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CO_2 enrichment and carbon partitioning to phenolics: do plant responses accord better with the protein competition or the growth-differentiation balance models?

W. J. Mattson, R. Julkunen-Tiitto and D. A. Herms

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Rising levels of atmospheric CO_2 can alter plant growth and partitioning to secondary metabolites. The protein competition model (PCM) and the extended growth/differentiation balance model (GDB_e) are similar but alternative models that address ontogenetic and environmental effects on whole-plant carbon partitioning to the phenylpropanoid biosynthetic pathway, making many divergent predictions. To test the validity of the models, we compare plant responses to one key prediction: if CO_2 enrichment simultaneously stimulates both photosynthesis and growth, then PCM predicts that partitioning to phenolic compounds will decline, whereas GDB_e generally predicts the opposite. Elevated CO_2 (at 548 ppm) increased the biomass growth (ca 23%) as well as the net photosynthesis (ca 13%) of 1-year-old potted paper birch, *Betula papyrifera* Marsh., in a free air carbon dioxide enrichment study (FACE) in northern Wisconsin. Concomitantly, elevated CO_2 increased carbon partitioning to all measured classes of phenolics (Folin-Denis phenolics, HPLC low molecular weight phenolics (i.e. cinnamic acid derivatives, flavonol glycosides, and flavon-3-ols), condensed tannins, and acid-detergent lignin) in leaves. In stem tissues, tannins and lignin increased, but F-D phenolics did not. In root tissues, F-D phenolics, and tannins increased, but lignin did not. The data suggest that CO_2 enrichment stimulated pathway-wide increase in carbon partitioning to phenylpropanoids. High CO_2 plants had 11.8% more F-D phenolics, 19.3% more tannin, and 10% more lignin than ambient plants after adjusting for plant mass via analysis of covariance. In general, the results unequivocally support the predictions of the GDB_e model. By way of contrast, results from many parallel studies on FACE trembling aspen, *Populus tremuloides* Michx., suggest that although CO_2 enrichment has consistently stimulated both photosynthesis and growth, it apparently did not generally stimulate pathway-wide increases, or decreases, in carbon partitioning to phenylpropanoids in leaves and wood, but rather has specifically, though not consistently, increased partitioning to foliar phenolic glycosides. Likewise, in this case, GDB_e's predictions better accord with the FACE aspen data than PCM's. If further tests of the two models also support GDB rather than PCM, then PCM's main assumption (whole-plant N rather than C is limiting partitioning to phenolic synthesis) may be incorrect.

W. J. Mattson, Forestry Sciences Laboratory, 5985 Highway K, Rhinelander, WI 54501, USA (wmattson@fs.fed.us). – R. Julkunen-Tiitto, Biology Dept, Univ. of Joensuu, FIN-80101 Joensuu, Finland. – D. A. Herms, Dept of Entomology, The Ohio State Univ., Ohio Agricultural Research and Development Center, 1680 Madison Ave., Wooster, OH 44691, USA.

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Understanding how plants allocate and partition resources (sensu Dickson and Isebrands 1993) to their myriad physiological processes over the course of ontogeny and in response to environment ranks as the Holy Grail of plant physiology (Dickson 1991, Geiger et al. 1996, Sturm and Tang 1999). With the realization that the world is undergoing substantial climate change due to natural global cycles and anthropogenic alterations of the atmosphere (Jefferies and Maron 1997, Rozema et al. 1997, Vitousek et al. 1997, Dickson et al. 2000), the question of how environment affects plant resource partitioning has become ever more timely (Körner 2003). In particular, explaining partitioning to secondary metabolites has been the subject of ecological interest for more than two decades (Mattson 1980, Tuomi et al. 1991, Matsuki 1996, Haukioja et al. 1998, Koricheva et al. 1998, Penuelas and Estiarte 1998, Kleiner et al. 1999).

Two phytocentric conceptual models have been advanced to address the effects of environmental factors, including elevated CO_2, on whole-plant carbon partitioning to secondary metabolites: the growth/differentiation balance hypothesis (GDB_e) as extended by Herms and Mattson (1992), and the protein competition model (PCM, Jones and Hartley 1999). Though having many overlapping assumptions, the two models make different predictions about elevated CO_2 effects on partitioning to secondary metabolism.

GDB_e is a developmental systems model that posits that resource variability impacts constitutive secondary metabolism by means of physiological tradeoffs between growth (G) and storage (S) processes and various secondary metabolism pathways that are intricately coupled with environmental effects on the highly dynamic source–sink balance (Herms and Mattson 1992, Kause et al. 1999, Arnold and Schultz 2002, Riipi et al. 2002, Haukioja 2003). GDB_e cautiously asserts that CO_2 enrichment effects on carbon-based secondary metabolism will vary by species, and environment, depending on how CO_2 affects the relative strengths of each species' carbon sources and sinks, and their assimilate transport and storage systems (Farrar and Williams 1991, Körner et al. 1995, Sturm and Tang 1999). Generally, however, elevated CO_2 will increase carbon partitioning to secondary metabolism in proportion to the vagile surplus between photosynthesis (Ps) up-regulation and G stimulation and S needs, availing more carbon for facultative secondary metabolite synthesis up to the point where Ps may be down-regulated because of excess capacity, reflected in diminished rates of uploading and/or downloading of transport sugars and build-ups of S reserves (Dickson 1991, Ward and Boyd 1999). In general, if changing resources stimulate G and S without simultaneously stimulating Ps, then partitioning to secondary metabolite synthesis is predicted to decline toward base-line levels, resulting in a negative correlation between G and secondary metabolites. On the other hand, if changing resources stimulate both G and Ps, then partitioning to secondary metabolism is predicted to covary with the C surplus at any particular stage of development. Integrating over time, this can eventually lead to a positive correlation between G and secondary metabolism (Herms and Mattson 1992). Storage demands for C may also take precedence over some facultative increases in partitioning to secondary metabolism owing to an evolutionary history that may have selected for keeping the "tank full" to ameliorate ever looming catastrophes (Sage and Coleman 2001, Körner 2003).

The PCM (Jones and Hartley 1999) is also a developmental systems model, but strictly focused around a particular mechanism, and accordingly makes more exact predictions about how elevated CO_2 effects on source/sink interactions impact partitioning to phenolics and their tissue specific concentrations. The PCM differs from GDB_e by explicitly proposing that the widely observed tradeoff between G and phenolic synthesis (Margna 1977, da Cunha 1987, Margna et al. 1989) results not from competition for a limited pool of available carbon, but rather from competition for the specific aromatic amino acid, phenylalanine (PHE). As a branch point in the shikimic acid pathway, PHE is considered a rate-limiting precursor for phenylpropanoid synthesis, and at the same time is an essential amino acid for protein synthesis. PCM proposes that as G increases the total demand for protein synthesis, a smaller pool of PHE is available for partitioning to the total demand for phenolic biosynthesis, the first step of which depends on catalysis by the enzyme pheynlalanine amonia lyase (PAL). Because of its zero sum emphasis on PHE per se, PCM and GDB_e make different predictions about elevated CO_2 effects.

For example, PCM makes four contingent predictions: (1) when both Ps and G increase in response to elevated CO_2, phenolic concentrations will decline because increased protein demand for G will decrease partitioning to phenolics, and increased dry matter accumulation will dilute phenolic concentrations. (2) If Ps increases but G is static, phenolic partitioning will not change because there is no change in protein demand, and thus no change in phenolic partitioning. However, phenolic concentrations decline as a result of passive dilution from dry matter accumulation. (3) If Ps is static and G increases, higher photosynthetic nitrogen use efficiency (PNUE) can allow some carboxylation protein to be released for G increases. Phenolic partitioning is predicted to remain constant because total protein demand is unchanged. Although phenolic concentration is predicted to increase slightly due to passive concentration as leaf dry matter is exported from source leaves to support the active sinks. (4) If Ps and G are both static but PNUE increases, PCM predicts an increase in phenolic

partitioning and concentration. Total protein demand is predicted to decrease as growth demand remains unchanged, but carboxylation protein demand declines, thereby resulting in increased diversion of PHE to phenolic partitioning. Carbon storage is unchanged, causing phenolic concentrations to increase.

None of the predictions is consistent with GDB_e which makes the following tentative predictions about partitioning to phenolics in the above four cases: (1) increases from base-line in proportion to the surplus between G and S demands and Ps supply, (2) increases from base-line in proportion to the surplus between S demands and Ps supply, (3) decreases toward base-line levels, and (4) no change. GDB_e predictions are more tentative because it posits that partitioning to phenolics depends on the highly vagile C balance between sink demands and source supplies. The "balance" is elusive to measure and its effects are difficult to precisely predict because as a concept it simultaneously refers both to a time and tissue specific dynamic balance occurring at crucial points in ontogenetic development as various biosynthetic pathways and processes initiate and cycle, as well as to a seasonal balance, i.e an integrated total for an entire plant over an entire growing season.

Jones and Hartley (1999) point out that most studies of CO_2 effects on phenolic synthesis are difficult to interpret in light of these models because most have not measured photosynthesis nor corrected for dry matter accumulation, and have measured only a few classes of leaf phenolics, rather than whole-plant concentrations of all phenylalanine-derived phenolics, including lignin which is often a major structural constituent.

The primary objective of this study was to document CO_2-induced phenotypic plasticity in whole-plant carbon partitioning to (a) major groups of phenolic compounds (e.g. condensed tannins, lignin, and total Folin-Denis phenolics) in all organs, and (b) specific species of low molecular weight phenolics in the foliage of paper birch, *Betula papyrifera* Marsh. and to evaluate the results with the fundamental predictions of GDB_e and PCM.

Methods

Birch seedlings, germinated in spring 1997, were grown in a shade house in 6 l pots containing a potting medium of peat:sand:vermiculate (2:1:1 vol.) and a timed release Osmocote fertilizer 17-6-12 NPK (4 g l^{-1}). On 18 May 1998, 120 vigorous plants were randomly assigned to two elevated (ca 548 ppm) or two ambient (ca 362 ppm) CO_2 treatments at the open-air $CO_2 \times O_3$ FACE II study in northern Wisconsin (Dickson et al. 2000, King et al. 2001b, Karnosky et al. 2003). Seedlings were watered to saturation at least 2–3 times per week over 140 days, until 6–8 October, when they were removed from the FACE rings for study before autumnal color changes and abcission had begun.

Trees were measured for height, and diameter at 7.6 cm above root collar, and then ranked by diameter, after which a subset of 20 with comparable diameters from each CO_2 treatment was selected at random. This was done by eliminating from the selection pool the very smallest ambient plants (those with diameters < than the smallest CO_2 seedling) and the very largest high CO_2 plants (those with diameters > than the largest ambient seedling). The objective was to control for possible size differences in resource allocation and carbon partitioning that may have been stimulated by higher CO_2 regimes (Coleman et al. 1993, Gebauer et al. 1998).

To quantify carbon and nitrogen allocation, we measured whole plant biomass. All 40 study trees were separated into leaves, stems, and roots, the latter being washed carefully to remove the potting media. Leaves were counted, measured for surface area, and then oven dried at 50°C to constant weight, except for ca 10% (selected at random) which was air dried in the laboratory in paper bags in preparation for low molecular weight phenolic analyses by HPLC. Stems and roots were oven dried at 50°C to constant weight.

All tissues were ground to pass through a 40 mesh screen and stored in opaque vials in the dark until aliquots were taken at random and analyzed for concentrations of C and N (on a Carlo-Erba carbon–nitrogen analyzer), energy density (J g^{-1}) using a Parr microbomb calorimeter, total starch using an enzymatic method (Elliott 1999), neutral detergent fiber, and acid-detergent lignin, using an ANKOM automated fiber digester/analyzer (Komarek et al. 1993, 1996), and total Folin-Denis phenolics (using tannic acid standard), and total condensed tannins (using quebracho tannin standard) were analyzed on Rapid-Flow analyzer after Nitao et al. (2001). Low molecular weight phenolics (cinnamic acid derivatives: neochlorogenic acid, chlorogenic acid, p-OH-cinnamic acid derivative; flavonol glycosides: myricetin-3-galactoside, myricetrin, hyperin, quercetin-3-glucoside + glucuronide, quercetrin, and kaempherol-3-rhamnoside; and flavon 3-ols: (+)- catechin) were analyzed via HPLC using air dried leaf samples following the methods of Julkunen-Tiitto and Sorsa (2001) and Keinanen et al. (1999).

The limitations of Folin phenolic assays in ecological studies have been thoroughly reviewed by Appel et al. (2001). To be a valid index to the general pool of phenolics, the F-D assay should correlate with other, independent measures of phenolic products, especially if the latter make up a significant proportion of the overall phenolic milieu. To test the strength of this purported relationship, we regressed the paper birch F-D assays on three different assays of phenolic products in the same

tissue (leaves), i.e. the sum of HPLC low molecular weight phenolics, condensed tannins, and acid-detergent lignin.

The FACE experimental design is a blocked split-plot with CO_2 effects being tested with main-plot error (Dickson et al. 2000). However, only two CO_2 replicates were operating at the start of the experiment in 1998, thereby providing virtually no power ($error_a$ df = 1) for detecting CO_2 effects. To overcome this design limitation, the data were analyzed employing a 2×2 completely randomized block design: 2 CO_2 treatments (C_i), 2 blocks (B_j), and 10 experimental units (tree seedlings) per treatment-block cell: $Y_{ijk} = \Phi + C_i + B_j + C_iB_j + e_{ijk}$. Treatment and block effects were considered fixed. Because block effects and block by treatment interactions were usually insignificant, as expected for potted plants, the design essentially collapses to a one-way, completely randomized ANOVA. Analysis of covariance was used to test for treatment effects on several whole plant variables, after adjustment to the mean covariate value.

Results

Drymass allocation

Elevated CO_2 increased the numbers of buds (16.4%), and leaves (23.1%), and leaf mass (23.1%). However, total leaf area, and mean leaf size did not differ between treatments (Table 1). Neither did leaf area ratio (leaf surface area/woody plant mass). As expected, leaf mass per area was significantly higher (7.39 vs 6.70 mg cm^{-2}) under elevated CO_2. Elevated CO_2 also increased root mass (38.9%) but not stem mass (Table 1). Therefore, total plant mass was 23% higher under elevated CO_2. The root/shoot ratio (root mass/aboveground mass) increased significantly (0.68 vs 0.56) under elevated CO_2 (Table 1).

Energy allocation

To verify that dry mass of plant organs was a reliable indicator of energy allocation, we measured organ-specific energy densities (J g^{-1}) which can vary because of partitioning differences to fats, waxes, oils, and secondary compounds (Griffin et al. 1993). Elevated CO_2 decreased energy densities of leaves (19.03 kJ g^{-1}vs 19.87 kJ g^{-1}), increased energy densities (20.52 kJ g^{-1} vs 20.32 kJ g^{-1}) of stems, but did not change root energy densities (Table 2). Elevated CO_2 plants had more total energy invested in leaves (422.26 kJ vs 358.75 kJ), and roots (699.69 kJ vs 509.81 kJ), but similar quantities of stem energy (Table 2). However, both elevated and ambient CO_2 trees invested 24% of their total energy into leaves, whereas the elevated group invested 39.4% of its energy into roots and 36.4% into stems, nearly the mirror image of control plants (34.9% in roots, and 40.4% in stems). High CO_2 plants accumulated 14% more energy (209.3 kJ) than ambient plants, after adjusting for stem diameter via analysis of covariance (Table 5). This translates to an enhanced energy accretion of 1.49 kJ/da, implying that high CO_2 plants could build over the summer an additional 69 leaves, or 10.8 g of roots (given that a leaf averaged ca 3.02 kJ, and roots averaged ca 19.3 kJ g^{-1}).

Nitrogen allocation

N concentrations in leaves and roots were ca 30% and 14% lower, respectively, under elevated CO_2, but stem N was unaffected (Table 3). At the whole plant level, both elevated and ambient seedlings had the same total

Table 1. Comparing mean trait values (± se) of birch seedlings grown under ambient and elevated CO_2 after 140 days, and their associated P values from ANOVA.

Plant trait	CO_2 ambient	CO_2 elevated	P value
diameter (cm)-all seedlings	0.95±0.02	1.06±0.02	<0.001
diameter (cm)-study seedling	1.08±0.02	1.10±0.02	0.187
height (cm)-all seedlings	84.76±1.89	82.17±2.26	0.390
height (cm)-study seedlings	89.54±3.32	83.32±3.64	0.203
buds	137.8±4.6	160.4±6.9	0.012
leaf numbers	112.2±5.8	140.5±7.4	0.002
leaf mass (g)	18.07±0.91	22.24±1.12	0.008
leaf size (cm^2)	24.61±1.06	22.24±1.12	0.110
leaf area/canopy (cm^2)	2710±132	3042±167	0.137
leaf mass per area (mg cm^{-2})	6.70±0.14	7.39±0.16	0.002
leaf area ratio (cm^2 g^{-1} woody mass)	49.91±2.36	45.70±2.29	0.227
N specific leaf mass (mg N cm^{-2})	0.15±0.006	0.12±0.004	<0.001
stem mass (g)	28.93±1.36	31.60±1.79	0.246
root mass (g)	26.07±1.13	36.22±2.00	<0.001
total mass (g)	73.08±2.77	90.05±4.36	0.003
root/shoot ratio (mass)	0.56±0.02	0.68±0.02	<0.001

Table 2. Mean energy densities of and total energy (± se) allocation to different tissues under ambient and elevated CO_2, and their associated P values from ANOVA.

Plant trait	CO_2 ambient	CO_2 elevated	P value
kJ g^{-1} leaves	19.87±0.08	19.03±0.09	<0.001
kJ g^{-1} stems	20.32±0.09	20.52±0.06	0.039
kJ g^{-1} roots	19.51±0.13	19.32±0.08	0.180
kJ total leaves	358.75±17.96	422.26±20.55	0.029
kJ total stems	588.20±27.74	648.78±36.77	0.191
kJ total roots	509.81±22.94	699.69±38.94	<0.001
kJ total plant	1,456.76±55.67	1,770.73±85.71	0.005
KJ gN^{-1} leaves	895.42±23.21	1227.00±37.24	<0.001
KJ gN^{-1} stems	2281.47±69.49	2401.80±67.39	0.211
KJ gN^{-1} roots	1834.77±48.06	2135.26±89.58	0.006
KJ gN^{-1} whole plant	1554.96±37.75	1862.30±50.12	<0.001
root/shoot ratio (J)	0.54±0.02	0.66±0.02	<0.001

N_g content (Table 4). However, high CO_2 plants invested a significantly smaller percentage of their N_g in foliage (36.5 vs 42.7%), but a larger share in roots (35.0 vs 29.6%, Table 4, 5).

Carbon partitioning

Starch levels increased almost 3-fold in leaves, but did not change in stems, nor in roots in response to high CO_2 (Table 3). At the whole plant level, high CO_2 plants had 15.7% more starch than ambient plants after adjusting for plant mass (Table 4, 5).

Levels of neutral detergent fiber (i.e. the sum of hemicellulose, cellulose, and lignin fractions) did not change in leaves nor in roots, but declined ca 2% in stems in response to elevated CO_2 (Table 3). At the level of the whole plant, CO_2 enrichment had no effect on total NDF content after adjusting for plant mass (Table 4, 5)

Lignin levels increased in leaves and stems by 14% and 4%, respectively, but not in roots in response to elevated CO_2 (Table 3). At the level of the whole plant, high CO_2 plants had about 10% higher lignin content after adjusting for plant mass (Table 5).

Total F-D phenolics increased in leaves and roots by ca 15% and 7.5%, respectively, but did not change in stems in response to elevated CO_2 (Table 3). Because the stems contain a high proportion of woody tissue of low physiological activity, except for the surface layers, we calculated the g of F-D phenolics per surface area (m^2) of the stems by using their heights and diameters in the formula for the surface of a right circular cone. Elevated CO_2 elicited higher levels (62.53 vs 51.03 g m^{-2}) of F-D phenolics per unit area of stem surface. Likewise, FD-phenolics per unit leaf surface (5.71 vs 4.47 g m^{-2}) increased under elevated CO_2 (Table 3). At the level of the whole plant, high CO_2 increased partitioning to F-D phenolics by 11.8% after adjusting for plant mass (Table 4, 5).

Condensed tannins increased by about 37%, 15% and 8% in leaves, stems, and roots, respectively under elevated CO_2 (Table 3). At the level of the whole plant,

Table 3. Comparing mean concentrations (mg g^{-1}+se) of starch, netural detergent fiber, N, F-D phenolics, low molecular weight HPLC phenolics, condensed tannins, acid-detergent lignin, and C/N ratios in different tissues under ambient and elevated CO_2, and their associated P values from ANOVA.

Plant trait	CO_2 ambient	CO_2 elevated	P value
Starch leaves (mg g^{-1})	11.5±1.45	30.6±4.00	<0.001
Starch stems (mg g^{-1})	40.3±2.03	40.3±2.03	0.154
Starch roots (mg g^{-1})	141.8±4.22	147.4±3.64	0.659
NDFiber leaves (mg g^{-1})	176.4±5.21	180.3±3.22	0.526
NDFiber stems (mg g^{-1})	649.4±5.43	636.2±3.83	0.043
NDFiber roots (mg g^{-1})	520.2±5.91	525.6±4.82	0.484
N leaves (mg g^{-1})	22.5±0.60	15.8±0.50	<0.001
N stems (mg g^{-1})	9.1±0.27	8.7±0.26	0.319
N roots (mg g^{-1})	10.8±0.30	9.3±0.37	0.004
FD phenolics leaves (mg g^{-1})	66.7±1.78	77.4±3.78	0.043
FD phenolics stems (mg g^{-1})	31.5±1.43	32.7±0.62	0.162
FD phenolics roots (mg g^{-1})	41.6±1.32	44.7±0.85	0.050
FD phenolics g m^{-2} leaves	4.5±0.16	5.7±0.30	<0.001
FD phenolics g m^{-2} stem	51.03±12.44	62.53±4.46	0.034
HPLC phenolics leaves (mg g^{-1})	49.7±2.00	58.3±2.66	0.004
Cinnamic acid derivatives (mg g^{-1})	13.3±0.74	15.9±1.00	0.026
Flavonol glycosides (mg g^{-1})	35.3±2.28	41.1±1.96	0.019
Flavon 3-ols: (+)- catechin (mg g^{-1})	1.1±0.09	1.4±0.11	0.007
Tannins leaves (mg g^{-1})	270.5±8.28	379.5±21.05	<0.001
Tannins stems (mg g^{-1})	271.5±16.62	317.7±6.57	0.016
Tannins roots (mg g^{-1})	403.7±13.31	450.1±16.74	0.026
Lignin leaves (mg g^{-1})	92.6±6.49	113.9±3.10	0.018
Lignin stems (mg g^{-1})	148.0±1.58	154.2±1.84	0.014
Lignin roots (mg/g)	132.0±3.56	137.6±3.44	0.277
C/N ratio leaves	21.8±0.55	30.1±0.89	<0.001
C/N ratio stems	55.6±1.69	56.9±1.50	0.584
C/N ratio roots	41.6±1.28	49.7±2.15	0.003

Table 4. Comparing mean levels (g DW ± se) of whole organ and whole plant N, starch, neutral detergent fiber, F-D phenolics, condensed tannins, and acid detergent lignin under ambient and elevated CO_2, and their associated P values from ANOVA.

Plant trait (g DW)	CO_2 ambient	CO_2 elevated	P value
N total foliage	0.41 ± 0.03	0.35 ± 0.02	0.113
N total stems	0.26 ± 0.01	0.27 ± 0.02	0.499
N total roots	0.28 ± 0.01	0.34 ± 0.03	0.065
N total whole plant	0.95 ± 0.04	0.97 ± 0.06	0.800
Starch total foliage	0.21 ± 0.03	0.67 ± 0.10	<0.001
Starch total stems	1.15 ± 0.06	1.26 ± 1.26	0.307
Starch total roots	3.72 ± 0.22	5.34 ± 0.32	<0.001
Starch whole plant	5.08 ± 0.26	7.28 ± 0.40	<0.001
NDF total foliage	3.14 ± 0.14	3.98 ± 0.18	<0.001
NDF total stems	18.86 ± 0.97	20.14 ± 1.19	0.407
NDF total roots	13.58 ± 0.62	18.96 ± 0.97	<0.001
NDF whole plant	35.58 ± 1.48	43.08 ± 2.09	0.006
FD phenolics total foliage	1.21 ± 0.07	1.73 ± 0.13	0.002
FD phenolics total stem	0.91 ± 0.06	1.03 ± 0.06	0.140
FD phenolics total roots	1.08 ± 0.05	1.61 ± 0.09	<0.001
FD phenolics whole plant	3.20 ± 0.15	4.37 ± 0.22	<0.001
Tannins total foliage	4.87 ± 0.27	8.49 ± 0.66	<0.001
Tannins total stems	7.19 ± 0.66	9.16 ± 0.58	0.034
Tannins total roots	10.49 ± 0.52	16.20 ± 0.95	<0.001
Tannins whole plant	22.55 ± 1.16	33.85 ± 1.84	<0.001
Lignin total foliage	1.67 ± 0.16	2.53 ± 0.14	<0.001
Lignin total stems	4.26 ± 0.17	4.83 ± 0.24	0.062
Lignin total roots	3.42 ± 0.16	4.91 ± 0.20	<0.001
Lignin total plant	9.36 ± 0.33	12.27 ± 0.47	<0.001

high CO_2 increased partitioning to tannins by 19.3% after adjusting for plant mass (Table 4, 5).

Detailed analysis of the low molecular weight phenolic constituents of leaves via HPLC revealed, as in Eurasian white birches (Lavola 1998, Saleem et al. 2001), that there were 11 major low molecular weight compounds derived from three different side branches of the phenlypropanoid pathway (PPP), i.e. cinnamic acid derivatives, flavonol glycosides, and flavon-3-ols (Fig. 1). The summed concentration of all 11 compounds increased ca 16% in response to elevated CO_2. Likewise, the pooled concentrations of compounds derived from each of the three PPP side branches, all increased significantly. Although eight compounds showed apparent increases in response to CO_2, only 5 were significantly greater than ambient (Fig. 1).

The Folin assay as an index to the plant phenolic pool

Regressing the paper birch F-D assays on three different assays of phenolic products (the sum of HPLC low molecular weight phenolics, condensed tannins, and acid-detergent lignin) in the leaves, resulted in a significant, linear multiple regression: $P_{FD\ (mg/g)} = 0.436\ P_{HPLC\ (mg/g)} + 0.107\ Tan_{(mg\ g^{-1})} + 19.14$, $R^2 = 0.62$, $n = 40$. The magnitudes of the partial regression coefficients reflect both the abundance of the particular classes of compounds as well as their collective redox capacity. Lignin, though a large phenolic component of leaf tissue, failed to have a significant partial regression coefficient. The unexplained variation in the regression model can be attributed, in part, to variation in unmeasured phenolic products (e.g. hydrolyzable tannins, etc.), as well as other possible reducing agents in the leaf tissue (Appel et al. 2001). Similarly, we tested the F-D assay in FACE trembling aspen, *Populus tremuloides* Michx., where we had measured the same four variables in small branch wood (Table 6): $P_{FD\ (mg\ g^{-1})} = 0.114\ P_{HPLC\ (mg\ g^{-1})} + 0.129\ Tan_{(mg\ g^{-1})} + 4.52$, $R^2 = 0.66$, $n = 80$. The significant regressions validate that the F-D assay reflects at least the levels of both condensed tannins and low molecular weight phenolics in paper birch leaves and aspen branch wood.

Discussion

CO_2 effects on birch partitioning to phenolics: comparing GDB_e and PCM predictions

Both the GDB_e and PCM are premised in a resource-based tradeoff between primary and secondary metabo-

Table 5. Analysis of covariance for evaluating ambient and elevated CO_2 effects on whole plant traits, showing least square means and their associated P values.

Whole plant trait	Covariate	Least square means		P values	
		CO_2 ambient	CO_2 elevated	CO_2 effect	covariate
kJ roots	kJ plant	573.87	645.63	0.003	<0.001
kJ plant	leaf area	1510.69	1716.80	0.013	<0.001
kJ plant	plant diam.	1509.1	1718.4	0.005	<0.001
NDF g	plant mass	39.64	39.01	0.415	<0.001
Starch g	plant mass	5.73	6.63	0.006	<0.001
F-D phenolics g	plant mass	3.63	4.06	0.012	<0.001
F-D phenolics g	N g per plant	3.23	4.43	<0.001	<0.001
Tannin g	N g per plant	22.85	33.21	<0.001	<0.001
Tannin g	plant mass	26.46	31.57	<0.001	<0.001
Tannin g	kJ plant	25.58	30.48	<0.001	<0.001
Lignin g	plant mass	10.24	11.38	<0.001	<0.001

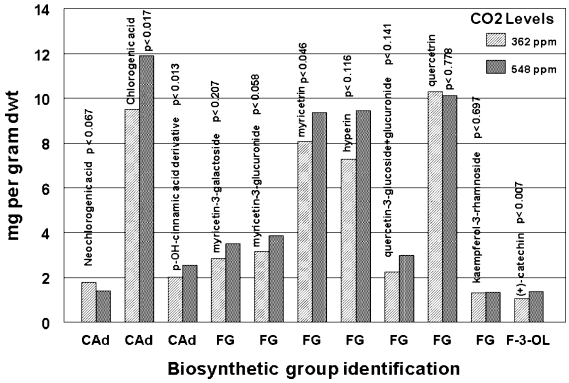

Fig. 1. Comparing the effects of ambient and elevated CO_2 on the concentrations (mg g^{-1} DW) of low molecular weight phenolics in the foliage of potted paper birch seedlings in FACE experiments. End products belonging to the same biosynthetic pathways are identified as follows: cinnamic acid derivatives (CAd), flavonol glycosides (FG), and flavon 3-ols (F-3-OL).

lism, and predict that phenolic production in response to elevated CO_2 is contingent on source–sink balance. GDB_e proposes that the tradeoff results from competition for carbon skeletons at all of the several entry points to phenolic synthesis (Koricheva et al. 1998, Keinanen et al. 1999, Riipi et al. 2002, Haukioja 2003). On the other hand, PCM assumes that carbon is not limiting, and the tradeoff results strictly from competition for PHE per se (Jones and Hartley 1999). These differing postulates lead to divergent predictions. Specifically, in this study where elevated CO_2 increased both Ps and G and S (estimated by starch), GDB_e predicts that phenolic partitioning and concentrations will increase, whereas PCM predicts the opposite.

There is no question that CO_2 increased phenolics as well as total G and S in this study (Table 1, 3, 4, 5). However, did it truly increase photosynthesis? Although, we did not directly measure Ps, two lines of evidence suggest unequivocally that it increased under elevated CO_2. First of all, CO_2 enrichment increased light-saturated Ps 50–72% in other FACE paper birch (Karnosky et al. 2003). Secondly, if two treatment groups start out at the same size but one grows larger and accrues more energy (Table 1, 5), it can only be due to increased photosynthesis and/or increased total leaf area. However, total leaf area did not differ significantly between the CO_2 treatments (Table 1). Moreover, covariance analysis showed that the mean total energy accrued per plant (kJ) was 13.7% higher (1717.8 vs 1510.8 kJ) for elevated CO_2 plants even after adjustment to common leaf surface area (Table 5). Therefore, all evidence unequivocally suggests that Ps increased substantially under elevated CO_2.

Increased carbon acquisition in elevated CO_2 treatments was not associated with increased foliar nitrogen, suggesting that levels of carboxylation protein did not increase. And furthermore, foliar N per leaf decreased under elevated CO_2, as shown by the significantly lower N specific leaf mass (0.12 vs 0.15 mg N cm^{-2}). Therefore, increased carbon acquisition was associated with

Table 6. An overview of the effects of elevated CO_2 on carbon partitioning in FACE aspen based on several, simultaneous parallel studies examining aspen wood and foliage. Mean values of traits under ambient and elevated CO_2, and their associated P values from ANOVA.

Plant trait	CO_2 ambient	CO_2 elevated	P value
	FACE aspen branch wood: means		
F-D phenolics mg g^{-1}	12.9	15.0	0.305
Tannins mg g^{-1}	23.89	23.08	0.588
A-D lignin mg g^{-1}	90.82	91.17	0.837
HPLC total phenolics mg g^{-1}	76.32	86.03	0.153
phenolic glycosides	73.28	82.24	0.318
cinnamic acid deriv.	2.89	3.52	0.063
(+)- catechin	0.10	0.12	0.448
NDF mg g^{-1}	661.7	641.3	0.032
Starch mg g^{-1}	33.23	43.34	0.034
N mg g^{-1}	5.3	5.1	0.036
KJ g^{-1}	20.08	19.55	0.549
	FACE aspen studies on foliage and stemwood		Citations
Tannins (foliage)	no ($\Leftrightarrow$) differences between CO_2 treatments		1,2,4,5,7,8
Phenolic glycosides (foliage)	increased ($\Uparrow$) under elevated CO_2		4,6,7
Phenolic glycosides (foliage)	no ($\Leftrightarrow$) differences between CO_2 treatments		5,8
Starch (foliage)	increased ($\Uparrow$) under elevated CO_2 treatments		2,7
Starch (foliage)	no ($\Leftrightarrow$) differences between CO_2 treatments		1,4,5,8
PAL transcript levels (foliage)	decreased ($\Downarrow$) under elevated CO_2		3
CHS transcript levels (foliage)	no ($\Leftrightarrow$) differences between CO_2 treatments		3
Lignin (stem wood)	no ($\Leftrightarrow$) differences between CO_2 treatments		6
Cellulose (stem wood)	no ($\Leftrightarrow$) differences between CO_2 treatments		6

Citations: 1) Lindroth et al. 2001, 2) Oksanen et al. 2001, 3) Wustman et al. 2001, 4) Lindroth et al. 2002, 5) Holton et al. 2003, 6) Karnosky et al. 2003, 7) Kopper and Lindroth 2003a, 8) Kopper and Lindroth 2003b.

increased photosynthetic NUE. Unequivocally, there was more energy gain per unit N because high CO_2 plants had 20% higher kJ N^{-1} (Table 2). Moreover, the free growing birches and aspens at the FACE site also showed evidence of higher PNUE (Takeuchi et al. 2001, Karnosky et al. 2003). If the well documented inverse relationship between growth and phenolic production results from nitrogen in PHE being incorporated into proteins as proposed by PCM, rather than competition from carbon-based substrate, then it would not be possible for both growth and phenolics to increase.

The failure of the PCM to predict phenolic accumulation in this study may be based on its assumption that the tradeoff between growth and phenolic production results from competition for PHE per se rather than for carbon, as assumed by GDB_e. Razal et al. (1996) found that PHE is continuously regenerated from a limited nitrogen pool during phenolic biosynthesis, suggesting that the pool of PHE, while critical, is not limited by N but instead by the carbon skeletons entering into its biosynthesis. Furthermore, the tradeoff between protein synthesis and phenylpropanoid synthesis reflects a more general substrate competition for the particular carbon skeletons funneled through PHE. This explains how substantial phenolic accumulation can occur in nitrogen-limited environments, where the accumulation of N-based compounds based upon PHE would be constrained (Margna 1977, Razal et al. 1996, Riipi et al. 2002). It also suggests that energy committed to whole pathways of secondary metabolite biosynthesis will be unavailable for primary metabolism and vice versa, no matter where the location of the branch point between competing pathways.

Clearly there was more dry matter accumulation under elevated CO_2 as evidenced by the higher leaf specific mass and nearly 3-fold higher leaf starch concentrations, which did not dilute the phenolics, as predicted by PCM. In parallel FACE experiments, Lindroth et al. (2001), and Oksanen et al. (2001) have likewise found that elevated CO_2 increased both starch and tannin levels in the foliage of the paper birches, but Kopper et al. (2001) did not. In biotron studies with potted, 2-year-old paper birch, McDonald et al. (1999) reported similar CO_2 enrichment effects under high light: 3–4 fold increases in foliar starch, and >2 fold increases in condensed tannins, coupled with substantial increases in growth. Agrell et al. (2000) reported the identical pattern, though the magnitudes of changes were less. Therefore, most of the evidence from paper birch saplings demonstrates that elevated CO_2 increased both growth and phenolic concentrations. GDB_e, in fact, predicts the possibility of a positive correlation between growth and secondary metabolism, even though there exists a carbon-based tradeoff between them, so long as Ps outpaces both G and S demands throughout development (Herms and Mattson 1992).

CO_2 effects on aspen partitioning to phenolics: comparing GDB_e and PCM predictions

Although, there is no fully equivalent data set on whole plant partitioning to phenolics by trembling aspen at the FACE site, there is never the less a substantial body of piecemeal data that can be assembled to permit one to compare the known facts against the predictions of the two source–sink balance models (Table 6). GDB_e and PCM assume explicitly that plant species will differ in their resource partitioning patterns to secondary metabolism as a function of their fundamental life history strategies (Herms and Mattson 1992, Jones and Hartley 1999). However, because paper birch and trembling aspen are both fast growing, short-lived, pioneering tree species, one might expect their responses to elevated CO_2 to be very similar. But, mounting evidence suggests otherwise (Table 6). For example, even though elevated CO_2 has increased light-saturated Ps (20–33%), though mostly in upper canopy leaves (Takeuchi et al. 2001, Karnosky et al. 2003), and G (40%) in FACE aspen (Isebrands et al. 2001, King et al. 2001b), increased partitioning to foliar phenolic glycosides, tannins, lignin, carotenoids, and starches has not been consistently evident (Lindroth et al. 2001, Oksanen et al. 2001, Wustman et al. 2001, Karnosky et al. 2003, Holton et al. 2003). Moreover, elevated CO_2 decreased the activity of PAL, but had no effect on chalcone synthase (a downstream PPP enzyme) in FACE aspen leaves (Wustman et al. 2001). Our own studies on carbon partitioning in FACE aspen branch wood in 2000 also indicates only minor partitioning changes due to elevated CO_2 (Table 6): there was no effect on concentrations of lignin, tannins, several species of low molecular weight HPLC phenolics (including phenolic glycosides), and F-D phenolics, but a small but significant increase in starch, and a slight decrease in NDF. In a nutshell, the FACE aspen data suggest that in spite of consistently increased Ps and G, there was either no significant change or a very selective increase in partitioning to phenolics, specifically phenolic glycosides. However, there was a general tendency for concentrations of FACE aspen phenolics to be higher in the CO_2 treatment, but it was seldom significantly different from controls. PCM specifically predicts a decrease in partitioning to phenolics, whereas GDB_e generally predicts the opposite, but asserts that changes in partitioning to phenolics will covary with the C surplus between sink (G and S) demands and source supplies.

In open-top chamber studies with aspen, King et al. (2001a) likewise reported that CO_2 enrichment had no effects on tannins and total non-structural carbohy-

drates in newly senesced aspen foliage. On the other hand, in biotron studies, McDonald et al. (1999) found that CO_2 enrichment elicited increased partitioning to condensed tannins but not to foliar starch. Agrell et al. (2000), studying the same system, but measuring foliage several weeks later, found the opposite, i.e. no increase in partitioning to tannins, but increased levels of starch. Both biotron studies, however, reported increased partitioning to foliar phenolic glycosides, as has Lindroth et al. (2001), but not Holton et al. (2003) in FACE aspen foliage. In conclusion, aspen unlike paper birch, apparently does not generally and robustly increase partitioning to phenolics under elevated CO_2, but rather specifically, though not always, increases partitioning to phenolic glycosides.

Can PCM and/or GDB_e reconcile apparent differences in partitioning patterns between between apens and paper birches growing under nearly identical conditions? One possible explanation derived from the FACE data (Karnosky et al. 2003), is that because aspen's Ps increased (20–33%) much less than did birch's Ps (50–72%) in response to CO_2 enrichment, it therefore generated a smaller increment in source strength. But source strength also depends on leaf canopy size. The literature suggests and the FACE whole tree harvest data corroborate that aspen and birch of equivalent size generally have equivalent leaf mass (Stanek and State 1978, Roussopoulos and Loomis 1979, W. J. Mattson et al., unpubl.), but birch supports a larger leaf surface area because it leaves have a lower leaf mass per area (ca 7.2 vs 8.6 mg cm^{-2} for birch and aspen leaves, respectively in 2002, W. J. Mattson et al., unpubl.). Clearly, elevated CO_2 has not yet changed the leaf surface or leaf mass allometry of aspens or birches (Karnosky et al. 2003, W. J. Mattson et al., unpubl.). Given that FACE aspens have also produced smaller annual G increments (d^2h) than birch in response to elevated CO_2 (Isebrands et al. 2001, Karnosky et al. 2003), the evidence indicates that CO_2 enrichment has increased aspen's source–sink balance less than that of birch.

Aspen's source capacity at FACE may also have been significantly compromised by its high apparent natural susceptibility to herbivory (Mattson et al. 2001, Newcombe et al. 2001). For example, since 1998, the first full summer of FACE treatments, herbivory pressure on aspen has been significant and relentless (W. J. Mattson, unpubl.). A variety of insects, pathogens and fall frost branch dieback, especially in the CO_2 plots, have contributed annually to substantive reductions (averaging ca 50% in 2001) in functional leaf surface area, and altered canopy architecture due to terminals being repeatedly killed back, especially by *Venturia* spp. shoot blight (Isebrands et al. 2001, W. J. Mattson, unpubl.). Very likely, herbivory has contributed to the source limitation of the aspens, though there is no evidence of heightened leaf area reductions under CO_2 enrichment, except for fall frost-induced shoot dieback (Isebrands et al. 2001, W. J. Mattson unpubl.). Nothing of the equivalent has so far impacted the expansion and functioning of FACE birch canopies.

Aspen's apparent lesser and more selective partitioning to phenolic secondary metabolism under elevated CO_2 may also generally reflect its deeper commitment to a growth strategy in response to herbivory and catastrophe (Dickson et al. 2001, Gielen and Ceulemans 2001). For example, Loehle (1988), and Enquist et al. (1999) have argued that the growth and life history strategies of woody plants are related to how they allocate to and partition resources within their stems, affecting a constellation of stem wood traits that are reflected in such proxies as wood specific gravity and volumetric heat yield. Aspen, for example, has a very low (relative to other angiosperms) specific gravity (0.35) and low volumetric heat (7.33 J cm^{-3}), whereas paper birch has a 37% higher wood specific gravity (0.48), and 32% higher volumetric heat (9.68 J cm^{-3}) (Loehle 1988). In other words, the proxies are integrated indices that reflect (though inversely) the relative fraction of a plant's resources that are invested in resource gathering growth processes (Herms and Mattson 1992). Therefore, aspen may on average partition a higher percentage of its carbon gains for canopy and root expansion than does paper birch, at least up to the point where it becomes sink limited.

In conclusion, PCM did not, but GDB_e did correctly predict that paper birch, responding with increased Ps and G under elevated CO_2, would generally increase its whole-plant partitioning to the phenylpropanoid pathway (PPP). Likewise, PCM did not, but GDB_e did at least partially correctly predict aspen's partitioning to the PPP under the same environmental conditions. GDB_e predictions were generally in the right direction because some of aspen's phenolic products did actually increase, and others tended to increase, while none decreased (as PCM predicts) in response to elevated CO_2. GDB_e predictions that facultative partitioning to phenolics will covary with the surplus between G and S demands and source supply over the growing season are straight forward, but the temporal pattern of the highly vagile source–sink balance is likely to be important in the actual partitioning to the myriad secondary metabolism demands that vary day by day over ontogeny (Kause et al. 1999, Kleiner et al. 1999, Haukioja 2003). If Körner's (2003) prediction that the continued CO_2 enrichment of the atmosphere is likely to generally enhance the disparity between source and sink capacities of trees is correct, can one also predict that C partitioning to the phenylpropanoid pathway will also generally rise?

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