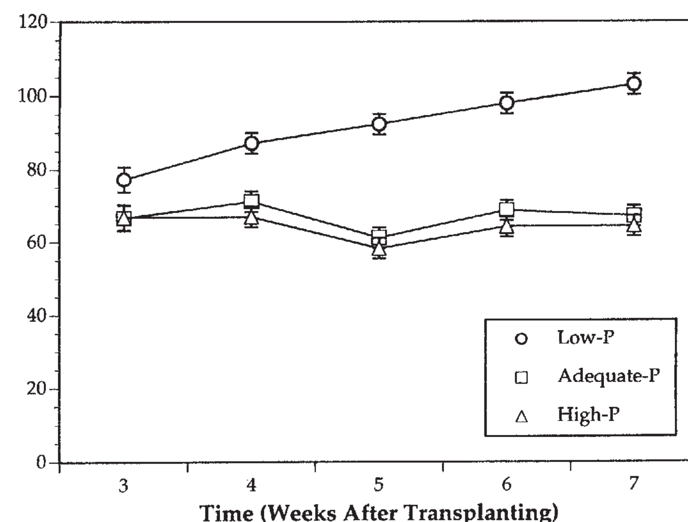

Fig. 6. Specific leaf mass (SLM) expressed as the total leaf mass per unit leaf area. Each point represents the mean of six plants (three replications × two daylength treatments). Error bars represent SES of the mean.

Participation of the alternative nonphosphorylating respiratory pathway was suggested by Hoefnagel et al. (1992) to occur primarily during nutrient deficiency in the presence of excess sugar and may have contributed to the relatively high root respiration in our low-P plants. If P is not deficient, the cytochrome pathway is mainly involved in root respiration (Lambers and Rychter, 1990; Rychter and Mikulska, 1990). Although the contribution of the alternative pathway to root respiration varies with plant species (Poorter et al., 1991), it indicates some metabolic changes are induced by P deficiency to accommodate the shortage of P. This allows respiration to proceed even under very low P conditions (Theodorou and Plaxton, 1993) and thereby permit temporary survival under unfavorable conditions.

Root respiration is known to be affected by root zone temperature (Rice and Eastin, 1986), and the relatively large variation in root respiration among replicate plants (Table 1) may have been due to measuring CO_2 exchange under greenhouse conditions where no attempt was made to control solution temperature. Later studies have shown considerably less variation when measuring under controlled solution temperature (unpublished data). Further improvement of the method may be obtained by matching cylinder size with root size.

The early and persistent overall C loss from the shoots of P-deficient plants was because root growth was favored over shoot growth, resulting in a substantial suppression of whole-plant photosynthesis and a high root respiration. Although phloem transport can occur over large distances, sinks are generally supplied with photosynthates from a nearby source (Wardlaw, 1990). Wilting of basal leaves in P-deficient plants may suggest that C supply to the roots was mainly translocated from basal leaves and support the findings of Palit (1985). Considering the overall C loss in P-deficient plants, we believe that our low-P treatment was very stressful. Most P-deficiency studies use P starvation or at least unbuffered conditions, which may result in even more severe stress symptoms than described here.

Several species of plants grown under P deficiency accumulated carbohydrate reserves in the form of starch in the stems and leaves (e.g., Heldt et al., 1977; Fredeen et al., 1989; Qiu and Israel, 1992; Terry and Rao 1991; Walker, 1980). This indicates that growth is retarded more than photosynthesis and that carbohydrate use rather than availability is limiting plant growth under P deficiency (Qiu and Israel, 1992). One of the major factors limiting growth of P-deficient plants is thought to be the regulatory function of orthophosphate (P_i) in photosynthesis and C metabolism of leaves (Heldt et al., 1977). Metabolic P_i declines after exposure to long-term P deficiency (Theodorou and Plaxton, 1993) and starch accumulation occurs in P-deficient plants as a result of reduced export–import of triose-P and P_i between the chloroplast and cytosol (Heldt et al., 1977; Terry and Rao 1991). The higher levels of starch accumulations found by Radin and Eidenbock (1986); Fredeen et al. (1989); Terry and Rao (1991); Qiu and Israel (1992) compared with our findings may be explained by P starvation or low and unbuffered P conditions used in these studies resulting in more severe P stress responses.

The substantial increases in SLM in low-P plants compared with nonstressed plants were of the same magnitude as in Rao et al. (1990), who found that only up to 9% of the increase was due to starch accumulation, suggesting that the rest of the increase was due to other C compounds such as cell wall polymers.

We conclude that, although P deficiency decreased photosynthesis and leaf area significantly, high SLM and leaf starch accumulation in P-deficient plants indicate that low P affected growth processes in chrysanthemum more than photosynthetic capacity.

Substantially less starch accumulated in leaves of reproductive than vegetative plants, reflecting increased sink demand by developing flowers and remobilization of stored photoassimilates from leaves to developing flowers.

We showed that it is possible to maintain a high root activity without affecting the shoot biomass considerably by exposing plants to reduced and more buffered compared with high-P conditions. This indicates that the high-P concentration used in this study exceeded the concentration required for maximum growth of chrysanthemum and may be considered as excess P availability. Critical P concentrations down to 3 μM have been shown to be adequate for maximum growth of different species grown in hydroponic (First et al., 1987) and sand culture systems (Lynch et al., 1991). Although the external P concentration required for maximum growth may vary considerably with the growth medium used, we believe that high-P conditions (in the mM range) often used in scientific studies and in modern horticultural systems are well above critical P concentration required for maximum growth and may even be deleterious. If so, this suggests that reduced P fertilization could be of economic importance to the horticultural industry, in addition to reducing environmental pollution.

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