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Photosynthetic responses of loblolly pine (*Pinus taeda*) needles to experimental reduction in sink demand

DAVID A. MYERS,[1] RICHARD B. THOMAS[1] and EVAN H. DeLUCIA[2]

[1] *Biology Department, West Virginia University, P.O. Box 6057 Brooks Hall, Morgantown, WV 26506, USA*

[2] *Department of Plant Biology, University of Illinois, Urbana, IL 61801, USA*

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Summary Sink strength in loblolly pine (*Pinus taeda* L.) was experimentally manipulated on two sun-exposed branches on each of two neighboring trees by excising the emerging terminal cohort (second flush of 1996) during a period of rapid needle expansion. In addition, export of photosynthate was restricted on one of these branches from each tree by removal of bark and phloem just below the second flush of 1995. Treatment-induced changes in needle biochemistry were measured in 3-month-old (first flush of 1996) and 1-year-old (final flush of 1995) needles collected 1, 5 and 8 days after treatment. In 3-month-old needles, sugar concentration increased by 24% one day after leader excision, and increased by 86% on Day 8 after leader excision and girdling. Starch concentration increased by 33% in 3-month-old needles on Day 1 after leader excision, and by 400% in 1-year-old needles on Day 8 after leader excision and girdling. Physiological changes in 3-month-old and 1-year-old needles were measured by open-flow gas exchange and chlorophyll fluorescence on Day 8 after leader excision and girdling. Light- and CO_2-saturated net photosynthesis decreased following treatment in both 3-month-old and 1-year-old needles (23 and 17%, respectively). Maximum rate of carboxylation (V_{cmax}) decreased by 25% in 3-month-old needles and by 31% in 1-year-old needles in response to leader excision and girdling. The combined treatment resulted in a 38% decrease in maximum rate of electron transport (J_{max}) in 3-month-old needles and a 37% decrease in J_{max} in 1-year-old needles. Before leader excision and girdling, 2% oxygen in air stimulated photosynthesis by 17 to 19%, but this stimulation was only 3 to 4% at 9 days after treatment. These physiological responses indicate that experimentally lowered sink strength resulted in rapid feedback inhibition of leaf-level photosynthetic capacity in loblolly pine.

Keywords: girdling, rapid feedback inhibition, sink source, sink strength, terminal leader excision.

Introduction

Sink-limited reductions in photosynthetic capacity can result from a low capacity to translocate and store photosynthates (Neales and Incoll 1968, Socias et al. 1993). Physiological changes that frequently result from sink limitations include reduced photosynthetic capacity, reduced oxygen sensitivity of photosynthesis, changes in light energy dissipation and accumulations of starch and sucrose in leaves (Sharkey 1990). Although a definitive mechanistic link between carbohydrate accumulation and the regulation of photosynthetic capacity remains equivocal, several studies have related partitioning of photosynthate into excess starch or sucrose production as a result of decreased sink demand with a decrease in photosynthetic capacity (Nafziger and Koller 1976, Stitt and Quick 1989, Harley and Sharkey 1991, Stitt and Schulze 1994).

Numerous studies have examined changes in photosynthetic capacity by experimentally manipulating the souce–sink balance within plants. One tool used to investigate this phenomenon is to alter sink demand by excising the sink material from the plant. Kramer and Kozlowski (1979) describe the growth of pine trees as a system of sinks in competition for photosynthate from a shifting source as ontology proceeds. We examined changes in photosynthetic capacity after experimentally manipulating sink demand. Sink strength was manipulated in two ways. First, during a period of rapid needle expansion, the emerging terminal leader (the second flush of 1996) was excised resulting in reduced sink demand for that branch. A second treatment combined excision of the terminal leader with a girdle treatment involving removal of the bark and phloem from a 1-cm wide strip on the branch just below the final flush of the 1995 growth season. In addition to lowering sink demand on the branch, this treatment cut off flow of photosynthates between the experimental branch and the rest of the tree, resulting in removal of both major sinks for the remaining needle cohorts.

We used pulse amplitude modulated (PAM) chlorophyll fluorometry and open-flow gas exchange to obtain diagnostic evidence of down-regulation in photosynthetic capacity of loblolly pine needles *in vivo* in response to these treatments. Chlorophyll fluorescence quenching analysis by PAM fluorometry permits noninvasive examination of the energy dissipation and utilization qualities of chloroplast populations (Krause and Somersalo 1989, Krause and Weis 1991). Photochemical quenching (qP) measured by PAM fluorometry reflects light energy utilization through photochemistry. Experimentally decreasing sink demand should decrease qP as feedback regulation increases. During feedback regulation of

photosynthesis, increased amounts of excess light energy must be dissipated when photophosphorylation is limited by low phosphate concentrations in the stroma (Sharkey 1990). Non-photochemical quenching (qN) is a family of processes that dissipate energy by means other than linear electron transport, and is dominated by the buildup of a transthylakoid pH gradient (ΔpH) (Schreiber et al. 1986). Therefore, qN is expected to rise in leaves experiencing feedback regulation following experimental decreases in sink demand.

Open-flow infrared gas analysis (IRGA) permits rapid measurement of gas exchange characteristics of leaves in saturating light while the ambient CO_2 concentration is varied. The resulting CO_2 response curves can be analyzed by biochemically based equations describing potential limits to photosynthetic capacity such as the carboxylation rate of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and the rate of ribulose-1,5-bisphosphate (RuBP) regeneration mediated by electron transport (Harley and Sharkey 1991, Harley et al. 1992). We also used open-flow gas exchange analysis to measure the oxygen sensitivity of photosynthesis. Photosynthesis is normally very sensitive to O_2 concentration because Rubisco acts competitively as both a carboxylase and an oxygenase. Reduced photosynthetic sensitivity to O_2 when switching from 21 to 2% oxygen in air is indicative of a low rate of triose phosphate utilization (Sharkey 1990, Harley et al. 1992) that can result because of sink limited photosynthesis (Socias et al. 1993).

The relationship between photosynthetic capacity and sink demand in loblolly pine trees is complex because this species produces from one to seven distinct flushes of needle cohorts each growth season (Hellmers and Strain 1972). Growth of the new cohorts depends on photosynthate from the preceding cohorts to synthesize the substances needed for growth (Chung and Barnes 1977). In addition, studies that examined altered source–sink relationships in loblolly pine seedlings and saplings in response to CO_2 enrichment have suggested that changes in photosynthetic capacity may be related to reduced sink demand (Tissue et al. 1993, Thomas et al. 1994). Therefore, the objective of our study was to examine if decreased sink demand would result in altered carbohydrate concentration and rapid down-regulation of photosynthetic capacity in loblolly pine needles.

Materials and methods

Plant material and experimental treatment

Two, 15-year-old, approximately 11-m tall, loblolly pine (*Pinus taeda* L.) trees were selected from an even-aged stand from within the Blackwoods subdivision of the Duke Forest, west of Durham, NC. These trees grow in soils of the Enon series, a low-N soil common to the Piedmont of North Carolina. Two branches from the south side of each tree were used. The upper halves of all four of the branches were exposed to full sun between 1000 and 1500 h on cloudless days.

In late June 1996, the youngest needle cohort on four branches was excised with a razor blade (Figure 1). Terminal leader excision took place during a period of rapid expansion

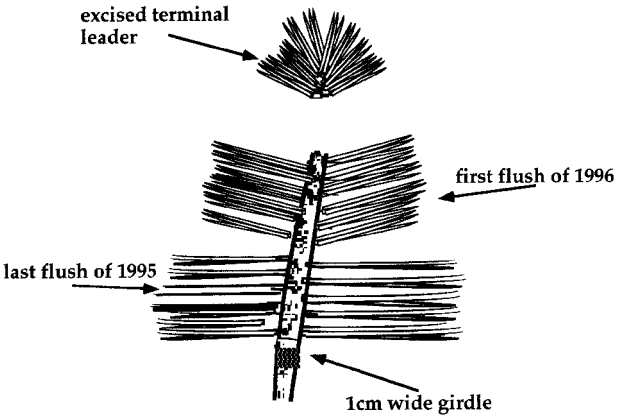

Figure 1. Schematic representation of sun branch needle cohorts, leader excision and girdling treatment location. Note that the terminal leader excised was the second cohort of 1996, therefore the terminal cohort remaining corresponded to the first cohort of 1996 (3-month-old needles) and the second cohort anterior to the excision was the second cohort of 1995 (1-year-old needles). Data were gathered from these two needle cohorts.

approximately 4 weeks after bud break. This was done to reduce sink demand on the remaining needle cohorts on the branch, yet not restrict export of photosynthate to the rest of the tree. The removed needle cohort was the second cohort of the 1996 growing season. Therefore, the new terminal cohort on each branch after leader excision was the first cohort of 1996 (3-month-old needles), with the next youngest cohort on the branch corresponding to the last flush of the 1995 season (1-year-old needles). In addition, a girdle treatment, involving removal of the bark and phloem from a 1-cm wide strip on the branch just below the 1-year-old needles, was applied to two of the four branches (one from each tree) thereby cutting the flow of photosynthate between the experimental branch and the rest of the tree.

Needle carbohydrate concentration

Following sink manipulation, photosynthetic down-regulation often takes up to 3–5 days to become apparent (Geiger 1976). Therefore, biochemical measurements were conducted on needles collected on Days 1, 5 and 8 following leader excision and girdling. Pre-treatment measurements were made on the same branches for two days before initiating treatments. Because of time constraints imposed by weather and canopy access, needles were collected after 1 and 5 days on the two branches from which the terminal leader was excised and after 5 and 8 days on the two branches from which the leader was excised and the branch girdled. Two fasicles were removed from the 3-month-old needle cohort and from the 1-year-old needle cohort at 1600 h on the days measurements took place. Needles were frozen in liquid N_2 and stored on ice until dried to constant mass at 60 °C. Dried needle samples were ground in a Tecator Cyclotec 1093 sample mill (Tecator, Hogänäs, Sweden) and nitrogen concentration was measured after tissue combustion in a Carlo Erba NA 1500 N, C, S elemental analyzer (Fisions Instruments, Valencia, CA). Needle starch

and soluble sugar concentrations were assayed spectrophotometrically as described by Tissue et al. (1993). Carbohydrate concentration of needles was expressed as percentage of dry weight attributable to sugars, starch and total nonstructural carbohydrates (TNC). Nitrogen concentration was expressed as a percentage of dry weight corrected for nonstructural carbohydrate accumulation (carbon-free basis).

Chlorophyll fluorescence quenching analysis

To monitor changes in light utilization, chlorophyll fluorecence quenching was measured by PAM fluorometry (Krause and Weis 1991) following leader excision and girdling treatments. The technique provides a sensitive nondestructive assay of the dissipation of excitation energy through both photochemical (qP) and non-photochemical (qN) pathways (Krause and Somersalo 1989). Chlorophyll fluorescence was measured with a modulated fluorometer (OS-500, OptiSciences, Inc., Boston, MA). A distance of 0.5 cm between the leaf surface and the end of the fiber optic cable was maintained in all measurements. The middle 2 cm of three attached needles from a single fasicle were dark adapted for 5 min before measurement. Quenching analysis of leaf-level light utilization was performed between 1300 and 1500 h at 8 days after the treatments on two fasicles from the two study cohorts on the branches that received both girdling and leader excision ($n = 4$). Quenching analysis was initiated by first measuring F_o (initial fluorescence; fluorescence with all photosystem II (PSII) traps open) and F_m (fluorescence maximum; fluorescence with all PSII traps closed) before the start of a 5-min actinic light period. Measurement of F_o was with a weak modulated beam. A 2-s saturating actinic flash followed, inducing variable fluorescence that peaked at F_m. Quenching analysis was performed under two actinic light regimes: 350 μmol $m^{-2} s^{-1}$ red light (the maximum possible with the OptiSciences LED source), and full sun (typically 1800 μmol $m^{-2} s^{-1}$). During exposure to actinic light, saturating flashes (≈8000 μmol $m^{-2} s^{-1}$) of 0.8 s duration, given at 20-s intervals, were used to cause transient reduction of Q_A, the electron acceptor of PSII, allowing measurement of the quenched state of F_m (F_m'). A weak 5-s far-red flash at the end of the run permitted measurement of the quenched state of F_o (F_o'). Photochemical and non-photochemical quenching parameters were calculated as described by van Kooten and Snel (1990). Fluorescence quenching values for a particular needle fasicle were obtained by averaging values obtained during three saturating flashes 60, 80 and 100 s after the actinic light was turned on.

Gas exchange

Gas exchange was measured on two randomly selected fasicles from both the 3-month-old and 1-year-old needle cohorts on branches subjected to both leader excision and girdling ($n = 4$). Measurements of needle gas exchange were conducted between 1300 and 1600 h during warm cloudless periods, 8 days after the leader excision and girdling treatments. Gas exchange parameters were measured with an open-flow gas-exchange system (LI 6400, Li Cor, Inc., Lincoln, NE). Three needles from a single fasicle for each measurement were placed across the long dimension of a 2 by 3 cm leaf cuvette. Total needle surface used in gas exchange measurements was calculated from a regression of needle length to total needle surface area (Naidu et al. 1999). Saturating light (1800 μmol $m^{-2} s^{-1}$) was provided by an LED red light source built into the top of the leaf chamber and CO_2 concentration was controlled by the Li Cor LI-6400 CO_2 injection system. The relationship between net assimilation (A) and the CO_2 partial pressure of the internal air space of needles (C_i) (A–C_i curve) was examined over a range of CO_2 (C_a) partial pressures from approximately 5 to 175 Pa, including 38 Pa CO_2 and 57 Pa CO_2. Relative humidity (RH) was maintained at 55 to 60% by controlling the air flow through a scrub of Li-Cor Dririte, resulting in a leaf-to-air vapor pressure deficit ranging between 1.25 and 1.75 kPa for all gas exchange experiments. Leaf temperatures were maintained within 0.5 °C of ambient (30–32 °C). Assimilation, C_i and stomatal conductance were calculated according to the equations of von Caemmerer and Farquhar (1981).

Maximum rates of carboxylation (V_{cmax}) and electron transport (J_{max}) were modeled by fitting the A–C_i data to a biochemically based linearization model (Wullschleger 1993; as modified by S.P. Long et al., University of Essex, U.K., personal comm.). In addition, assimilation data obtained at a C_i above 25 Pa were fitted to a second-degree polynomial to estimate A_{max}; i.e., net assimilation of the needles at saturating light and CO_2.

Sensitivity of photosynthesis to low oxygen air partial pressure was measured as the percent stimulation of light- and CO_2-saturated photosynthesis following exposure to 2% oxygen in balanced nitrogen as described by Harley and Sharkey (1991). Both 21 and 2% O_2 were provided by connecting the LI-6400 air inlet to bottled O_2 in air. This air was humidified to saturation before entering the LI-6400 by bubbling it through distilled water and it was then scrubbed to 55% RH to match the water content of the air used in the other gas exchange measures. First, assimilation was measured at 1800 μmol $m^{-2} s^{-1}$ of light, 175 Pa CO_2 and 21% O_2, and then the gas flow was switched to 2% O_2 air. Gas exchange was allowed to equilibrate for 10 min before assimilation was measured again.

All post-treatment values of gas exchange, fluorescence quenching and carbohydrate concentration were considered significantly different from the pre-treatment values if $P \leq 0.05$ in the student's t-test.

Results

Needle carbohydrate and nitrogen concentrations

Starch and total nonstructural carbohydrate concentrations increased significantly on Day 1 after leader excision (Table 1). A similar increase was observed for sugar concentration, except in 1-year-old needles from non-girdled branches where sugar concentration did not increase significantly even by Day 5 after leader excision. However, sugar concentration increased in 1-year-old needles when the branch was girdled. Sugar concentration in 1-year-old needles was twice that in

Table 1. Changes in needle biochemistry following candle excision and girdling treatments. Values are expressed as percent of needle dry weight ± standard error (SE). One fasicle was removed from each study cohort on 1 and 5 days post treatment from branches that received candle excision alone and on 5 and 8 days post treatment from branches that were also girdled. Values within the same column with a different letter following the SE values are significantly different at the $P \leq 0.05$ level in a student's *t*-test.

Needle flush	Treatment	Sugar	Starch	TNC	Nitrogen (carbon free)
3-Month-old needles	Pre-treatment	2.1 ± 0.1 a	4.0 ± 0.1 a	6.1 ± 0.1 a	3.32 ± 0.06 a
	1D After candle excision	2.6 ± 0.1 b	5.3 ± 0.1 b	7.9 ± 0.1 b	3.31 ± 0.02 a
	5D After candle excision	3.2 ± 0.1 c	6.0 ± 0.8 b	9.2 ± 0.6 c	3.53 ± 0.04 b
	5D After excision + girdle	4.1 ± 0.2 d	9.7 ± 0.6 c	13.8 ± 0.5 d	2.89 ± 0.03 c
	8D After excision + girdle	3.7 ± 0.4 d	12.5 ± 0.1 d	16.2 ± 0.3 e	2.90 ± 0.03 c
1-Year-old needles	Pre-treatment	5.0 ± 0.2 a	4.0 ± 0.2 a	9.0 ± 0.2 a	3.25 ± 0.08 a
	1D After candle excision	4.6 ± 0.01 a	6.9 ± 0.4 b	11.5 ± 0.3 b	3.26 ± 0.04 a
	5D After candle excision	4.4 ± 0.9 a	6.5 ± 0.4 b	10.9 ± 0.8 b	3.31 ± 0.09 a
	5D After excision + girdle	6.2 ± 0.1 b	11.1 ± 0.2 c	17.3 ± 0.2 c	2.64 ± 0.03 b
	8D After excision + girdle	7.0 ± 0.1 c	16.0 ± 0.5 d	23.0 ± 0.4 d	2.69 ± 0.07 b

3-month-old needles irrespective of the treatment. Increases in sugar concentration ranged from 24% at Day 1 after leader excision to a maximum of 86% at Day 8 after leader excision and girdling.

The experimentally induced reduction in sink demand resulted in a greater increase in starch concentration than in sugar concentration. The amount of starch accumulated was similar for both needle cohorts studied. The increase in starch concentration ranged from a low of 33% in 3-month-old needles, at Day 1 after leader excision, to a maximum increase of 400% in 1-year-old needles at 8 days after leader excision and girdling (Table 1). Patterns of changes in TNC were similar to those of starch because the relative increase in starch was much larger than the relative increase in sugar. The concentration of TNC was greater in 1-year-old needles than in 3-month-old needles because of their consistently higher sugar concentration.

Sugar, starch and TNC concentrations in 1-year-old needles did not continue to increase between Days 1 and 5 after leader excision, whereas sugar and TNC concentrations in 3-month-old needles increased between Days 1 and 5 after leader excision. Carbohydrate concentration was always greater in needles from branches that had the leaders excised and the branch girdled than in needles from branches that only had the leaders excised. The difference in starch concentration between the two treatments was greater than the treatment difference in sugar concentration. Between Days 5 and 8 after leader excision and girdling, sugar, starch and TNC concentrations increased significantly for both needle cohorts, except in the 3-month-old needles where the sugar concentration remained constant.

Nitrogen concentration was not affected by excision of the leader up to 5 days after treatment in 1-year-old needles, but it increased in 3-month-old needles (Table 1). However, when leader excision was combined with girdling, significant decreases in nitrogen concentration (% dry weight, calculated on a TNC-free basis) were observed on Day 5 after leader excision and girdling. These decreases ranged from a maximum of 17% in 1-year-old needles on Day 5 after treatment to a minimum of 13% in the 3-month-old needles on Day 8 after treatment.

Gas exchange

Stomatal conductance was unaffected by sink manipulation in both needle cohorts throughout the study period, indicating that stomatal closure was not a complicating factor in the interpretation of the photosynthetic response. For example, stomatal conductance measured in 3-month-old needles at saturating light and ambient CO_2 was similar ($P = 0.04$) on the day before treatment (92.2 ± 1.0 mmol m^{-2} s^{-1}) as on Day 8 after leader excision and girdling (89.9 ± 1.0 mmol m^{-2} s^{-1}).

Two potential biochemical limitations to photosynthesis were modeled from the A–C_i curves (Figure 2). Maximum Rubisco carboxylation rates (V_{cmax}) were estimated from the initial linear portions of the A–C_i curves (Table 2). The combined treatments caused the maximum rate of carboxylation to decrease by 25% in 3-month-old needles ($P = 0.02$) and by 31% in 1-year-old needles ($P = 0.05$). The maximum rate of electron transport, J_{max}, was estimated from linearization of the

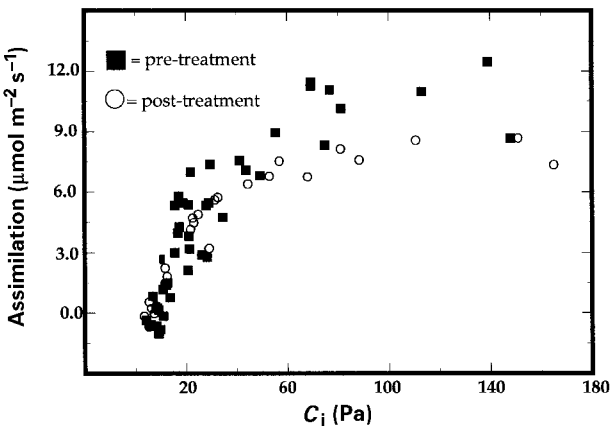

Figure 2. Plot of net assimilation versus internal CO_2 concentrations (C_i) for current-year needles before (■; six curves from six fasicles on two branches) and 8 days after terminal leader excision and girdling (○; four curves from four fasicles, two from each of two branches).

Table 2. Changes in photosynthetic characteristics of needles 8 days after girdling and terminal leader excision. The maximum rate of carboxylation (V_{cmax}) and electron transport (J_{max}) were modeled from A–C_i data by fitting the data to a biochemically based linearization model. The A_{max} was estimated from second-degree polynomial equations of best fit from data collected at a C_i of 25.0 Pa or above. During each A–C_i curve, the 175.0 Pa C_a measure made with 21% O_2 was repeated with 2% O_2. Percent stimulation was then calculated as $((A \text{ at } 2\% - A \text{ at } 21\%)/A \text{ at } 21\%) \times 100$. All post-treatment values were significantly different from the pre-treatment values at the $P \leq 0.05$ level in a student's t-test.

Needle flush	V_{cmax} (μmol m^{-2} s^{-1})	J_{max} (μmol m^{-2} s^{-1})	A_{max} (μmol m^{-2} s^{-1})	% stimulation by 2% O_2
3-Month-old needles				
Pre-treatment	33.05 ± 3.88 a	63.25 ± 4.78 a	11.45 ± 0.69 a	19.2 ± 7.7 a
Post-treatment	24.71 ± 2.13 b	39.45 ± 9.12 b	8.86 ± 0.57 b	3.3 ± 3.8 b
1-Year-old needles				
Pre-treatment	25.71 ± 3.55 a	59.63 ± 37.29 a	10.85 ± 1.03 a	16.5 ± 7.4 a
Post-treatment	17.70 ± 2.66 b	37.29 ± 3.81 b	9.03 ± 0.35 b	4.4 ± 5.1 b

entire A–C_i curve (Table 2). The combined treatments resulted in a 38% decrease in J_{max} in 3-month-old needles ($P = 0.01$) and a 37% decrease in J_{max} in 1-year-old needles ($P = 0.01$).

There was a reduction in A_{max} following leader excision and girdling in both the 3-month-old and 1-year-old needles (Table 2). The decrease for 1-year-old needles was smaller than for 3-month-old needles (17 and 23%, respectively).

Assimilation rates during exposure to saturating light and CO_2 and 21% oxygen in air were recorded before the O_2 concentration in the air supply was switched to 2% O_2. Before sink removal, low oxygen stimulated assimilation rates by 17 to 19%. Nine days after leader excision and girdling, low O_2 air stimulated assimilation rates by only 3 to 4% (Table 2).

Chlorophyll fluorescence quenching analysis

Photochemical quenching of chlorophyll fluorescence (qP) was not affected by leader excision and girdling when 350 μmol m^{-2} s^{-1} of red light was used as the actinic source (Table 3). However, when full sunlight was used as the actinic light source, post-treatment qP values were similar for both cohorts and were 24–27% lower than before the treatments. Needles from both cohorts exhibited increased non-photochemical quenching (qN) on Day 8 after treatment regardless of actinic intensity (Table 3). Large changes in qN were associated with measurements conducted with 350 μmol m^{-2} s^{-1} of red light as the actinic source (Table 3). Non-photochemical quenching in three-month-old needles increased 24% following leader excision and girdling, whereas qN increased 124% in 1-year-old needles. Use of full sunlight as the actinic source resulted in high qN values before treatment and consequently there was little capacity for further increases in qN in either the 3-month-old or 1-year-old needles in response to leader excision and girdling (Table 3). The increase in qN under full sunlight conditions was small (4%) although significant in 3-month-old needles, and there was a 40% increase in qN in 1-year-old needles during full sun irradiation on Day 8 after leader excision and girdling.

Discussion

When photosynthetic rate exceeds export of photosynthate, allocation of newly fixed carbon to transitory storage is critical for achieving a balance between source production and export to sinks (Geiger and Servaites 1991). In our study, experimentally reducing sink demand by leader excision and branch girdling caused decreases in leaf-level photosynthetic capacity and carbohydrate storage, indicating an imbalance between production and export, and resulting in photosynthetic down-

Table 3. Pre- and post-treatment chlorophyll fluorescence quenching analysis 8 days after terminal leader excision and girdling. All post-treatment values of photochemical quenching (qP) and non-photochemical quenching (qN) were significantly different from the control values at the $P \leq 0.05$ level in a student's t-test, except for the qP values measured with 350 μmol m^{-2} s^{-1} of red actinic light ($n = 4$, two fasicles from two different branches from each treatment and each needle flush).

Needle flush	Actinic light			
	350 μmol m^{-2} s^{-1} red light		Full sun ≈1800 μmol m^{-2} s^{-1}	
	qP	qN	qP	qN
3-Month-old needles				
Pre-treatment	0.92 ± 0.01 a	0.33 ± 0.01 a	0.89 ± 0.03 a	0.77 ± 0.02 a
Post-treatment	0.92 ± 0.03 a	0.41 ± 0.02 b	0.68 ± 0.04 b	0.80 ± 0.01 b
1-Year-old needles				
Pre-treatment	0.91 ± 0.00 a	0.21 ± 0.01 a	0.84 ± 0.02 a	0.52 ± 0.02 a
Post-treatment	0.90 ± 0.02 a	0.47 ± 0.02 b	0.61 ± 0.04 b	0.73 ± 0.01 b

regulation.

Export and storage of phosynthate following sink demand manipulation

The pathways for photosynthate storage tend to be species specific. In sugar beets, for example, the rate of sucrose export is similar to the rate of photosynthate production under normal conditions and carbohydrates are stored as starch if production exceeds export (Fondy et al. 1989). In barley (Gordon et al. 1980) and spinach (Gerhardt et al. 1987), excess photosynthates are stored as sucrose and starch. In loblolly pine, alterations in source–sink relationships in response to CO_2 enrichment resulted in excess carbohydrates being stored as soluble sugars and starch, with the increase in starch concentration being approximately twice that of the sugars (Griffin et al. 1996). Similarly, we found that excess carbohydrates were stored both as sugar and starch and the increase in starch concentration was often 2 to 10 times greater than the increase in sucrose concentration.

Chung and Barnes (1980) modeled photosynthate allocation in 15-year-old loblolly pine trees and concluded that the first flush of the current year consumed more photosynthate than it produced until mid-June, a date corresponding with bud break of the second flush. Concurrently, the two flushes of the previous year lost surplus carbon at a steady rate throughout the growing season. Their study predicts that, at the time of our leader excision and girdling treatments, 1-year-old needles were still a source for the expanding terminal leader (second flush of 1996). Based on the low carbohydrate concentration of the 3-month-old needles before the treatments, we conclude that these needles were already a major source of photosynthate for the emerging leader. The 1-year-old needles were probably also a source of photosynthate for the new leader because total relative carbohydrate accumulation following either sink reduction treatment was similar in extent in both the 1-year-old and 3-month-old needles.

Our data indicate that needles were exporting photosynthate to both the new growth on that branch and to the rest of the tree. Five days after leader excision, there was a 51% increase in TNC in the 3-month-old needles and a 21% increase in TNC in the 1-year-old needles. Five days after leader excision and girdling, TNC concentration increased 126% in the 3-month-old needles. In the 1-year-old needles, TNC concentration increased 92% in response to leader excision and girdling. Thus, at this point in the growing season, photosynthate was being exported to the growing leader as well as being transported out of the branch.

Noninvasive diagnosis of sink-limited photosynthetic capacity

Non-photochemical quenching measured with 350 μmol m^{-2} s^{-1} of red light as the actinic source provided a sensitive indicator of feedback regulation. The low amounts of qN measured before the treatments with 350 μmol m^{-2} s^{-1} of red light as the actinic source can be explained on the basis that the cohorts studied were experiencing a high demand for photosynthate; therefore, when given sub-saturating irradiance, there was little excess energy to dissipate. Following leader excision and girdling, sink demand was greatly reduced and the requirement for excess energy dissipation greatly increased. These results are in agreement with Sharkey's (1990) interpretation that feedback regulation of photosynthetic capacity requires increased amounts of excess light energy to be dissipated because photophosphorylation is limited by low phosphate concentrations in the stroma.

During full sunlight trials, few treatment effects on qN were observed in 3-month-old needles. It is known that there are species specific differences in the capacity for excess energy dissipation (Johnson et al. 1993). Also, it has been shown that qN can be saturated and that qN is correlated with actinic irradiance (Myers et al. 1997). Full sunlight may have saturated the ability of 3-month-old needles to dissipate energy by non-photochemical pathways. However, in 1-year-old needles, the treatments increased qN by 40% during full sunlight. The increase in qN in these needles can be explained on the basis of the finding that qN measured in full sunlight was low before the treatments were imposed, indicating that these older needles were fully acclimated to saturating light.

When full sunlight was used as the actinic source during analysis, qP decreased to a similar extent in both flushes. This is compatible with similar decreases in A_{max} for both cohorts measured by gas exchange. On the other hand, qP values were unchanged following leader excision and girdling when examined with 350 μmol m^{-2} s^{-1} of red light as the actinic source. Because this irradiance is well below the saturating value for these needles, it may not maintain electron transport rates high enough to drive ATP production at rates that would exhaust P_i (inorganic phosphorus) availability. Consequently, qP measured during 5-min exposures to 350 μmol m^{-2} s^{-1} of red light was insensitive to decreases in triose phosphate utilization resulting from feedback regulation, indicating that such measurements do not provide the diagnostic value of qP measurements made at full sunlight.

It has been hypothesized that low sink strength regulates photosynthetic capacity through a variety of direct and feedback mechanisms (Krapp and Stitt 1995). The most direct possibility is physical damage to the chloroplast as a result of an excess of stored starch grains (Nafziger and Koller 1976, Cave et al. 1981, Schaffer et al. 1986). Gezelius et al. (1981) reported that removal of the apical meristems from one- and two-year-old Scots pine seedlings resulted in large increases in needle starch concentration. Although Rubisco activity was higher following meristem removal, light-saturated photosynthesis was lower, indicating that starch buildup resulted in thylakoid damage. Schaffer et al. (1986) concluded that, in orange tree branches that were both girdled and defruited, storage of a large excess of starch resulted in physical damage to the thylakoid membranes leading to decreased photosynthetic capacity. In their study, only a combination of both girdling and defruiting resulted in starch grains of a size that induced chlorosis. The large increase (up to 400%) in starch concentration in our pine needles is similar to that measured in orange tree leaves and cotton and suggests that physical damage to the chloroplast contributed to the loss of photosynthetic capacity.

Less direct feedback mechanisms that regulate photosynthetic capacity include down-regulation of photosynthetic gene expression mediated by carbohydrate content (Sheen 1989, Krapp et al. 1993, Jang et al. 1997) and the limitations imposed by a lack of photosynthetic raw materials related to the inhibition of sucrose synthesis and recycling of hexoses (Foyer 1987, Goldschmidt and Huber 1992). In our study, measured changes in needle biochemistry suggest that both feedback mechanisms could have been responsible for the observed down-regulaton of photosynthetic capacity. Decreases in needle nitrogen content, carboxylation rates and saturated photosynthetic rates indicate reduced Rubisco content. The expression of the transcript for the small subunit of Rubisco (*rbcS*) has been shown to be regulated by sucrose concentration through a signal transduction pathway initiated at the assimilatory enzyme hexokinase (Jang et al. 1997). The large increases in sucrose and starch concentrations and the time line of a few days are consistent with a feedback control of Rubisco content associated with decreased *rbcS* transcription as has been observed in spinach (Krapp and Stitt 1995).

On the other hand, the insensitivity of photosynthesis to low concentrations of oxygen in air, low J_{max} and the fluorometric data strongly indicate that photosynthetic capacity was being limited by low concentrations of cytosolic inorganic phosphorus, P_i, following the removal of sink demand. Reduced rates of sucrose production may be caused by the accumulation of sucrose as a result of end-product feedback control on sucrose phosphate synthase (SPS) (Huber and Huber 1992). This feedback control results in an accumulation of phosphorylated intermediates (Stitt and Quick 1989, Sawada et al. 1990, Suwigno et al. 1995) and an inadequate supply of photosynthetic raw material (RuBP, cytosolic P_i) that can limit photosynthetic capacity (Herold 1980, Sharkey 1990). Reduced triose phosphate utilization may inhibit photosynthetic capacity by decreasing the cytosolic P_i available for ATP production (Harley and Sharkey 1991, Harley et al. 1992). Plaut et al. (1987) removed buds and girdled seven crop species and concluded that, for three of the seven species, decreased photosynthesis was attributable to feedback regulation resulting from a decrease in cytosolic P_i resulting from an accumulation of phosphorylated sugars. In our study, J_{max} decreased by 38% on Day 8 following the removal of sink demand. In addition, there was a large decrease in relative sensitivity of photosynthesis to 2% oxygen in air and a large increase in non-photochemical quenching. These findings indicate that RuBP and P_i supply were limiting to photosynthetic capacity following the removal of sink demand (Sharkey 1990, Harley and Sharkey 1991). If this interpretation is correct, low P_i concentrations may help explain the lower V_{cmax} measured following removal of sink demand, because low cytosolic P_i concentrations have been shown to regulate Rubisco activity (Sharkey 1990). As estimated from the A–C_i data, carboxylation rates in 3-month-old needles decreased 31% after sink removal, whereas decreases of 25% were observed in 1-year-old needles. Similarly, decreases in photosynthetic capacity following sink removal were a result of decreased Rubisco activity in both single-rooted soybean leaves (Sawada et al. 1990) and in whole plants (Sawada et al. 1995) in which free P_i concentrations were low. By artificially increasing the concentration of P_i available to bind to CO_2, regulation of Rubisco activity has been directly linked to the concentration of free P_i in the stroma (Anwaruzzaman et al. 1995).

In summary, a reduction in sink demand resulted in an imbalance between photosynthate production and export. Just before leader excision and girdling, both current-year and previous-year needles were exporting photosynthate to the emerging terminal leader as well as out of the branch. Following the experimental reduction in sink demand, carbohydrates accumulated as both sucrose and starch. In addition, noninvasive techniques identified changes in physiological characteristics indicative of feedback down-regulation of photosynthetic capacity. We conclude that photosynthetic capacity in loblolly pine needles can be rapidly down-regulated when sink limitations develop.

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