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Partitioning of ^{14}C-labeled photosynthate to allelochemicals and primary metabolites in source and sink leaves of aspen: evidence for secondary metabolite turnover

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Abstract Theories on allelochemical concentrations in plants are often based upon the relative carbon costs and benefits of multiple metabolic fractions. Tests of these theories often rely on measuring metabolite concentrations, but frequently overlook priorities in carbon partitioning. We conducted a pulse-labeling experiment to follow the partitioning of $^{14}CO_2$-labeled photosynthate into ten metabolic pools representing growth and maintenance (amino acids, organic acids, lipids plus pigments, protein, residue), defense (phenolic glycosides, methanol:water and acetone-soluble tannins/phenolics), and transport and storage (sugars and starch) in source and importing sink leaves of quaking aspen (*Populus tremuloides*). The peak period of ^{14}C incorporation into sink leaves occurred at 24 h. Within 48 h of labeling, the specific radioactivity (dpm/mg dry leaf weight) of phenolic glycosides declined by over one-third in source and sink leaves. In addition, the specific radioactivity in the tannin/phenolic fraction decreased by 53% and 28% in source and sink leaves, respectively. On a percent recovery basis, sink leaves partitioned 1.7 times as much labeled photosynthate into phenolic glycosides as source leaves at peak ^{14}C incorporation. In contrast, source leaves partitioned 1.8 times as much ^{14}C-labeled photosynthate into tannins/phenolics as importing sink leaves. At the end of the 7-day chase period, sink leaves retained 18%, 52%, and 30% of imported ^{14}C photosynthate, and labeled source leaves retained 15%, 66%, and 19% of in situ photosynthate in metabolic fractions representing transport and storage, growth and maintenance, and defense, respectively. Analyses of the phenolic fractions showed that total phenolics were twice as great and condensed tannins were 1.7 times greater in sink than in source leaves. The concentration of total phenolics and condensed tannins did not change in source and sink leaves during the 7-day chase period.

Key words Phenolic glycosides · Phenolics · Plant chemical defense · Metabolic turnover · Aspen

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Introduction

Carbon-based allelochemical content can be an important aspect of tree leaf quality for insect herbivores. A diversity of abiotic and biotic factors, such as drought, nutrient availability, elevated atmospheric CO_2, light, and defoliation can alter the concentrations of carbon-based allelochemicals, and thus change the suitability of foliage to insect herbivores (Mattson and Haack 1987; Rossiter et al. 1988; Waterman and Mole 1989; Waring and Cobb 1992; Lindroth et al. 1993). The cost of carbon-based allelochemicals, relative to the demands for photosynthate by other metabolic functions, has been the basis for theories on the ecological and evolutionary production of plant defenses (Feeny 1976; Rhoades and Cates 1976; Bryant et al. 1983; Coley et al. 1985; Herms and Mattson 1992). However, the lack of empirical studies on the relative costs of plant defenses has led to inconsistent predictions of the distribution or change in plant defenses in response to environmental conditions (Larsson 1989; Reichardt et al. 1991; Stamp 1992; Gershenzon 1994).

The carbon/nutrient balance (CNB) hypothesis (Bryant et al. 1983; Gershenzon 1984; Tuomi et al. 1984) has provided an effective theoretical framework for experimentally testing the effects of resource availability on the relative responses of carbon-based allelo-

chemical concentrations in plants (Reichardt et al. 1991; Fajer et al. 1992; Herms and Mattson 1992). The basis of this theory recognizes that plants acquire carbon, allocate it to regions of growth (leaves, stem, roots), reproduction, or storage. Moreover, plants partition this carbon among various metabolic fractions that can be broadly defined as supporting growth (protein, amino acids, organic acids, lignin, and hemicellulose), defense (carbon-based defenses), or storage (starch) (Mooney and Chu 1974; Dickson and Larson 1975; Bloom et al. 1985). When environmental conditions increase the availability of carbon beyond the requirements for growth, the allocation and partitioning of photosynthate to carbon-based defenses and storage increases. Although the CNB hypothesis has been supported by numerous experimental studies and correlative evidence, empirical support for this hypothesis has not been consistent under all of the conditions tested (e.g., Reichardt et al. 1991; Fajer et al. 1992; Herms and Mattson 1992; Kinney et al. 1997).

The lack of consistent results from experiments testing the CNB hypothesis can be attributed, in part, to difficulties in explicitly demonstrating that carbon is allocated to growth at the expense of carbon-based allelochemicals. Moreover, ambiguous or erroneous conclusions may be drawn by inadequately accounting for plant responses at several levels of biological organization (Chapin 1991; Coleman et al. 1992), overlooking ontogenetic constraints on resource partitioning (Dickson and Larson 1975, 1981; Gershenzon 1994), or by not accounting for potential turnover of metabolic pools (Reichardt et al. 1991; Gershenzon 1994). Addressing the theoretical aspects of the CNB hypothesis has been hampered by limitations of experimental approaches for studying plant/insect interactions (Jones and Coleman 1991; Ågren 1994; Haukioja et al. 1994; Mole 541994). The use of ^{14}C has provided valuable insight into the allocation of carbon to plant structures and the partitioning to metabolic pools for trees growing under conditions that can alter resource availability (e.g., Roberts 1964; Edwards et al. 1992; Coleman et al. 1995; Samuelson and Kelly 1996). Radiotracer studies have also provided information on the relative metabolic costs, turnover and proportion of photosynthate partitioned to allelochemicals (Mooney and Chu 1974; Prudhomme 1983; Maufette and Oechel 1987; Mihaliak et al. 1991; Gershenzon 1994).

Plants from the genus *Populus* make excellent experimental models for the study of carbon allocation and partitioning because many of the relationships among physiological processes, chemical profiles, structural development and leaf ontogeny have been described in detail (Larson et al. 1972; Larson and Dickson 1973; Dickson and Larson 1975; Dickson and Shive 1982; Dickson 1986). However, little is known about the partitioning of recently fixed photosynthate to carbon-based allelochemicals. The objectives of this study were to examine ^{14}C partitioning to metabolic fractions representing growth, defense, and storage, and their turnover from (a) in-situ-produced photosynthate in mature aspen (*Populus tremuloides* Michx.) leaves and (b) imported photosynthate in young expanding leaves. Patterns of ^{14}C partitioning to defense fractions were compared with measures of total phenolics and condensed tannins.

Materials and methods

Plant material

Aspen plants were produced from half-sib seed obtained from the North Central Experiment Station at the University of Minnesota (Grand Rapids, Minn.). Seedlings were transplanted into 15-l pots with a 1:1:1 mix of sand:peat:vermiculite and 114 g of Osmocote 13-13-13 fertilizer (Sierra Chemical Corp., Milpitas, Calif.) and grown in a greenhouse (21/18°C), with a 11- to 15-h natural daylength, extended to 16 h with 400 W sodium vapor lamps.

^{14}C labeling

Plants were treated at a plastochron index (PI) of 24–26 (Larson and Isebrands 1971). An apical leaf that has attained a lamina length of 2 cm is designated as LPI 0, and the next older leaf is designated as LPI 1, and so on down the stem. Individual plants were preconditioned for 30 min in the treatment chamber beneath a sunbrella high-intensity light fixture (Environmental Growth Chambers, Chagrin Falls, Ohio) with high-pressure sodium and metal halide lamps (400 W each). Light intensities at the leaf surface ranged from 650 to 750 μmol m^{-2} s^{-1} (PAR). The most recently mature, non-expanding leaf (LPI 8–10) on each tree was enclosed in a water-cooled Plexiglas cuvette. These source leaves were treated with 1.85 MBq (50 μCi) $^{14}CO_2$ released from $Na_2{}^{14}CO_3$ with 20% lactic acid for 30 min in a closed system, with circulating, humidified air. Labeling was conducted between 6 and 10 h after the start of the photoperiod. After the labeling period, two punches of the ^{14}C-exposed leaf (0.5 cm diameter) were collected and oven dried, and the ^{14}C content was determined to estimate initial fixation. Treated plants were returned to the greenhouse, and the labeled source leaf, and 9–11 sink leaves above the source leaf were harvested from each of three separate plants at 0.5, 1, 2, 6, 24, 48, 72, and 168 h after the start of the labeling treatment. All leaves were frozen at −60°C, lyophilized and ground to 40 mesh with a Wiley mill. Leaf punches and subsamples from ^{14}C-labeled source leaves were solubilized with BTS-450 and the ^{14}C content determined by liquid scintillation spectrometry (LS-9800; Beckman, Fullerton, Calif.) using Ready-Organic scintillation cocktail (Beckman). Subsamples from ground sink leaves were hydrated and suspended in Ready-Gel for counting ^{14}C activity.

Temporal patterns of ^{14}C retention in source leaves and translocation to sink leaves were examined. By 6 h after labeling, the specific activity of sink leaves was great enough for partitioning analysis. Sink leaves that were eight positions above the labeled source leaf (LPI 0–2) had the highest ^{14}C activity and were selected for sequential separation into metabolic fractions.

Sequential separation of ^{14}C-labeled metabolic fractions

Leaf samples (7–20 mg) were separated into ten phytochemical fractions representing growth and metabolism (lipids plus pigments, amino acids, organic acids, protein, residue), storage and transport (starch and sugars), and carbon-based defenses (phenolic glycosides, methanol:water-soluble tannins/phenolics and acetone-soluble tannins/phenolics) using a modified sequential extraction method (Dickson 1979; Lindroth et al. 1986; Mauffette and Oechel 1987; Fig. 1). The name assigned to each separated

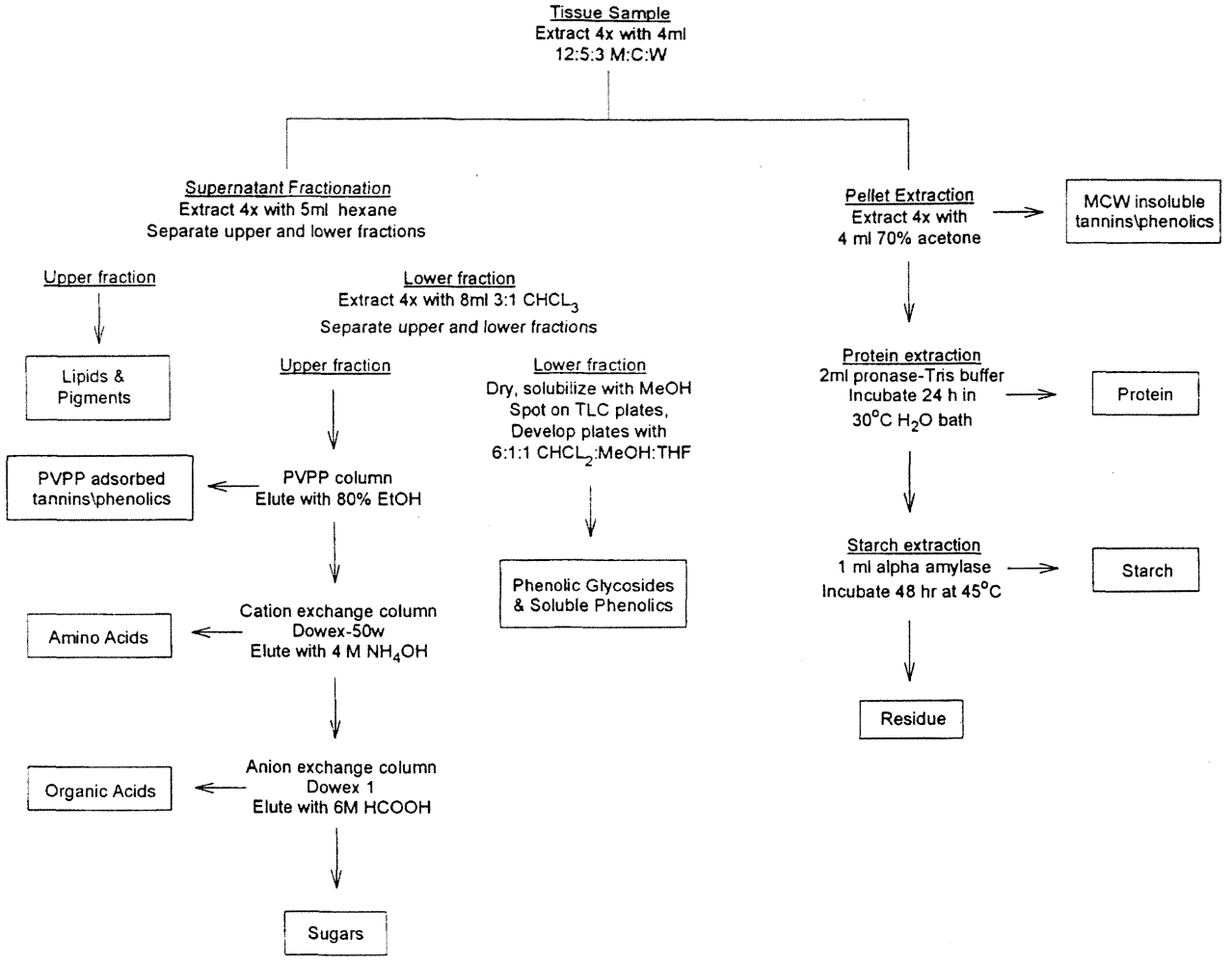

Fig. 1 Flow diagram for the sequential extraction and separation of ten metabolic fractions from aspen leaves (*M:C:W* methanol:chloroform:water, *PVPP* polyvinylpyrrolidone, *THF* tetrahydrofuran)

fraction reflects the dominant class of compounds contained in that fraction.

Subsamples of ground leaf material were extracted (four times) in 4 ml cold 12:5:3 methanol:chloroform:water (MCW) to isolate soluble constituents. Leaf material was mixed with solvent, sonicated in ice water for 15 min, then centrifuged at 2400 rpm for 10 min. The resulting supernatant was extracted with 5 ml hexane (4×) to isolate lipids plus pigments. Solvents from liquid/liquid extractions were separated by centrifuging at 1300 rpm for 5 min. Phenolic glycosides were not visible when the hexane fraction was reduced under vacuum, resolubilized in MeOH and spotted on thin-layer chromatography (TLC) plates (see below). The remaining methanol:chloroform:water fraction was extracted (four times) with 8 ml 3:1 chloroform:water to yield a lower chloroform:methanol fraction containing phenolic glycosides and simple phenolics and an upper methanol:water (MW) fraction. The chloroform:methanol fraction was dried under vacuum (25°C), solubilized in MeOH and spotted on silica gel TLC plates with fluorescent indicator. Plates were developed in 6:1:1 methylene chloride:methanol:tetrahydrofuran, the phenolic glycosides (salicin, salicortin, tremuloiden, and tremulacin were identified by standards) were visualized under UV light (254 nm) and scraped off the plate. The origin spot was counted separately and added to the soluble tannins/phenolics fraction isolated from the MW supernatant. When the MW fraction was reduced under vacuum, resolubilized in MeOH, and spotted on TLC plates, extremely faint phenolic glycoside spots were evident, indicating that not all of the phenolic glycosides could be extracted in the chloroform:methanol fraction.

Tannins and remaining phenolics were separated by pulling the MW fraction through a 8 × 100 mm glass column containing approximately 500 mg insoluble, cross-linked polyvinylpyrrolidone (PVPP; Polyclar AT, General Aniline and Film Corp., New York) fitted to a vacuum chamber (Speed-Mate 30, Applied Separations, Allentown, Pa.). After treatment with PVPP, absorption values of the MW fraction were equivalent to solvent blanks when tested for condensed tannins and total phenolics (see below for methods). The PVPP column was then rinsed with 60 ml 80% EtOH. The ethanol:methanol:water fraction from the PVPP column was then vacuum pulled through a tandem column (6-ml syringe columns; Applied Separations) consisting of a cation exchange resin (2.5 ml Dowex-50w) for adsorption of amino acids, and an anion exchange resin (2.5 ml Dowex 1) for adsorption of organic acids. Amino acids were eluted from the anion exchange resin with 4 M NH_4OH, and organic acids were eluted from the cation exchange resin with 6 M acetic acid. The supernatant passing through both columns contained neutral sugars.

The sample pellet was extracted (four times) in 4 ml 70% acetone to isolate residual tannins and phenolics, then treated with 2 ml pronase-Tris buffer (0.4 mg pronase/ml 0.05 M Tris adjusted to pH 7.4 with HCl; Calbiochem-Novabiochem, LaJolla, Calif.) in a 30°C water bath for 24 h to hydrolyze the protein. Following the protein extraction, the pellet was boiled in 2 ml H_2O to gelatinize the starch. Starch was hydrolyzed by adding 1 ml of mylase 100 to the pellet (20 mg alpha-amylase/ml 0.1 M sodium acetate adjusted to pH 5.5 with NaOH; G.B. Fermentation Industries, Charlotte, N.C.) and incubating at 45°C for 48 h.

The remaining pellet (residue) contained primarily hemicellulose, cellulose, and lignin.

The ^{14}C in the water- and organic-soluble fractions was counted in Ready-Safe and Ready-Organic scintillation cocktails, respectively. Silica gel, insoluble PVPP, and residue were suspended in Ready-Gel. The amount of ^{14}C is expressed as a percent of the total ^{14}C recovered (e.g., ^{14}C in sugars/total ^{14}C recovered in all chemical fractions), and as specific radioactivity (dpm/mg dry leaf weight).

Allelochemical analysis of leaves

Samples of ground source and sink leaves (45–55 mg) were extracted (three times) with a total of 4 ml, ice-cold 70% aqueous acetone containing 10 mM ascorbic acid. Proanthocyanidins were converted to anthocyanidins with BuOH:HCl and measured as condensed tannins using a modification of Porter et al. (1986). Total phenolics were estimated with a modification of the Prussian Blue assay (Price and Butler 1977). Condensed tannins from field-collected aspen foliage were isolated by absorption chromatography (with Sephadex LH-20 and ethanol and acetone as solvents; Hagerman and Butler 1978) and used to construct standard curves. There was not enough leaf tissue available after analyzing for ^{14}C to determine allelochemical concentration in sink leaves harvested at 6 and 24 h. Differences in allelochemical concentration among the sampling periods or between source and sink leaves were compared with one-way ANOVA (Proc AOV; SAS 1990).

Results

Transport of ^{14}C from source to sink leaves

Sink leaves with vascular connections to the labeled source leaf became apparent within 1 h after the start of the labeling period, and retained a high specific activity for up to 7 days (Table 1). The greatest specific activity was found in sink leaves eight positions above the source leaf. Leaves that were three, five, and seven leaves above the source leaf also had consistently high specific activity during the 7-day chase period. Maximum accumulation of ^{14}C into all harvested sink leaves occurred by 24 h. Sink leaves eight positions above the source leaf had, on average, one-third of the specific activity that was present in the source leaf at 0.5 h (Table 2). Within 24 h, the specific activity of the labeled source leaf decreased by

Table 1 Specific activity (dpm/mg leaf dry weight) in labeled source leaves and importing sink leaves during a 168-h sampling period. Each sample period is the average of three replicate plants. The bottom row of table shows the specific activity of leaf punches collected from the source leaves of replicate plants at 0.5 h. Samples from leaves marked with an *asterisk* were sequentially extracted into metabolic fractions

Position above source	Sampling Period (h)							
	0.5	1	2	6	24	48	72	168
11								5,125
10				30,836	99,528	–	46,699	9,102
9	14	148	48	338	4,561	4,299	4,975	922
8*	607	1,182	18,369	40,945	109,740	57,999	67,543	14,264
7	182	451	3,670	10,320	25,296	14,364	21,626	6,915
6	58	171	1,739	914	12,761	14,115	24,717	5,731
5	40	289	2,796	5,283	20,050	13,846	11,151	8,621
4	16	18	35	239	287	233	439	309
3	14	64	36	582	396	472	3,692	495
2	10	47	18	46	106	104	206	128
1	10	191	11	33	91	42	84	154
Source*	272,195	296,657	254,543	311,935	121,386	62,417	79,560	61,653
Source at 0.5 h	272,195	339,996	338,137	553,153	335,600	369,474	398,970	303,258

Table 2 Percent of specific activity (dpm/mg leaf dry weight) in source and sink leaves based upon the specific activity of leaf punches collected from labeled source leaves after the 30-min labeling period (0.5 h sample, Table 1). Columns do not sum to 100% because ^{14}C transported to stem and lower leaves was not accounted for. Each sample period is the average of three replicate plants. Samples from leaves marked with an *asterisk* were sequentially extracted into metabolic fractions

Position above source	Sampling Period (h)							
	0.5	1	2	6	24	48	72	168
11								1.69
10				5.57	29.66	–	11.70	3.00
9	0.01	0.04	0.01	0.06	1.36	1.16	1.25	0.30
8*	0.22	0.35	5.43	7.40	32.70	15.70	16.93	4.70
7	0.07	0.13	1.09	1.87	7.54	3.89	5.42	2.28
6	0.02	0.05	0.51	0.17	3.80	3.82	6.20	1.89
5	0.01	0.09	0.83	0.96	5.97	3.75	2.79	2.84
4	0.01	0.01	0.01	0.04	0.09	0.06	0.11	0.10
3	0.01	0.02	0.01	0.11	0.12	0.13	0.93	0.16
2	0.00	0.01	0.01	0.01	0.03	0.03	0.05	0.04
1	0.00	0.06	0.00	0.01	0.03	0.01	0.02	2.16
Source*	100.00	87.25	75.28	56.39	36.17	16.89	19.94	20.33

64%. By day 7 of the chase period, about 80% of the initial specific activity of the source leaves had been translocated or respired. Within sink leaves, declines in specific activity (Table 1) and in percent of initial source leaf specific activity (Table 2) were due to dilution of ^{14}C in rapidly expanding young leaves as well as to respiration.

Partitioning of ^{14}C among chemical fractions in labeled source leaves

The percentage of ^{14}C recovered during the first 24 h after labeling was greatest in metabolic pools representing transport and storage (sugars and starch, respectively) of photosynthate. The percentage of ^{14}C in the sugar fraction peaked 2 h after the labeling period, and then declined throughout the next two light and dark cycles before leveling off at 48 h (Fig. 2A). The specific activity of the sugar fraction peaked at 1 h and also declined until 48 h before leveling off (Fig. 2C). Both the percentage of ^{14}C and the specific activity of the starch fraction peaked toward the end of the first light cycle (6 h), followed by a steep decline during the subsequent dark cycle, then remaining relatively stable during the following 6 days (Fig. 2B,D). Nearly 15% of the ^{14}C recovered after the 7-day chase period was still in fractions representing transport and storage.

Metabolic pools reflecting the distribution of ^{14}C to the processes of growth and metabolism showed little change during the first 6 h after labeling, but increased during the first dark cycle and continued to increase within, and in some cases, beyond the first 48 h. The percentage of ^{14}C in the lipid + pigment fraction increased gradually throughout the 7-day sampling period, although the specific activity remained relatively constant (Fig. 2A,C). Both the proportion of ^{14}C and the specific activity of the amino acid fraction declined within the first 2 h after labeling, then remained constant (Fig. 2A,C). The percentage of ^{14}C in the organic acid fraction also declined during the first 6 h, then doubled at 24 h and remained constant for the next 48 h before gradually declining over the next 4 days (Fig. 2A). The proportion of ^{14}C in the protein and residue fraction increased after the end of the first light cycle (Fig. 2A), as activity in the starch and sugar fraction decreased. The percentage of ^{14}C in these two fractions continued to increase over the 7-day transport period. The specific activity of the protein fraction declined gradually after a peak at 6 h, whereas the specific activity of the residue fraction peaked at 48 h, then declined gradually during the next 5 days (Fig. 2D). Metabolic pools associated with growth and metabolism contained almost 66% of the ^{14}C recovered after 7 days.

Little ^{14}C was partitioned to metabolic pools representing plant defenses in source leaves within the first 6 h after labeling, but such partitioning increased rapidly at 24 h (Fig. 3A,C). Phenolic glycosides contained most of the ^{14}C, followed by MW-soluble tannins/phenolics. The proportion of ^{14}C in phenolic glycosides remained around 10% for 48 h, then increased to nearly 15% by day 7. MW-soluble tannins/phenolics declined for 24 h following a peak, then remained constant for the next 5 days. Acetone-soluble tannins/phenolics exhibited minor fluctuations that mirrored those of MW-soluble tannins/phenolics. Slightly more than 19% of the ^{14}C

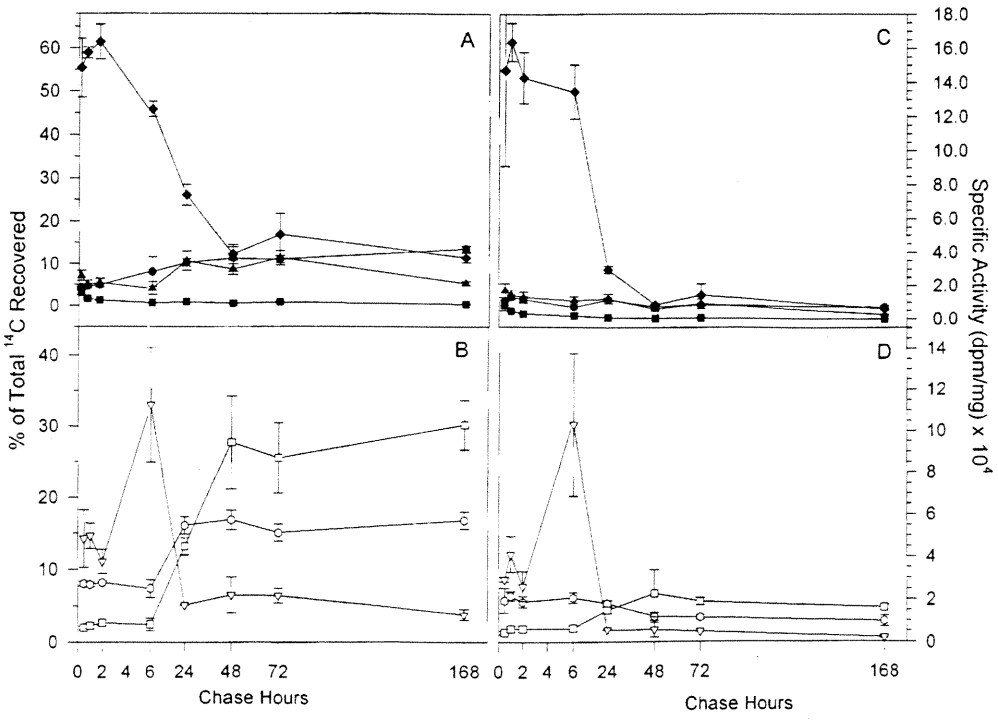

Fig. 2 Distribution of photosynthetically fixed ^{14}C among metabolic fractions in source leaves representing transport, storage and growth during a 168-h pulse-labeling experiment. Each point is the mean ± SE of three replicate plants. (*filled circles* lipids and pigments, *filled squares* amino acids, *filled triangles* organic acids, *filled diamonds* sugars, *open hexagons* protein, *open inverted triangles* starch, *open squares* residue) **A,B** Partitioning expressed as percent of total ^{14}C recovered. **C,D** Partitioning expressed as specific activity (dpm/mg leaf dry weight)

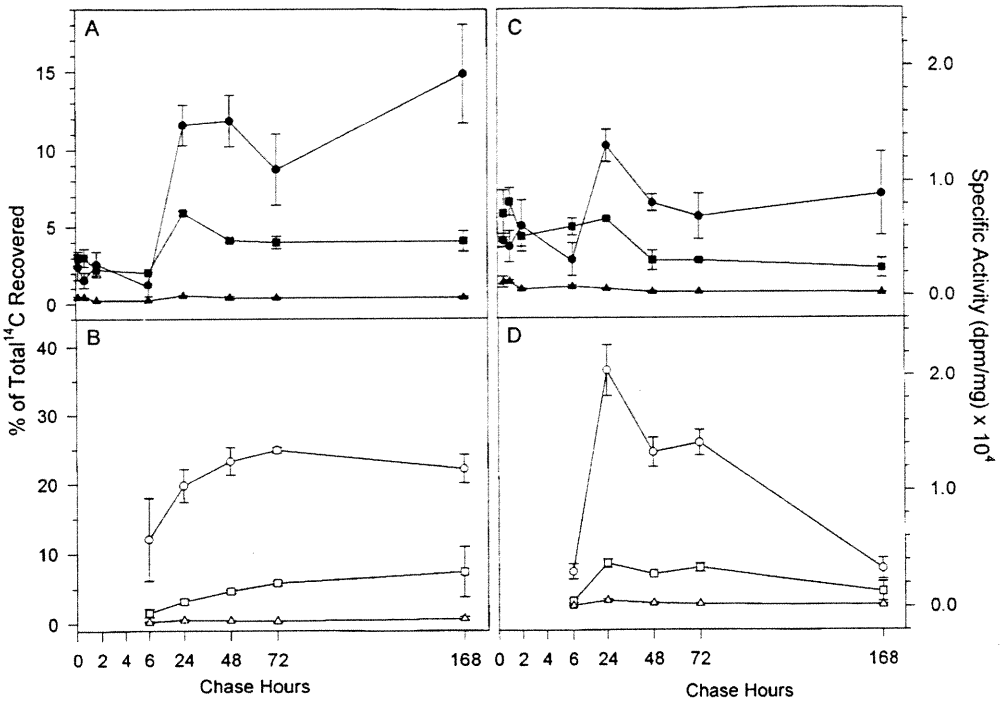

Fig. 3 Distribution of photosynthetically fixed ^{14}C among metabolic fractions representing defense compounds in aspen source (LPI 8–10) and sink (LPI 0–2) leaves during a 168-h pulse-labeling experiment. Partitioning expressed as percent of total ^{14}C recovered in source (**A**) and sink (**B**) leaves. Partitioning expressed as specific activity in source (**C**) and sink (**D**) leaves. Each point is the mean ± SE of three replicate plants (*circles* phenolic glycosides, *squares* methanol:chloroform:water-soluble phenolics/tannins, *triangles* acetone-soluble phenolics/tannins; *closed symbols* source. *open symbols* sink)

recovered after 7 days was found in these three defense fractions.

The time course of the percentage of ^{14}C recovered in any one of the metabolic fractions is dependent, in part, upon changes in the other nine, and therefore does not reflect pool turnover rates as accurately as specific activity does. The specific activity of phenolic glycosides in source leaves was the largest of the three defense fractions and peaked at 24 h (13,121 dpm/mg) declined by nearly 38% by 48 h (8,147 dpm/mg), then remained nearly constant until day 7 (Fig. 3C). ^{14}C in the combined MW- and acetone-soluble tannins/phenolics at 24 h was about half that found in phenolic glycosides (7,286 dpm/mg) and declined by 53% within 24 h (3,400 dpm/mg). By day 7 of the chase period, the specific activity of the combined tannin/phenolic pools had decreased by 64% from the peak at 24 h. The metabolic fractions representing growth and metabolism contained three times more ^{14}C than fractions representing defense (35,715 versus 11,456 dpm/mg) at the end of the 7-day chase period.

Partitioning of ^{14}C among chemical fractions in importing sink leaves

The distribution of imported ^{14}C-labeled photosynthate in chemical fractions of sink leaves differed from that of source leaves. Although percent ^{14}C recovered in the sugar fraction (transport) was nearly the same as for source leaves (compare Fig. 4A to Fig. 2A), the amount of ^{14}C found in starch (storage) was low and changed little with time compared to that in source leaves (compare Fig. 4B to Fig. 2B). The specific activity of the sugar fraction remained high during the first 24 h after labeling, as ^{14}C-labeled sugars continued to be imported (Table 1). Within 48 h, import of ^{14}C-labeled photosynthate into the sink leaf declined, as did the specific activity of the sugar fraction (Fig. 4C). The percentage of ^{14}C in the starch fraction remained low throughout the 7-day chase period. At the end of the 7-day chase period, approximately 18% of the recovered ^{14}C was in the transport and storage fractions.

Organic acids, amino acids and the lipid + pigment fraction all contained less than 10% of the ^{14}C recovered throughout the 7-day chase period (Fig. 4A). In contrast, the percent of ^{14}C recovered in the protein and residue fractions was much greater and increased during the 7-day chase period (Fig. 4B). The specific activity of all five fractions peaked at 24 h, then decreased (Fig. 4C,D). Over half (52%) of the ^{14}C recovered at the end of the 7-day chase period was in fractions associated with growth.

The percentage of ^{14}C in fractions associated with defense increased rapidly during the first 72 h of the chase period (Fig. 3B), then leveled off or decreased slightly for the remainder of the chase period. The proportion of ^{14}C recovered at 24 h in the phenolic glycoside fraction was five times greater than that recovered from both MW- and acetone-soluble tannins/phenolics. The three defense fractions contained just over 30% of the ^{14}C recovered at day 7.

Specific activity in all three defense fractions peaked at 24 h, with phenolic glycosides containing the most ^{14}C (Fig. 3D). Between the 24- and 48-h sampling periods, the specific activity of ^{14}C in phenolic glycosides decreased by 35%, and that of the combined MW- and acetone-soluble tannins/phenolics decreased by 28%. The specific activity

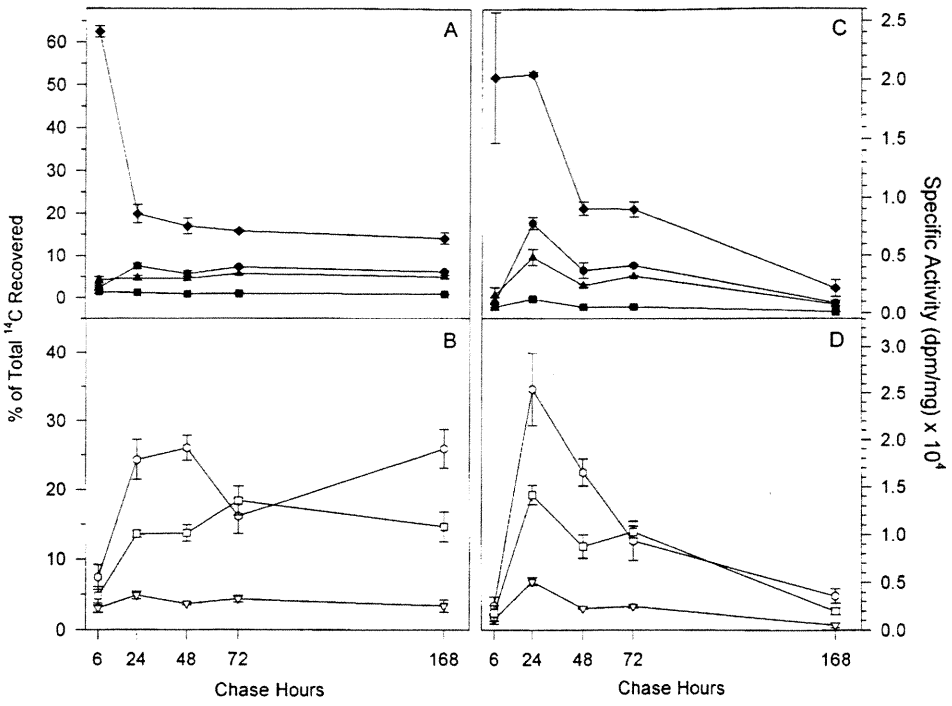

Fig. 4 Distribution of photosynthetically fixed ^{14}C among metabolic fractions representing transport, storage, and growth in aspen sink (LPI 0–2) leaves during a 168-h pulse-labeling experiment. Each point is the mean ± SE of three replicate plants. Symbols as in Fig. 2. **A,B** Partitioning expressed as percent of total ^{14}C recovered. **C,D** Partitioning expressed as specific activity

of both fractions remained constant between 48 and 72 h, then declined between 72 and 168 h. After 7 days of leaf growth, fractions representing growth and metabolism contained 1.5 times as much ^{14}C as the defense fractions (7,298 versus 4,638 dpm/mg), with the protein and phenolic glycoside fractions containing the most ^{14}C (3,595 and 3,242 dpm/mg, respectively).

Partitioning of ^{14}C to defense compounds differed between source and sink leaves. At 24 h, sink leaves partitioned nearly twice as much ^{14}C into phenolic glycosides compared to source leaves on a percentage basis (19.8 versus 11.6%), and 1.5 times as much on a specific activity basis (20,465 versus 13,121 dpm/mg). In contrast, source leaves partitioned approximately 1.8 times as much ^{14}C to MW- and acetone-soluble tannins/phenolics combined compared to sink leaves on both a percentage and specific activity basis (6.5 versus 3.6%, and 7,286 versus 4,268 dpm/mg, respectively).

Measures of allelochemicals in source and sink leaves

Concentrations of total phenolics and condensed tannin did not reflect the patterns of ^{14}C partitioning to defense fractions during the 7-day chase period. Total phenolics did not change significantly during the chase period for source leaves ($P = 0.25$; Fig. 5A) or sink leaves ($P = 0.61$; Fig. 5B). Condensed tannin concentrations were also constant during the chase period for both source ($P = 0.35$) and sink ($P = 0.15$) leaves. The concentrations of total phenolics were twice as great, and condensed tannins were 1.7 times greater in sink leaves than source leaves sampled between 48 and 168 h ($P < 0.04$ and $P < 0.03$, respectively).

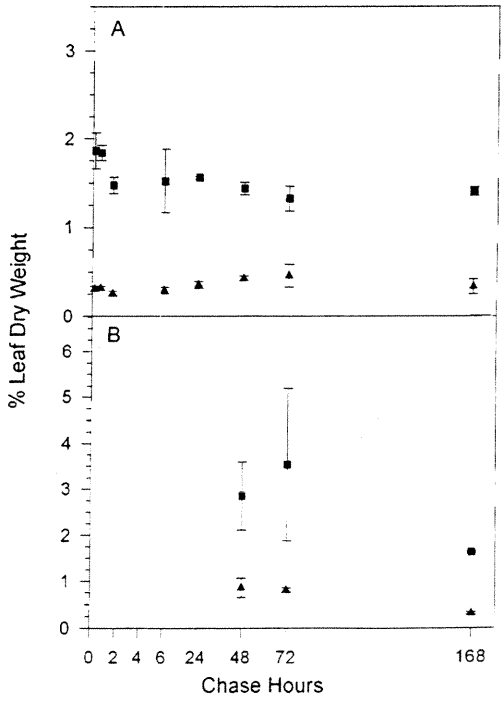

Fig. 5 Total phenolics (*squares*) and condensed tannins (*triangles*) in source (LPI 8–10) (**A**) and sink (LPI 0–2) (**B**) leaves of aspen sampled during a 168-h pulse-labeling experiment. Each point is the mean ± SE of three replicate plants

Discussion

Our results on the relative partitioning of recently fixed ^{14}C among metabolic pools representing growth and metabolism, and transport and storage in aspen are

similar to those reported for *P. deltoides* (Dickson and Larson 1975, 1981).

The steep decline in the specific activity of ^{14}C in both phenolic glycosides and tannins/phenolics within a short time period (between 24 and 48 h after labeling) suggests that these metabolic pools undergo turnover. The long-term decline (over 7 days) in the specific activity of phenolic glycosides in sink leaves reflects, in part, the increased weight gain of a young, expanding leaf. It is unlikely that the decline in activity of sink leaves reflects translocation out of the leaf. Sink leaves were LPI 0–2, and at this stage of development do not exhibit photosynthate export (Dickson and Shive 1982). Moreover, it is unlikely that phenolic glycosides were translocated from the source to the sink leaf. The specific activity in phenolic glycosides of both source and sink leaves peaked at the same time (24 h; Fig. 3C,D). Both peaks also followed sharp decreases in the activity of the sugar fractions (Figs. 2A, 4A), suggesting that phenolic glycosides were synthesized from the pool of sugars. Phenolic glycosides have been classified as a "dynamic" metabolite (Reichardt et al. 1991) due to short-term (24 h) and seasonal changes in concentration (Thieme 1965; Clausen et al. 1989). Our results support the designation of phenolic glycosides as labile metabolites.

The specific activity in the phenolic glycoside fraction of mature leaves increased, decreased, and then stabilized during the 7-day sampling period (Fig. 3C). In contrast, measures of total phenolics (which include the phenolic glycosides) did not change significantly during the same period (Fig. 5A). In the case of some secondary metabolites, such as phenolic glycosides, current rates of synthesis and turnover may not necessarily reflect metabolite concentration. This could partially explain why the results of some experiments that test the CNB hypothesis demonstrate a weak response in phenolic glycoside concentrations (Bryant et al. 1987; Reichardt et al. 1991; Lindroth et al. 1993; Kinney et al. 1997).

In mature leaves, the 50% decline in the specific activity of the tannin/phenolic fraction may reflect turnover of simple phenolics, such as cinnamic and *p*-coumaric acids. These simple phenolics do not accumulate, but are rapidly incorporated into larger compounds, such as lignin or flavonoids (Walker 1975; Barz and Köster 1981; Gross 1981). Flavonoids can also undergo turnover, but the metabolic fate of polymers, such as proanthocyanidins (condensed tannins) is uncertain (Barz and Hösel 1975), although they are generally considered stable (Barz and Hösel 1975; Swain 1979). However, proanthocyanidins may become bound to polysaccharides in the cell wall (Zucker 1983; Pizzi and Cameron 1986; Haslam 1988; Ya et al. 1989), which may partially explain the decrease in specific activity of the MW-soluble phenolic/tannin fraction and an increase in the residue fraction during the 7-day chase period. Some of the turnover in this pool may also reflect the small amount of phenolic glycosides retained in the MW fraction.

The incorporation of photosynthate into metabolic fractions can differ greatly depending upon the ontogenetic stage of the leaf (Dickson and Larson 1981; Wiermann 1981; Dickson and Shive 1982). The greater concentrations of total phenolics and condensed tannins that we found in young leaves versus recently mature leaves (Fig. 5) reflect an increase in the partitioning of photosynthate to defense metabolites in young leaves. Incorporation of ^{14}C at 24 h into phenolic glycosides and tannins/phenolics combined was nearly the same for sink and source leaves (24,733 versus 20,407 dpm/mg). However, more ^{14}C (dpm/mg) was incorporated into phenolic glycosides in sink leaves, whereas the specific activity of the tannin/phenolic fraction was greater in source leaves (Fig. 3C,D). These partitioning patterns only partially reflect the total investment of photosynthate into secondary metabolites of young, expanding leaves. We only examined the fate of imported photosynthate into young leaves. Dickson and Larson (1975) demonstrated differential incorporation of in-situ-produced and imported photosynthate in young leaves of cottonwood (*P. deltoides* Bartr.). A source leaf at LPI 4 incorporated nearly 40 times as much ^{14}C into the chloroform fraction (which contains lipids, pigments, phenolic glycosides, and simple phenolics), as did a sink leaf at LPI 4. In the present study, approximately 65% of the ^{14}C incorporated into chloroform-soluble metabolites could be attributed to phenolic glycosides and 10% to simple phenolics. Considering that young expanding leaves have two sources of photosynthate (in situ and imported) it is likely that the total amount partitioned to phenolic glycosides and tannins/phenolics would be greater than in recently mature leaves.

Although a greater proportion of labeled photosynthate was partitioned to the processes of growth than defense in both source and sink leaves, there was not a complete trade-off with the production of defense metabolites that could be expected in rapidly growing sink leaves (e.g., Herms and Mattson 1992). Defense metabolites in sink leaves contained about two-thirds of the ^{14}C found in the metabolic pools associated with growth. In addition, rapidly growing sink leaves contained greater concentrations of total phenolics and condensed tannins than recently mature leaves. However, not all of the secondary metabolites produced by a plant will contribute solely to the function of defense. Some of these secondary metabolites may have multiple roles, such as attracting pollinators, screening UV radiation, regulating metabolism, and providing structural support (Seigler and Price 1976; Bazzaz et al. 1987; Herms and Mattson 1992). Understanding how these multiple functions interact could provide more exact information about the costs of producing secondary metabolites and could clarify the concept of a metabolic trade-off between growth and defense.

Our results provide empirical support for the view that metabolic turnover must be considered when determining the relative costs of plant defense. Unlike compounds which are relatively stable once formed, the

costs of metabolically labile compounds must be considered over the lifetime of the leaf (Gulmon and Mooney 1986). The partitioning of carbon to different metabolic fractions within a leaf changes with leaf ontogeny and age, reflecting the demands of growth and physiological maturity, susceptibility to herbivores and the value of the leaf to the plant (Dickson and Larson 1975, 1981; Coley and Aide 1991; Aide 1993; Bryant and Julkunen-Tiitto 1995). For example, in *Quercus agrifolia*, ^{14}C partitioning to phenolics and total phenolic content was higher in young, growing leaves and declined as leaves aged (Mauffette and Oechel 1987, 1989). However, bimodality in the partitioning of photosynthate to defense complicates this further. Newly expanding leaves of *Heteromeles arbutifolia* initially partition a high proportion of photosynthetically fixed ^{14}C to phenolics, but no additional photosynthate was added to this pool until the growing season ended (Mooney and Chu 1974). Seasonal partitioning patterns may also reflect competitive interactions among metabolic pathways of defense compounds (Mole 1994). Both the deciduous *Betula glandulosa* and the evergreen *Ledum groenlandicum* partitioned carbon to phenolics during the early stages of leaf expansion, but as leaf expansion proceeded, carbon partitioned to phenolics declined, while partitioning to alkaloids increased (Prudhomme 1983).

The partitioning of photosynthate to plant defenses in *P. tremuloides* appears to follow an ontogenetically bimodal pattern, and may reflect the value of both young and old leaves to the whole plant (but see Meyer and Montgomery 1987 for alternate view). Partitioning of photosynthate to phenolic glycosides was greater from imported photosynthate in young foliage than from in situ photosynthate in mature foliage, whereas partitioning to tannins and phenolics was greater in recently mature source leaves. The ability of phenolic glycosides to decrease the feeding and growth of some insect folivores (e.g., *Choristoneura conflictana*, *Lymantria dispar* and *Malacosoma disstria*) is greater than that of condensed tannins (Bryant et al. 1987; Hemming and Lindroth 1995; Hwang and Lindroth 1997). The partitioning of photosynthate to putative "qualitative defenses" (phenolic glycosides) in young leaves and "quantitative defenses" (condensed tannins) in older leaves is consistent with the apparency theory of plants with indeterminate growth (Feeny 1976; Rhoades and Cates 1976; Mooney et al. 1983), and may indicate that phenolic glycosides are less costly to produce than condensed tannins. The change in partitioning from one defense compound to another may explain the increased susceptibility of *Populus* spp. leaves to herbivores and pathogens during their transition from metabolic sinks to sources (Coleman 1986).

Several theories have been developed to explain the distribution and concentrations of chemical defenses in plants, including the coevolution theory of Ehrlich and Raven (1964), the plant apparency theory (Feeny 1976; Rhoades and Cates 1976), the CNB hypothesis (Bryant et al. 1983), the resource availability hypothesis (Coley et al. 1985), and the growth-differentiation balance hypothesis (Loomis 1932; Lorio 1986). The utility of these hypotheses for making realistic predictions about specific systems has been questioned (Courtney and Kibota 1990; Stamp 1992; Haukioja et al. 1994). Moreover, the extent to which these hypotheses are testable is limited by a lack of understanding of, and an inability to directly assess the mechanisms involved. Continued investigation of defense metabolite turnover with radiotracers (Mihaliak et al. 1991; Gershenzon et al. 1993; Baldwin et al. 1994) is one means to improving our understanding of the ecology, evolution, and costs of defense in plants.

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