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Growth and physiology of aspen supplied with different fertilizer addition rates

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Variable internal plant nutrient content may confound plant response to environmental stress. Plant nutrient content may be controlled with relative addition rate techniques in solution culture. However, because raising large numbers of plants in flowing solution culture is difficult, we investigated the feasibility of raising plants in soil mix using relative fertilizer additions. Aspen (*Populus tremuloides* Michx.) clones (216, 259 and 271) planted in pots containing a peat, sand and vermiculite (2:1:1, v/v/v) soil mix were grown with exponentially increasing fertilizer concentrations and harvested periodically to assess growth. Addition rate treatments ranged from 0.01 to 0.05 day^{-1}. The lag phase of growth, in which plants adjusted to the fertilizer regime, lasted 40 days after which plants entered the experimental period characterized by constant relative growth rates equivalent to applied fertilizer addition rates. Total plant nutrient concentration was (1) unique for each addition rate, (2) linearly related to addition rate and growth rate, and (3) tended to increase at the highest, and decrease at the lowest addition rates. Regardless, the plants appeared to have attained steady-state conditions. Allocation of carbon to roots increased with lower addition rate treatments and was not dependent upon ontogeny. There were no treatment differences in growth response among aspen clones. Yet there were treatment differences in leaf chlorophyll and photosynthesis within the clones. For the 0.05 day^{-1} addition rate treatment, chlorophyll, leaf N concentration and photosynthetic rate were strongly correlated with one another, were at a maximum in recently mature leaves, and rapidly declined with leaf age. The rate of decline in these leaf characteristics was slowest in clone 271, consistent with the leaf longevity stress response reported elsewhere. Plant responses from these relative fertilizer addition trials in soil mix agree closely with those run in hydroponics, indicating that steady-state nutrition can be achieved with a technically simple experimental assemblage.

Key words – Allometry, aspen, chlorophyll, nitrogen, photosynthesis, plant growth analysis, *Populus tremuloides*, steady-state nutrition.

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Introduction

Examining the response of plants to interacting environmental factors is often complicated in greenhouse or controlled environment studies by changes in plant growth rates and tissue nutrient concentrations. For instance, experiments assessing plant response to trace gases such as elevated CO_2 and ozone show that plant nutritional status is an important interacting factor; yet, many such experiments use traditional nutrient application regimes where plant growth rate and/or internal nutrient concentration are not constant over the experimental period (Pell et al. 1990, 1995, Brown 1991, Tjoelker and Luxmoore 1991, Sinclair 1992, Bazzaz and

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Miao 1993, Coleman et al. 1993). Steady-state plant growth and nutrition can be maintained with relative nutrient addition rates (Ingestad and Lund 1979, 1986, Ingestad 1982, Jia and Ingestad 1984, Ågren 1985, Ingestad and Kähr 1985). With relative addition techniques, changes in relative growth rate or internal nutrient concentration during an experiment are minimized, making it possible to examine directly the effects of other environmental factors on plant morphology and physiology (cf. Ingestad and McDonald 1989, Pettersson et al. 1993).

Relative nutrient addition rate techniques typically involve technically complex hydroponic systems. Nutrient solutions are circulated past the root system and through automatic monitoring and replenishment devices that maintain relative addition rate while tabulating nutrient usage (Ingestad and Lund 1979, 1986, Freijsen and Otten 1987, MacDuff et al. 1993). Such systems are not easily assembled without specialized knowledge, and do not readily accommodate large numbers of replicate plants required to test simultaneously multiple interacting stress factors. Relative addition rate techniques have not been widely adopted because of these requirements. Furthermore, root systems grown in hydroponics are not representative of soil-grown roots because of differences in soil resistance encountered and the availability of nutrients at the root surface (cf. Taylor 1974, Nye and Tinker 1977). Although there have been relative addition rate experiments with solid media (Granhall et al. 1983, Burgess 1990, 1991, Timmer et al. 1991, Imo and Timmer 1992, Stewart and Lieffers 1993, Wait et al. 1996), there is a need to develop less complex relative addition rate techniques that apply appropriate fertilizer treatments to soil-grown plants.

The present paper reports the successful application of relative fertilizer additions to potted plants using a simple trickle irrigation technique. Because these experiments were designed to establish treatment protocol for subsequent experiments with atmospheric trace gases, plant growth and physiological responses were analyzed to establish plant response to steady-state nutrition.

When relative fertilizer addition rates were applied to potted plants, steady-state growth and nutrition were obtained, as well as strong linear relationships between nutrient addition, plant nutrient content and plant growth rate. The growth and nutrition responses observed were equivalent to those found in the more complex hydroponic systems, although response variability was greater in this potting-soil-based system.

Abbreviations – K, allometric constant; LPI, leaf plastochron index; R_A, relative addition rate; R_G, relative growth rate; R_U, relative uptake rate; s, start day; t, application day.

Materials and methods

Plant material

Greenwood cuttings (approximately 10 cm long) of three trembling aspen (*Populus tremuloides* Michx.) clones (216, Bayfield Co., WI, USA; 259, Porter Co., IN, USA; 271, Porter Co., IN, USA) were rooted under mist in 40 × 20 × 8 cm trays containing perlite and peat (3:1, v:v) (Karnosky et al. 1996). Following rooting and hardening, individual cuttings were transplanted into 6-l pots (15 cm diameter) containing peat, sand and vermiculite (2:1:1, v:v:v), and established with a single application of full-strength, complete nutrient solution containing 16 m*M* nitrogen (modified from Johnson et al. 1957). Concentrations for each chemical used in our full-strength experimental fertilizer solution were: $Ca(NO_3)_2$, 2.5 m*M*; KNO_3, 3 m*M*; $CO(NH_2)_2$, 4 m*M*; KH_2PO_4, 3 m*M*; $MgSO_4$, 3 m*M*; KCl, 3 m*M*; Sequestrene 330 (10% Fe, Ciba-Geigy Corporation, Greensboro, NC, USA), 50 g l^{-1}; H_3BO_3, 0.075 m*M*; $MnSO_4$, 0.015 m*M*; $ZnSO_4$, 0.006 m*M*; $CuSO_4$, 0.0015 m*M*; H_2MoO_4, 0.00026 m*M*.

Plants were established and grown in a greenhouse with a maximum photosynthetic photon flux density of 400 to 600 µmol m^{-2} s^{-1} and a 16-h photoperiod, temperature 24 ± 3°C day/20 ± 2°C night and relative humidity 40 to 80%.

Fertilizer application

Once the cuttings were established (10 days after planting), complete nutrient solution was applied daily at concentrations that increased exponentially according to the following formula (Ingestad and Lund 1979):

$$N_t - N_s = N_s(e^{R_U(t-s)} - 1)$$

where e is the base of the natural logarithm and N_t is the total amount of nitrogen (mg) in the seedling on the application date (t) and N_s is total seedling N the day the experiment started (s). For the two replicate experiments conducted, N_s was based on estimated dry weights and the assumption that the average N concentration of cuttings was 30 mg N g^{-1} dry weight. In Experiment 1, N_s was estimated to be 20 mg N for all plants, and in Experiment 2, N_s was estimated to be 44 mg N. The rate of N increase in the seedling (R_U, day^{-1}) was presumed to be equivalent to the relative addition rate (Ingestad and Lund 1979).

Five relative addition rates were used in Experiment 1 (0.01, 0.02, 0.03, 0.04 and 0.05 day^{-1}) and three were used in Experiment 2 (0.03, 0.04 and 0.05 day^{-1}). Enough complete Johnson's nutrient solution was taken from concentrated stock to provide the daily increment of N (i.e. $N_t - N_s$). Each pot received this increment in 600 ml of water because this was equiva-

lent to the minimum volume required to saturate the soil on a daily basis. Nutrient solutions were dispensed through trickle irrigation plumbing (Stuppy, Inc., North Kansas City, MO, USA). Each plumbing assemblage had one manifold line (19 mm inside diameter) supplying 60 trickle tubes (1.5 mm inside diameter); one for each pot in the treatment. The total volume required for all the pots in each treatment was dispensed through the manifold supply line by using a siphon mixer (A. H. Hummert Seed Co., St Louis, MO, USA). Solution stocks were diluted to the desired daily concentration, taking into account the dilution rate of the siphon injector and the total volume required for all pots in the treatment. Because flow rate of each emitter on the assemblage was not the same, each pot was watered with enough solution to drain through. It was assumed that upon draining, the required 600 ml of solution was retained in the soil column. Thus each pot was flushed daily and, following drainage, all pots received equivalent amounts of solution.

Growth measurements

Non-destructive measurements of height and basal diameter were taken each week. Destructive harvests occurred every 2 to 3 weeks within 1 day of non-destructive measurements. Each of the five destructive harvests in Experiment 1 included four trees per treatment. Each of the four harvests in Experiment 2 included five trees per treatment. Leaf, stem and root tissues were separated, dried (70°C for 72 h), weighed and analyzed for N content (Carlo Erba NA 1500 NC, Fisons Instruments, Danvers, MA, USA).

Chlorophyll and photosynthesis

Chlorophyll concentration and photosynthetic rates were measured on the 11-week-old plants of Experiment 2. Chlorophyll content was determined at eight evenly spaced leaf positions (every five to seven leaves) using a portable chlorophyll meter (SPAD-502, Minolta Camera Co., Osaka, Japan). Because it is necessary to calibrate SPAD meter values to actual chlorophyll content (Campbell et al. 1990), punches were taken from a subsample of leaves, chlorophyll was extracted with N,N-dimethylformamide and absorbance was measured with a spectrophotometer (Model 690, Turner Corp., Mountain View, CA, USA) (Inskeep and Bloom 1985). Light-saturated, ambient-CO_2 photosynthetic rates were measured (LI-6200, Li-Cor, Lincoln, NE, USA) on a single representative leaf from the recently mature, mature and overmature age classes on each tree.

Statistics

Plants were arranged in completely random three-way factorial designs. Experiment 1 included five relative addition rates (0.01, 0.02, 0.03, 0.04 and 0.05 day^{-1}), three clones (216, 259 and 271) and five harvests. Experiment 2 included three addition rates (0.03, 0.04 and 0.05 day^{-1}), three clones and four harvests. However, the first harvest was not included in statistical analyses because the plants had not equilibrated to the fertilizer treatments. There were four replicate plants in Experiment 1 and five in Experiment 2 for each treatment by clone by harvest combination. Plant morphology and tissue N were analyzed with a three-factor analysis of variance using SAS (SAS Institute, Inc., Cary, NC, USA).

The effect of treatments and clones on relative growth rate was evaluated with analysis of variance using natural log-transformed total dry weight as the dependent variable (Cain and Ormrod 1984, Poorter and Lewis 1986). Due to loss of trees and the resulting design imbalance, clone 216 was not included in Experiment 1 for this analysis. The main effect of time was tested for linearity using orthogonal polynomials. A significant linear effect indicates that the data are adequately explained by a straight line and relative growth rate is constant. Significant higher order polynomials indicate that the data are curvilinear and relative growth is not constant with time. The effect of either treatment or clone on relative growth rate was tested using two-way interactions with time. The three-way interaction term tested if the effect of addition rate on relative growth rate differed among clones. The time by treatment effect was partitioned with orthogonal polynomials. A significant linear effect indicates constant relative growth rate during the experimental period while a significant higher order effect indicates variable relative growth rate (Cain and Ormrod 1984, Poorter and Lewis 1986). Relative growth rate was calculated for each treatment by clone combination from the slope of log-transformed plant dry weight vs time using least-squares linear regression. Relative uptake rate was similarly calculated from log-transformed plant N content. The linear correlations among relative growth rate, relative uptake rate and relative addition rate were compared between Experiments 1 and 2 using tests for parallelism and coincidence (Kleinbaum and Kupper 1978). SAS was used for analysis of variance and orthogonal polynomial contrasts for relative growth rate and relative uptake rate, and SYSTAT (SYSTAT, Inc., Evanston, IL, USA) was used for regression analysis.

A repeated-measures analysis of variance was used for the chlorophyll, photosynthesis and leaf N data of Experiment 2. Chlorophyll data were from eight leaf positions. Photosynthesis and leaf N data were from three leaf positions. The design compared the response of these variables to three relative addition rates and three clones. SYSTAT was used for the analysis.

Results

Initial trees

The initial N content of rooted cuttings differed from estimates based on preliminary experiments of similar sized material where dry weights and N concentrations were determined. The total N content of plants in Experiment 1 was 58% less than the estimated 20 mg plant^{-1} and N content for plants in Experiment 2 was 16% greater than the estimated 44 mg plant^{-1} (Tab. 1). The discrepancy between predicted and actual N content resulted from a lower than estimated 30 mg N g^{-1} dry weight and underestimates of initial dry weight in Experiment 1 and a greater plant N concentration in Experiment 2. These discrepancies likely extended the equilibration period prior to reaching steady-state.

Growth response

Non-destructive measurements of stem volume were obtained by multiplying the square of basal diameter by height. Stem volume reached constant growth rates after the 40th day of relative addition rate treatments as indicated by linear growth response in semi-log plots (Fig. 1). The period of adjustment before 40 days is termed the lag phase and the period after 40 days is the experimental period (Ingestad and Lund 1986). During the experimental period, plant diameter and height growth, thus stem volume, increased with increasing relative addition rates to a maximum growth rate of 0.021 day^{-1} in the 0.05 day^{-1} treatment of Experiment 1. To define fully whole plant response to fertility regime and confirm non-destructive measurements, it was necessary periodically to harvest replicate plants for dry weight evaluation.

Dry weights collected from destructive harvests showed that the non-destructive measurements were accurate predictors of plant dry weight ($r^2 = 0.962$, $P < 0.001$). The transition between lag and experimental phases of growth was also evident in the dry weight data after 40 days (Fig. 2). During the experimental period, relative growth rate was constant for each relative addition rate and increased with increasing addition rate. Relative growth rate linearity and differences among treatments and clones were evaluated with analysis of variance on natural log-transformed dry weights collected during the experimental growth phase (Cain and Ormrod 1984, Poorter and Lewis 1986). For the time main effect, more than 99% of the variation in log-transformed weight was explained by a linear response; therefore, relative growth rate was constant during the experimental period (Tab. 2). The interactions with time indicate differences in relative growth rate for the factor involved. Relative growth rate was significantly affected by treatment (significant Time × Treatment interaction). Relative growth rate differences among treatments were constant during the experimental period because virtually all of the variation among treatments (91% in Experiment 1; 99% in Experiment 2) was explained by a linear response. Relative growth rates did not differ among clones, as shown by the non-significant Time × Clone interaction, even though there were significant clonal differences in weight (Experiment 2). Furthermore, there were no differences among clones in the relative growth rate response to addition rate treatments (non-significant Time × Treatment × Clone interaction). Analysis of variance using natural log-transformed total N content gave very similar results (Tab. 2), indicating that relative uptake rate was highly dependent on addition rate treatment and independent of clonal differences.

Relative growth rate was calculated from the slope of the lines shown in Fig. 2, and relative uptake rate was similarly calculated from the slope of log-transformed plant N content plotted as a function of time. Close linear correlations were found for relative growth rate and relative uptake rate vs relative addition rate (Fig. 3). Relative growth rate data for both experiments fell on statistically the same line (Fig. 3A), the slope was near 1, and the intercept was close to zero (compare the regression lines to the 1:1 line). Relative uptake rate data for the two experiments fell on statistically different lines (Fig. 3B). Experiment 1 uptake rates lay near the 1:1 line, but Experiment 2 data were offset ($P < 0.001$). The slopes were not significantly different ($0.1 > P > 0.05$).

Nitrogen content

Nitrogen concentration of harvested plants from both experiments also showed distinct treatment effects (Fig. 4). During the experimental period, N concentration varied with harvest time, treatment and clone ($P < 0.01$ for each factor in both experiments). For Experiment 1,

Tab. 1. Initial dry weight and nitrogen content for a subsample of rooted cuttings used in relative addition rate experiments.

	Clone	Total DW (mg)	N concentration (mg N g^{-1} DW)	Total N content (mg plant^{-1})
Exp. 1	216	594 ± 113	19 ± 1	11.2 ± 2.2
	259	344 ± 103	19 ± 1	6.3 ± 1.7
	271	464 ± 167	17 ± 2	7.7 ± 3.0
	Average	467 ± 127	18 ± 1	8.4 ± 2.3
Exp. 2	259	1 454 ± 415	36 ± 1	51.0 ± 14.0

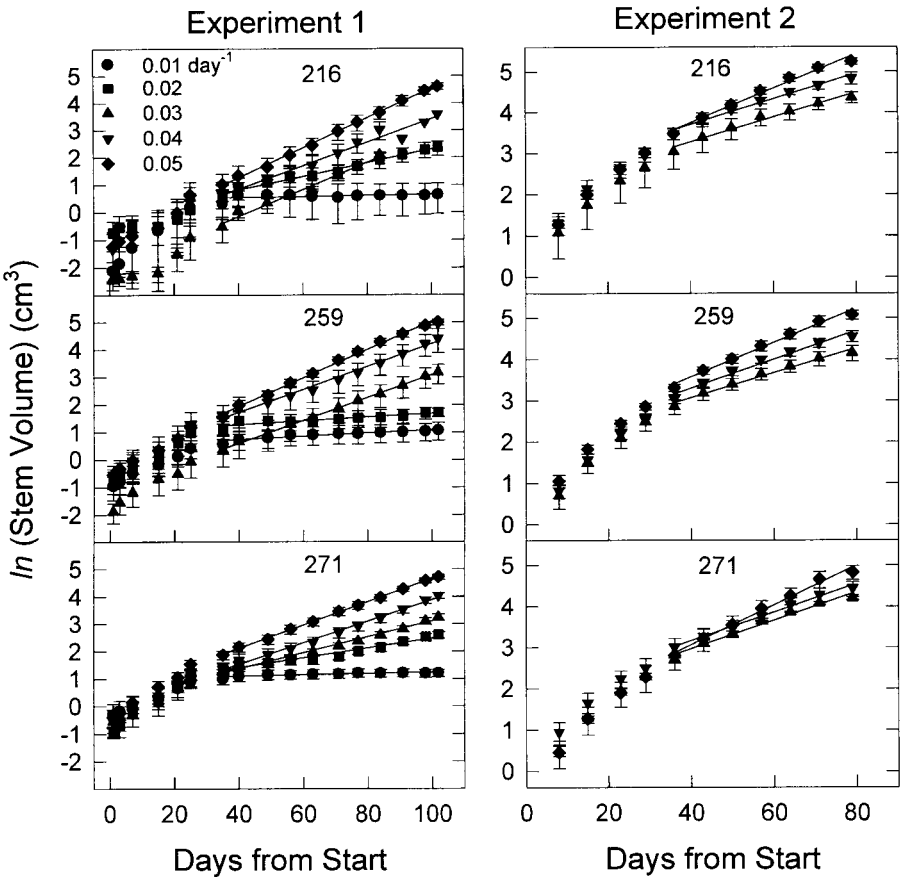

Fig. 1. Stem volume growth during relative addition rate experiments. Stem volume was obtained by multiplying height by the square of basal diameter. Three clones of aspen (216, 259 and 271) were compared for their response to five addition rates (0.01, 0.02, 0.03, 0.04 and 0.05 day^{-1}) in Experiment 1 and three addition rates in Experiment 2 (0.03, 0.04 and 0.05 day^{-1}). Regressions were for the experimental period only. Each point represents the mean ± SE of four trees in Experiment 1 and five trees in Experiment 2.

N concentration increased with time at higher relative addition rates and decreased at lower rates (Time × Treatment interaction, $P < 0.001$). For Experiment 2, no time interaction occurred. The effect of clone on N concentration was not consistent. In Experiment 1, N concentration of clone 216 was equal to that of clone 259 and significantly higher than that found in clone 271. However, in Experiment 2, N concentration of both clones 216 and 259 was significantly lower than that of clone 271. There were no significant interactions involving clones.

Relative growth rate was linearly dependent on average plant N content, and for a given N concentration, relative growth rates were higher in Experiment 2 than in Experiment 1 (Fig. 5). The slope of this relationship, termed N productivity, is the rate of dry weight produced per unit of N in the plant when the line passes through the origin (Ingestad 1981). The slope for both experiments was statistically the same, averaging 2.2 g dry weight g^{-1} N day^{-1}. However, since the line for Experiment 1 did not pass through the origin, it is not a true measure of the growth rate per unit N. Alternatively, N productivity (P_N) can be calculated as $P_N = (e^{RG} - 1)/n$ where n is nitrogen concentration in g N g^{-1} dry weight (Ingestad 1981). N productivity calculated in this manner increased with increasing addition rates, and was consistently higher in Experiment 2 compared with common treatments in Experiment 1 (Tab. 3).

The absolute rate of N uptake per unit of root weight similarly depended on the rate of N addition (Fig. 6) or plant N concentration (not shown). As addition rate (and plant N concentration) increased, net uptake rate also increased. Uptake of N, either on an absolute (Fig. 6) or a relative (Fig. 3B) basis, was greater in Experiment 1 than in Experiment 2 for a given addition rate.

Allocation patterns

The proportion of root weight to total plant weight (root weight ratio) was affected by relative addition rate treatment and harvest time, but there was no clonal effect. After 42 days of treatment, root weight ratio decreased with increasing addition rates ($P < 0.001$) (Fig. 7A). These treatment differences became greater with time, because at higher addition rates (0.03 to 0.05 day^{-1}), root weight ratio decreased with harvest time, and at lower addition rates it increased with time. As a

result of the temporal shifts in root weight ratio in response to relative addition rate, there was a significant Time × Treatment interaction in Experiment 1 ($P < 0.001$). This interaction was not significant in Experiment 2 because only the higher addition rate treatments were included.

Developmental shifts in dry weight allocation are important to note, but it is possible that observed treatment differences may simply be due to allocation shifts with plant size (Coleman and McConnaughay 1995). To check for this possibility, root weight ratio was plotted against log of total dry weight (Fig. 7B). At low nutrient addition rates, root weight ratio increased with total dry weight, whereas at high nutrient addition rates, root weight ratio decreased with increasing total dry weight. The allometric constant (K) can be calculated from the slope of a double log plot for two tissue types and is a concise way to express differential growth between the two components (Hunt 1978). Any value other than unity shows that the growth of one tissue is different relative to the other. In this case, the allometric constant between root weight and total plant weight was less than one for the lower addition rate treatments and greater than one for the higher addition rate treatments (0.01 day^{-1}, K = 0.65; 0.02 day^{-1}, K = 0.80; 0.03 day^{-1}, K = 0.93; 0.04 day^{-1}, K = 1.08; 0.05 day^{-1}, K = 1.90). The large differences in allocation patterns between addition rates (Fig. 7B and K values) show that patterns observed through time (Fig. 7A) could not be explained by developmental differences.

Physiology

To assess the effect of fertility and clone on chlorophyll concentration, leaf N and photosynthesis in different-aged leaves, detailed information was collected on 11-week-old plants (70 to 80 days) grown in Experiment 2. Measurements made with the SPAD meter were correlated with total chlorophyll measured by extraction (total chlorophyll = 12 × SPAD − 141, $r^2 = 0.78$, $P < 0.001$). With this correlation, it was possible to use SPAD measurements to define patterns of treatment and clonal response along a leaf age series. The chlorophyll content significantly increased ($P < 0.001$) with higher relative addition rate (Fig. 8), but there were no significant clonal effects. Chlorophyll concentrations also increased with leaf expansion (LPI 0 to 10), then either decreased or remained constant in older leaves.

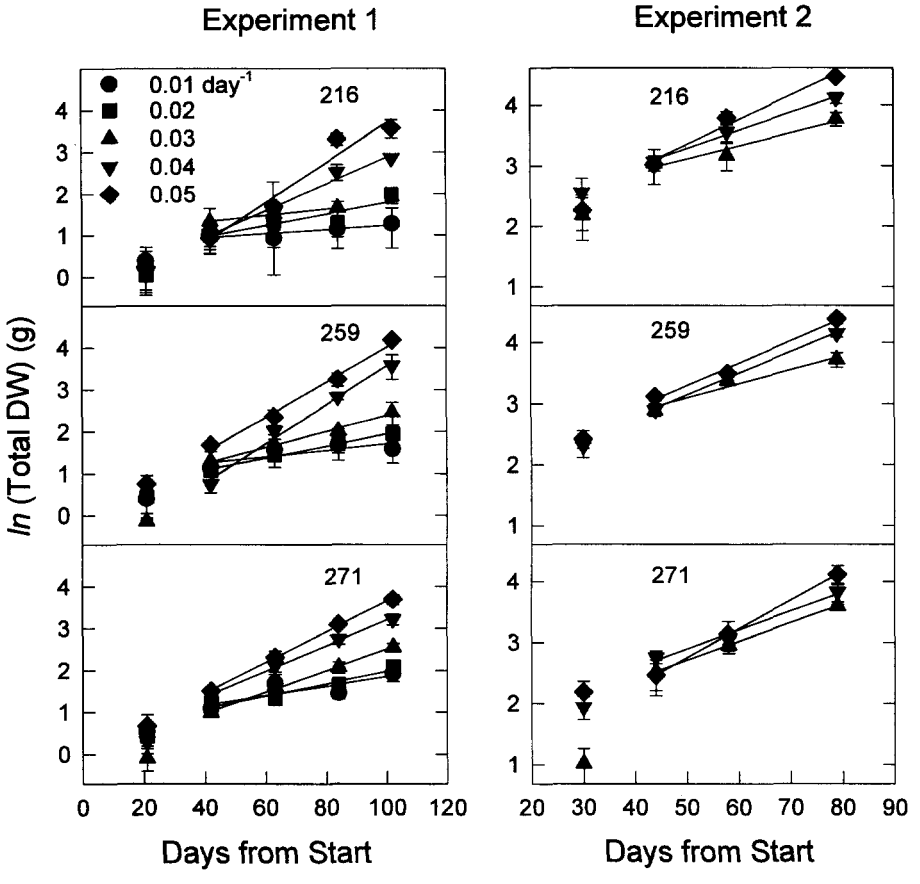

Fig. 2. Total dry weight accumulation during relative addition rate experiments. Experiment and clone descriptions as in Fig. 1. Regressions were for the experimental period only. Each point represents the mean ± SE of four trees in Experiment 1 and five trees in Experiment 2.

Tab. 2. Analysis of variance results for natural log-transformed total dry weight and natural log-transformed total N content in response to time of harvest, relative addition rate treatment, and clonal origin. The analysis for Experiment 1 included two aspen clones (259, 271) and five relative addition rate treatments (0.01, 0.02, 0.03, 0.04 and 0.05 day^{-1}) with harvests 2–5 included in the experimental phase. Clone 216 was excluded from the analysis for Experiment 1 because of the imbalance created by lack of trees in the 0.03 day^{-1} treatment at the fifth harvest. Experiment 2 included three clones (216, 259, 271) and three treatments (0.03, 0.04 and 0.05 day^{-1}) with harvests 2–4 included in the experimental phase. Level of F-test significance: ***, $P < 0.001$; **, $0.001 < P < 0.01$; *, $0.01 < P < 0.05$; NS, non-significant, $0.05 < P$.

Source	Experiment 1					Experiment 2				
	df	DW		N content		df	DW		N content	
		SS	F-test	SS	F-test		SS	F-test	SS	F-test
Time	3	41.66	***	48.07	***	2	28.05	***	15.86	***
Linear	1	41.55	***	47.56	***	1	27.96	***	15.75	***
Residual	2	0.11	NS	0.51	*	1	0.09	NS	0.11	NS
Treatment	4	33.15	***	99.39	***	2	2.54	**	15.95	***
Clone	1	0.01	NS	0.13	NS	2	4.46	***	0.87	*
Ti × Tr	12	12.22	***	20.63	***	4	2.55	*	5.84	***
Linear	5	11.11	***	19.63	***	3	2.53	**	5.82	***
Residual	7	1.11	NS	1.00	NS	1	0.02	NS	0.02	NS
Ti × C	3	0.07	NS	0.08	NS	4	0.07	NS	0.11	NS
Tr × C	4	0.44	NS	0.81	*	4	0.19	NS	0.06	NS
Ti × Tr × C	12	1.59	NS	1.63	NS	8	1.74	NS	1.08	NS
Error	119	13.87		9.63		97	23.59		13.42	

The leaves containing maximum chlorophyll concentration varied with treatment and clone. At the 0.05 day^{-1} addition rate, chlorophyll concentration peaked in younger leaves (lower LPI) than at other addition rates (Treatment × Position interaction, $P < 0.01$). The chlorophyll concentration peaked in older leaves in clone 271 and remained high as the leaves aged compared with the other clones (Clone × Position interaction, $P < 0.01$). The second and third order interactions involving both treatment and clone were not significant.

Chlorophyll was linearly correlated with both photosynthesis and leaf N concentration (photosynthesis vs total chlorophyll, $r^2 = 0.64$, $P < 0.001$; photosynthesis vs leaf N, $r^2 = 0.70$, $P < 0.001$; total chlorophyll vs leaf N, $r^2 = 0.81$, $P < 0.001$). As a consequence of these linear correlations, treatment, clone and positional responses of leaf N concentration and photosynthesis were quite similar to those of chlorophyll (Fig. 8). Both leaf N concentration and photosynthetic rate increased with relative addition rates and decreased with leaf age ($P < 0.001$). In addition, differences in leaf N and photosynthetic rate associated with different addition rates decreased with leaf age (significant Treatment × Position interaction, $P < 0.05$). Leaf N was greater in clone 271 than in the other clones ($P < 0.001$), and leaf N and photosynthesis did not decrease as much with leaf age in clone 271 as in the other clones (Clone × Position interaction, $P < 0.01$). Treatment differences were consistent among leaf positions for each clone as indicated by non-significant two- and three-way interactions involving treatment and clone.

Discussion

Experimental approach

Experiments examining the effects of multiple stresses on tree growth require large numbers of potted plants. Plant growth may be influenced by internal nutrient concentration as well as by the experimental variables. Therefore, it is necessary to control internal plant nutrient concentration to evaluate the influence of other variables. Standard nutrient regimes involving periodic applications of fixed fertilizer amounts will result in declining growth rates and uncontrolled N concentrations. However, growth rate and internal nutrient concentration may be controlled with relative addition rate techniques. It was technically simple to apply mineral nutrients to large numbers of potted plants in exponentially increasing daily amounts with trickle irrigation. Although control over plant growth was not as precise as found for circulating solution culture, the plants did reach constant growth rates in accordance with relative addition rate theory (cf. Ingestad and Lund 1979, 1986, Ingestad 1982, Jia and Ingestad 1984, Ågren 1985, Ingestad and Kähr 1985). Evidence for steady-state nutrition includes constant relative growth rate that is equivalent to nutrient supply rate and to nutrient uptake rate (i.e. $R_G \cong R_A \cong R_U$), as well as stable internal nutrient concentration. Indeed, during the experimental period, growth rate remained constant in both experiments for each addition rate (Fig. 2), and relative growth rate was comparable to the applied relative addition rate treatment (Fig. 3A), indicating that nutrient supply was appropriate to maintain exponential growth. Additionally, relative uptake rate was equivalent to the addition

rate, especially in Experiment 1, although uptake rate was lower than addition rate in Experiment 2.

Plant N concentrations varied over time and plants in each treatment had a unique pattern of change (Fig. 4). Concentrations generally increased with higher addition rates and decreased with lower addition rates. These shifts in concentration result from relative growth rate being less than relative uptake rate for higher treatments and growth rate being greater than uptake rate for lower treatments. Although plant N concentration changed during the experimental period, mean standard errors for the experimental period only ranged from 0.3 to 0.6 mg N g^{-1} dry weight in Experiment 1 and from 0.6 to 0.7 mg N g^{-1} dry weight in Experiment 2 for the individual relative addition rates. Standard errors less than or equal to 0.7 mg N g^{-1} dry weight in relative addition rate experiments are considered adequate evidence of stable internal nutrient concentration (Jia and Ingestad 1984, Ingestad and Kähr 1985). Although plant N concentration was not constant, the deviation from steady-state nutrition was small compared with shifts in concentrations observed in other nutrition experiments applying constant fertilizer amounts to potted plants (Pell et al. 1990, 1995, Brown 1991, Tjoelker and Luxmoore 1991, Sinclair 1992, Bazzaz and Miao 1993, Coleman et al. 1993) or in other tests of relative addition rate in solid potting media (Burgess 1990, 1991, Wait et al. 1996).

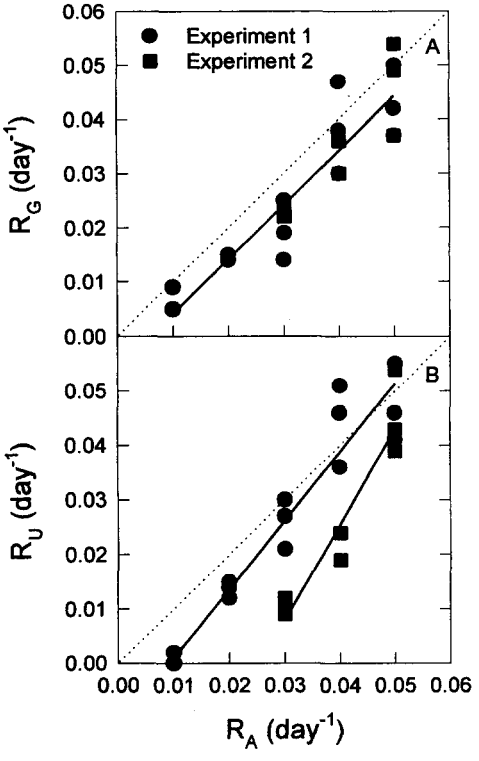

Fig. 3. Relative growth rate (R_G) and relative uptake rate (R_U) plotted as a function of relative addition rate (R_A). Relative growth rates (A) are taken from the slope of semi-log plots of dry weight vs time shown in Fig. 2. The R_G line is the least-squares regression for both experiments combined ($R_G = 1.00 \times R_A - 0.59$, $r^2 = 0.86$, $P < 0.001$). Relative uptake rate (B) is similarly obtained from plots of total N content as a function of time. Two lines were necessary to describe the R_U vs R_A data for Experiment 1 ($R_U = 1.26 \times R_A - 1.16$, $r^2 = 0.92$, $P < 0.001$) and Experiment 2 ($R_U = 1.75 \times R_A - 4.56$, $r^2 = 0.88$, $P < 0.001$). Three clones (216, 259 and 271) were examined with up to five relative addition rate treatments.

The supply of nutrients for root uptake differs between soil and nutrient solutions. In soil, nutrient supply is provided through root growth, diffusion and mass flow. Root growth and extension into unexploited soil are most important for supply of highly immobile nutrients, such as phosphorus; diffusion and mass flow are important for more relatively mobile nutrients such as nitrate and ammonium (Clarkson 1985). For the more mobile N compounds, depletion zones develop quite a distance from roots because N uptake is greater than supply at the root surface and the established gradient encourages diffusion further from the root. Overlap of depletion zones and root competition occur at high rooting density (Nye and Tinker 1977). Yet, young plants growing exponentially in the field have sufficient unexploited soil volume to avoid root competition. It is expected that exponential growth into unexploited soil volumes will meet exponential nutrient uptake required to achieve steady state nutrition (Ingestad 1982). In solution culture, roots are completely immersed in a liquid medium that is mixed rapidly to avoid all but a small boundary layer of lower concentration at the root surface (Nye and Tinker 1977). To achieve steady-state nutrition, exponentially increasing nutrient demand is supplied by exponential increases in solution concentration (Ingestad 1982). Our experimental system with potting soil mix had elements of both field-grown soil and solution culture. The roots had some limited new soil volume to explore, but depletion zones most likely developed between fertilizer applications. Depletion zones were replenished daily with exponentially increasing fertilizer solution concentrations. Our study shows that steady-state nutrition can be achieved for potted plants grown in artificial soil media with a significant cation exchange capacity.

Nitrogen productivity

Nitrogen productivity in our study was calculated from relative growth rate and plant N concentration (Tab. 3, Fig. 5). The N productivity range of 0.65 to 2.39 g dry weight g^{-1} N day^{-1} obtained here for aspen is low compared to reported values ranging from 2.4 to 6.7 for other tree species including poplar and willow grown in solution culture experiments (Ericsson 1981, Ingestad 1981, Jia and Ingestad 1984, Ingestad and Kähr 1985). Although the N concentrations found in our aspen overlapped that of the other species grown in solution culture (cf. Ågren and Ingestad 1987), the growth rates were much lower. However, solution culture experi-

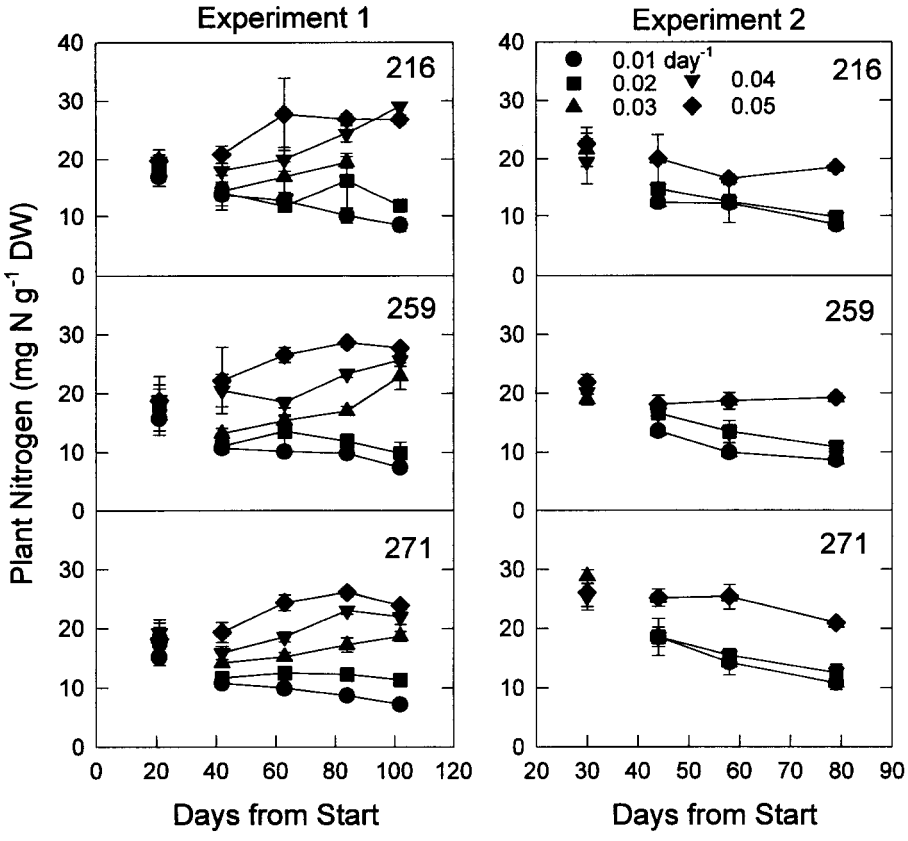

Fig. 4. Total plant nitrogen concentrations for two relative addition rate experiments. Experiment and clone descriptions as in Fig. 1. Symbols connected by lines indicate the experimental period. Each point represents the mean ± SE of four trees in Experiment 1 and five trees in Experiment 2.

ments reporting higher growth rates and thus higher N productivity were all carried out with 24-h photoperiods, so they would be expected to have higher growth rates. The total daily irradiance used in our experiments was not sufficient to saturate the N productivity vs light response curve for birch (*Betula pendula* Roth.) (Ingestad and McDonald 1989, McDonald et al. 1991) or pea (*Pisum sativum* L.) (MacDuff et al. 1993). Therefore, low N productivity found here for aspen may be attributed to low daily cumulative light levels relative to solution culture experiments. Our plants were also much larger than those growing in solution culture, therefore more physiologically inactive N was bound in structural components.

Despite the low N productivity found for aspen, it is important to note that this parameter indicates that small changes in internal N content will result in large gains in production. A change in plant N concentration from 20 to 25 mg N g^{-1} dry weight (leaf N concentration from 30 to 40 mg N g^{-1} dry weight) results in over a 0.01 day^{-1} increase in relative growth rate (Fig. 5). If these data can be extrapolated to field conditions, a 0.01 day^{-1} change in growth could double the annual productivity over a 100-day growing season. Therefore, one practical objective of a forest fertilization regime should be to increase the internal N concentration to realize these significant increases in productivity. Optimal nutrition trials designed to match increasing demand for N during the growing cycle by regular fertilizer additions have increased internal N concentration and demonstrated dramatic increases in growth (Linder 1989, Pereira et al. 1989). Similar experiments have not been described for poplars. Based on the results presented here, increased productivity in short-rotation intensive culture poplar plantations will most likely be obtained by adopting such fertilization regimes.

Allocation patterns

Allocation of carbon within the plant is strongly controlled by nutrient availability (Robinson 1986, Hilbert 1990, Mooney and Winner 1991). The relative weight of roots increases and that of leaves decreases as N availability decreases. Our results agreed with this general response (Fig. 7). In addition, root weight ratio differences among relative addition rate treatments got larger with time. Other experiments have shown that plants grown at steady-state nutrition in solution culture have constant allocation patterns over time (Inges-

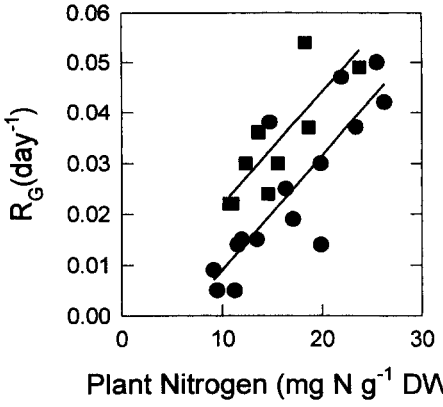

Fig. 5. The dependence of relative growth rate (R_G) on the whole plant nitrogen concentration. Relative growth rates are taken from the slope of semi-log plots of dry weight vs time shown in Fig. 2. Nitrogen concentrations are the average of all harvest dates during the experimental period for each clone by treatment combination. Three clones (216, 259 and 271) were examined with up to five relative addition rate treatments. The lines are the least-squares linear regression for Experiment 1 ($r^2 = 0.72$, $P < 0.001$, ●) and Experiment 2 ($r^2 = 0.65$, $P < 0.001$, ■). They are parallel but not coincident ($P < 0.001$).

tad and Ågren 1995). The temporal shifts in allocation observed in our experiments may be associated with the drift of internal nutrient concentration (Fig. 4) resulting from imperfect steady-state conditions. These allocation changes occurred even though plants were growing at a constant rate. Changing allocation in response to other environmental variables has been attributed to differences in plant size among treatments (Coleman and McConnaughay 1995). However, our allometric constant data and graphical analysis (Fig. 7B) do not support such a conclusion for the various relative addition rate treatments applied. Although allocation changes may be associated with imperfect steady-state conditions, changing allometric relationships have been reported in other relative addition rate experiments conducted with solution culture where whole plant relative growth rate and internal nutrient concentrations are constant (Freijsen and Otten 1984, MacDuff et al. 1993). Therefore, a closer look at allocation changes in relative addition rate experiments is called for.

Tab. 3. Nitrogen productivity for each relative addition rate treatment used in Experiments 1 and 2. Values are the mean ± SE for the three clones examined.

Relative addition rate (day^{-1})	Nitrogen productivity (g DW g^{-1} N day^{-1})	
	Experiment 1	Experiment 2
0.01	0.65 ± 0.17	
0.02	1.20 ± 0.04	
0.03	1.13 ± 0.24	1.92 ± 0.13
0.04	1.86 ± 0.33	2.38 ± 0.22
0.05	1.75 ± 0.13	2.39 ± 0.32

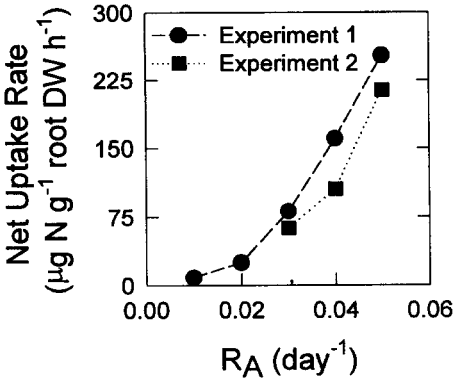

Fig. 6. The net nitrogen uptake rate per unit root weight expressed as a function of relative addition rate (R_A).

Leaf nitrogen, chlorophyll and photosynthesis

Leaf chlorophyll content, leaf N content and photosynthetic rates are positively correlated and are highly interdependent (Wong et al. 1985, Field and Mooney 1986, Evans 1989, Green and Mitchell 1992, Reich et al. 1994). Thus, the measurement of one component has predictive value for the others. The SPAD meter takes advantage of this interdependence, not only for estimating chlorophyll content, but also for rapidly estimating leaf N and photosynthetic capacity. The patterns of chlorophyll response to various leaf positions that were generated from SPAD measurements are, therefore, good predictors of leaf N and photosynthetic capacity. Our data show that changes in these three parameters were closely related with one another, and each was dependent upon addition rate treatment as well as leaf position (Fig. 8).

Changes in these physiological responses related to nutrient availability may have important impacts on carbon fixation and plant growth. In our study with aspen, the changes in leaf N and photosynthesis with leaf position were much greater at the 0.05 day^{-1} addition rate treatment than with lower treatment rates. Nitrogen concentration usually decreases from upper to lower leaves of terminal shoots and from upper to lower leaves in the crown. The changes in leaf N concentration are related to leaf age, light environment during leaf formation, leaf weight per unit area, N availability, changes in allocation between leaf N pools and retransport of N within the plant (Evans 1989, Chen et al. 1993, Aerts and deCaluwe 1994, Hikosaka et al. 1994, Hollinger 1996). When light exposure during leaf formation is similar (current terminals or single stem seedlings), leaf N concentration is related to N availability or supply rate. With low N availability, N concentration commonly decreases along a gradient from young leaves to older leaves. With high N availability, this gradient is decreased or absent (Hikosaka et al. 1994). In contrast to this pattern, N

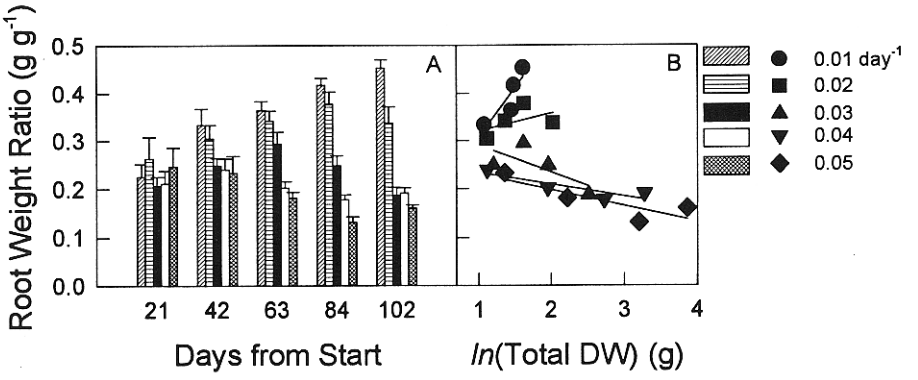

Fig. 7. Root weight ratio (root weight/total plant weight) for five relative addition rate treatments in Experiment 1. The data are presented as a function of age (A) and plant size (B). No clonal differences were observed so the data presented are the mean ± SE of 12 individual plants.

concentration decreased in our aspen clones with leaf age only at the higher relative addition rates (Fig. 8). Similarly N concentration decreased from upper to lower leaves of terminal shoots of hybrid poplar with high N fertility levels (35 to 28 mg N g^{-1} dry weight from upper to lower leaves), but not with low N fertility levels (18 mg N g^{-1} dry weight for all leaves) (E. A. Hansen and D. N. Tolstead, unpublished data from field and greenhouse fertility trials).

Even though decreases in N concentration and photosynthetic rate were greater from young to old leaves in the highest relative addition rate treatment (0.05 day^{-1}), final leaf N concentration and photosynthetic rates were greater in all leaf age classes (Fig. 8) thus increasing carbon fixation and productivity. Plants allocate carbon and nitrogen to maximize resource gain (Bloom et al. 1985). Therefore, there may be an advantage in maintaining some minimal N concentration in

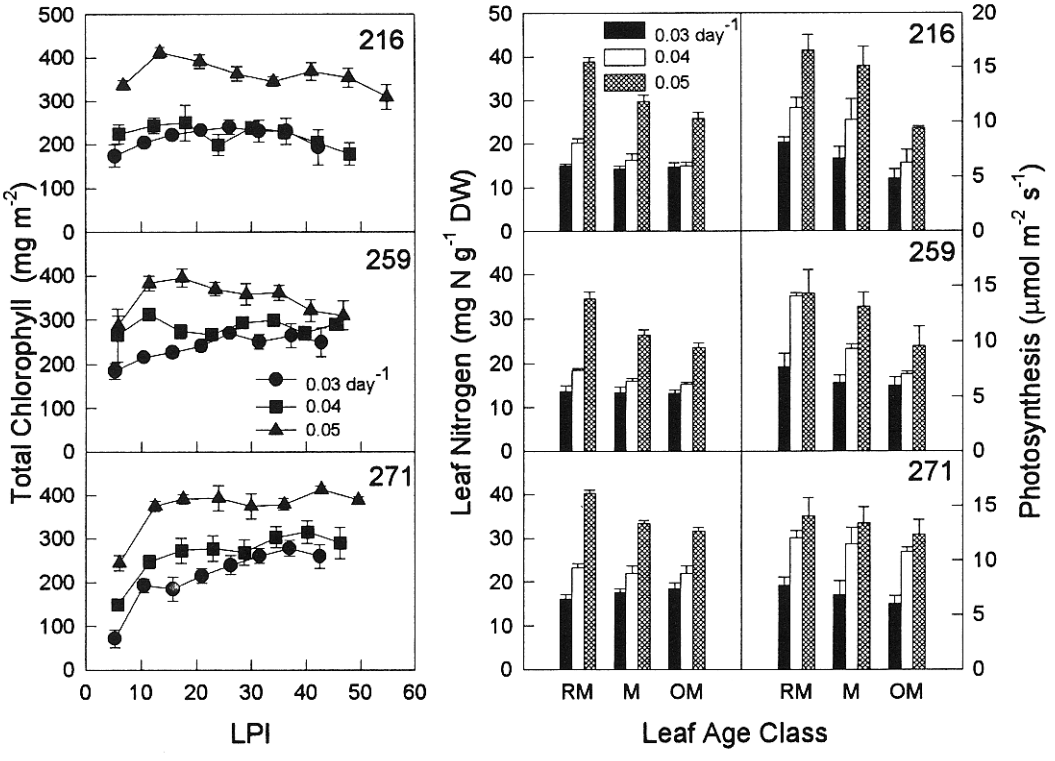

Fig. 8. The dependence of leaf chlorophyll, leaf nitrogen and photosynthesis on leaf position in Experiment 2. Chlorophyll is shown for several positions expressed as leaf plastochron index (LPI, with the youngest leaves and lowest numbers at the top of the plant). Nitrogen and photosynthesis are shown for three leaf age classes that include recently mature (RM), mature (M) and overmature (OM). Photosynthesis values were collected at 30 ± 1°C, 51 ± 5% relative humidity and 1 058 ± 156 μmol m^{-2} s^{-1} PPFD. Each measurement is the mean ± SE of four replicate measurements.

leaves throughout the canopy. In addition, disturbance-adapted early successional species such as aspen and other poplars have greater increases in photosynthetic rates with increasing N concentration compared to late successional species (Reich et al. 1994) leading to rapid growth on fertile sites. Thus allocation of surplus N to upper high-light leaves would be an advantage.

Clonal differences in nutrient use efficiency, carbon allocation to roots or leaves, nutrient uptake capacity, and growth rates are well documented (Heilman 1985, Heilman and Stettler 1986, Coleman et al. 1995a, Stettler et al. 1996, Scarascia-Mugnozza et al. 1997). Steady-state nutritional experiments have the potential to greatly improve clonal comparisons because variable nutrient regimes often complicate comparisons. Clonal differences in nutrient uptake and in physiological gradients with leaf age may also be important not only in productivity but in response to environmental stresses. Clone 271 was able to maintain greater function in older leaves compared to the other clones. For example, with the 0.05 day^{-1} addition rate, photosynthetic rate for the overmature leaf class in clone 271 was 88% of the recently mature leaf class compared with 62% averaged for the other clones (Fig. 8). Chlorophyll content of older leaves did not decline in any of the addition rate treatments for clone 271, but for the other clones there was a distinct decline with age especially in the 0.05 day^{-1} treatment. Maintenance of greater physiological activity in older leaves is consistent with clone 271's greater ability to retain older leaves and minimize accelerated senescence due to ozone stress in comparison to the other clones (Coleman et al. 1995b, Karnosky et al. 1996). Conversely, the decreases in leaf N concentration and photosynthetic rate in older leaves of clone 259 even with high fertility may be related to its high sensitivity to ozone stress.

Conclusions

The close correspondence between these relative addition rate experiments conducted in soil compared to those previously conducted in solution culture (Ingestad 1982) is supportive of steady-state nutrition theory and the applicability of the described techniques for such work. Plant relative growth rate was constant during the experimental period, and equivalent to applied fertilizer addition rates. Each addition rate resulted in plant nutrient concentrations that were unique and linearly related to the growth rate.

Results from this technically simple experimental technique demonstrate that it is possible to maintain growth and physiological responses of plants in steady-state conditions for large scale studies. The main differences between the results of this relative addition rate experiment with plants grown in potting soil compared to those grown in flowing solution culture were lower N productivity as well as more variable internal nutrient content and root weight ratios with time. Such differences may result from the longer experimental time and larger plants in our experiments. Despite these differences, morphological and physiological responses of plants were consistent with steady-state nutrition theory.

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