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Estimation of Tissue Construction Cost from Heat of Combustion and Organic Nitrogen Content

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Abstract. We present a method for estimating the construction costs of plant tissues from measurements of heat of combustion, ash content, and organic nitrogen content. The method predicts glucose equivalents, the amount of glucose required to provide carbon skeletons and reductant to synthesize a quantity of organic product. Glucose equivalents have previously been calculated from the elemental composition of tissue. We define construction cost as the amount of glucose required to provide carbon skeletons, reductant and ATP for synthesizing the organic compounds in a tissue via standard biochemical pathways. The fraction of the total construction cost of a compound or tissue (excluding costs of transporting compounds) that is reflected in its glucose equivalents is the biosynthetic efficiency (E_B). This quantity varies between 0.84 and 0.95 for tissues with a wide range of compositions. Using the new method, total construction cost can be estimated to $\pm 6\%$ of the value obtained from biochemical pathway analysis.

Construction costs of leaves of three chaparral species were estimated using the proposed method and compared to previously published values, derived using different methods. Agreement among methods was generally good. Differences were probably due to a combination of inaccuracy in the estimated biosynthetic efficiency and technical difficulties with biochemical analysis, one of the older methods of determining construction cost.

Key-words: Lepechinia calycina (Benth.) Epl.; *Diplacus aurantiacus* (Curtis) Jeps.; *Heteromeles arbutifolia* (Ait.) Roem.; production value; glucose equivalent; construction cost; heat of combustion; biosynthetic efficiency; growth efficiency.

Introduction

The concept of cost is fundamental to understanding how plants function in their environments (Mooney, 1972; Orians & Solbrig, 1977; Mooney & Gulmon, 1982; Bloom, Chapin & Mooney, 1985). Any evaluation of the benefit a plant accrues from a particular structure or compound (in terms of increased resource gain, fitness, etc.) must include an estimate of the structure's 'cost'. The simplest measure of cost is the amount of resources allocated to a structure's formation and maintenance. The manner in which a plant allocates resource expenditures among different organs and different metabolic functions affects its overall growth and performance in a particular environment.

Penning de Vries, Brunsting & van Laar (1974) reported a method for determining the carbon costs of constructing biological materials that was based on analysis of the metabolic pathways used for producing the organic components of a sample. From the biochemical composition of the sample they calculated its 'production value' (*PV*); the amount of biomass of that composition that could be formed from one gram of glucose. The production value includes both the glucose required for carbon skeletons and that consumed in respiration to supply reductant and ATP for energy-requiring steps in the biosynthesis of the tissue's constituent molecules. *PV* also includes an estimate of the ATP required for importing monomers and ions for growth. *PV* directly predicts an organism's true growth yield (i.e. the amount of biomass of plant produced per gram of substrate utilized for its production; Pirt, 1975; Thornley, 1976). The inverse of *PV* (grams glucose required per gram biomass) is an estimate of the construction cost of the tissue.

Although the method of Penning de Vries *et al.* (1974) is elegant in principle, the multiple biochemical analyses make it laborious, particularly for processing large numbers of samples. A simpler approach was developed by McDermitt & Loomis (1981) who estimated growth yields from the elemental composition of a sample. Using elemental composition, they calculated the glucose equivalent' for a sample, defined as the number of moles of glucose required to provide the carbon and electrons contained in a mole of product. Glucose equivalent (*GE*) is given by

$$GE = \frac{c}{6} + \frac{h - 2x + kn + ms}{24}, \qquad (1)$$

where *GE* is in moles glucose/mol product, *c*, *h*, *x*, *n* and *s* are moles of carbon, hydrogen, oxygen,

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nitrogen and sulphur, respectively in a mole of product (or 100 g of plant dry matter), and k and m are the oxidation states of the substrate forms of nitrogen and sulphur (-3 for ammonium, $+5$ for nitrate, -2 for sulphide and $+6$ for sulphate). *GE* constitutes most of a tissue's construction cost and can be used to estimate *PV*, as defined by Penning de Vries *et al.* (1974). The method of McDermitt & Loomis (1981) has the advantage of requiring fewer analyses, but is still limited by the high cost of elemental analysis.

McDermitt & Loomis (1981) also related *GE* values of organic compounds to their heats of combustion. Kharasch & Sher (1925) and Kharasch (1929) showed that the heat of combustion of a compound appears to be related to the number of electrons displaced from a position in a methane-type bond (C–H) to one in a carbon dioxide-type bond (C=O). For compounds containing only carbon, hydrogen and oxygen, the number of valence electrons experiencing this transition (N_e) was:

$$N_e = 4c + h - 2x, \quad (2)$$

where c, h and x are as in eqn (1). Equation (2), divided by 24 (the number of available electrons in a glucose molecule), is identical to McDermit & Loomis' formulation for *GE* (eqn (1)) when k and m equal zero. McDermitt & Loomis (1981) showed that, for compounds containing nitrogen and sulphur, as well as carbon, hydrogen and oxygen, *GE* still gave a good prediction of heat of combustion when calculated with $k = 0$ and $m = +4$. These values of k and m correspond to the oxidation values of nitrogen and sulphur in N_2 gas and SO_2 gas, the standard end-products for nitrogen and sulphur in combustion calorimetry. They obtained two regression lines for *GE vs.* heat of combustion: one for carbohydrates and glycerol and another for other organic compounds.

In this report we develop a method for estimating construction cost from the heat of combustion and to organic nitrogen content. We present a single equation for predicting $GE(k = 0, m = +6)$ from the heat of combustion of a tissue sample; thus, no knowledge of the sample's biochemical composition is required. Measurement of the organic nitrogen content of the sample then makes it possible to determine the sample's actual *GE* value. We examine the value of the efficiency factor, the factor relating *GE* to total construction cost, in order to determine the precision with which this method estimates total construction cost. Finally, we apply the method to estimate the construction costs of leaves of three chaparral species and compare our results with those obtained by other methods.

Materials and methods

Leaves of three chaparral shrub species, *Lepechinia calycina* (Benth.) Epl., *Diplacus aurantiacus* (Curtis) Jeps. and *Heteromeles arbutifolia* (Ait.) Roem., were collected at the Jasper Ridge Biological Preserve of Stanford University, San Mateo County, California. The samples were the same as those used for biochemical analysis by Merino, Field & Mooney (1984) who described the collection methods and the site. The dried, powdered samples were stored at room temperature in tightly stoppered vials.

Heat of combustion was determined using a Berthelot-type calorimeter, following the procedures of Lieth (1975). Determinations were performed in triplicate on samples weighing 1–1.2 g. For calculation of ash-free heat of combustion, ash values were taken from Merino *et al.* (1984).

Organic nitrogen content was measured as Kjeldahl nitrogen using a semi-automated procedure (Isaac & Johnson, 1976; Technicon Industrial Method No. 146-71A, Technicon Industrial Systems, Tarrytown, New York). Nitrate which may be partially detected in the Kjeldahl procedure, was determined using the colorimetric assay of Cataldo *et al.* (1975), modified for smaller sample mass. Carbon, hydrogen and nitrogen contents were determined using an elemental analyser (Carlo Erba, Milano, Italy). Oxygen was determined using a Perkin-Elmer Model 240C Elemental Analyser. All samples were redried at 40 °C for 24–48 h prior to analysis.

Biochemical pathway costs were calculated, taking glucose as the sole starting substance and using established pathways (Dagley & Nicholson, 1970; Lehninger, 1970; Robinson, 1980; Goodwin & Mercer, 1983; Luckner, 1984). All ATP, NADH and NADPH, aside from that produced during the biosynthesis of the compounds in question, was assumed to be generated through glycolysis, the tricarboxylic acid cycle, and the phosphogluconate pathway. The cost of making NADH was taken to be 0.0778 mol glucose/mol, based on a yield of 36 ATP molecules per glucose molecule, 28 of which are derived from oxidizing the 10 NADH molecules produced in plant cellular respiration. Glucose entering the phosphogluconate pathway requires one ATP and yields 12 NADPH, resulting in a cost of 0.0856 mol glucose/mol NADPH.

Results and Discussion

Development of the method

In order to use the heat of combustion and organic nitrogen content of a biological sample to estimate its construction cost, it is necessary to know (i) the quantitative relationship between the heat of combustion and *GE*, and (ii) the growth efficiency of the tissue. The term 'growth efficiency' has been used in different ways by different authors (e.g. Lambers, Szaniawski & de Visser, 1983). As defined by McDermitt & Loomis (1981), growth efficiency (E_G)

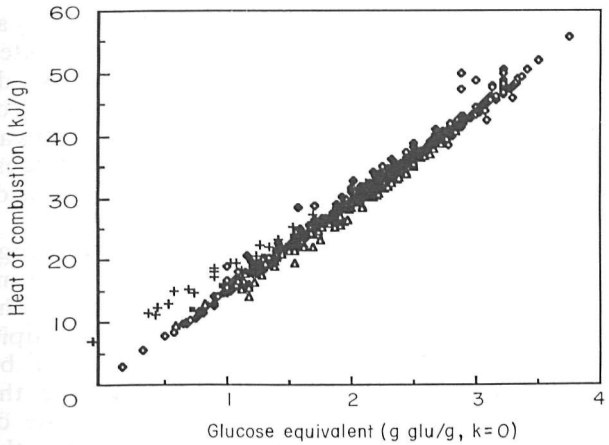

Figure 1. The relationship of heat of combustion (ΔH_C to GE' for 545 organic compounds. Data for compounds containing sulphur are from Cox & Pilcher (1970). All others from Weast (1975). GE' values for sulphur compounds were calculated both with SO_2 (g^Q) ($m = 4$) and H_2SO_4 (aq) ($m = 6$) as the sulphur-containing combustion product. Original data were tabulated with H_2SO_4 (aq) as the standard sulphur combustion product. The difference in heat release between the two reactions was calculated as 303.4 kJ/mol S (Barker *et al.*, 1955). Data for compounds containing nitrogen bonded directly to oxygen ($n = 22$), other forms of nitrogen ($n = 102$), or sulphur ($n = 134$) are displayed separately from those containing only carbon, hydrogen and/or oxygen ($n = 287$), as indicated. CH(O), ◇; Ch(O)N, ◆; CH(ON)–NOx, +; CH(ON)S ($m = 4$), △; CH(ON)S ($m = 6$), ■.

relates GE to construction cost (C):

$$C = \frac{GE}{E_G} = \left[\frac{c}{6} + \frac{h - 2x + kn + ms}{24}\right]\frac{1}{E_G} \qquad (3)$$

We determined the relationship between heat of combustion (ΔH_c) and GE from tabulated data on the heat of combustion of organic compounds (Weast, 1975) and GE'_o values calculated from eqn (1) (Fig. 1). We define GE' as the glucose equivalents of a substance expressed in grams glucose per gram of material (corresponding to the inverse of McDermitt & Loomis' 'glucose value') and GE'_o as GE' calculated using the oxidation numbers of the nitrogen and sulphur combustion products for k and m (eqn (1)).

Of the classes of compounds represented in Fig. 1, those containing carbon, hydrogen and possibly oxygen (CH(O)), as well as those containing nitrogen (CH(O)N) represent the most abundant compounds in plant tissue. The slopes of the regression lines for these two classes of compounds were not significantly different ($P > 0.10$). The regression line formed by the 389 compounds in both classes was:

$$\Delta H_C = 14.352 \times GE'_o + 0.929, \qquad (r^2 = 0.992) \qquad (4)$$

where ΔH_C is heat of combustion in kJ/g and GE'_o is calculated with $k = 0$. This equation is similar to that obtained by McDermitt & Loomis (1981) for a few biologically important compounds.

Compounds in which nitrogen is bonded directly to oxygen deviate from the general relationship between ΔH_C and GE'_o. (The slopes of the relationships are significantly different at $P < 0.001$.) Fortunately, this deviation does not present a substantial drawback to the use of ΔH_C as a predictor of the GE' of biomass, because the occurrence of nitro compounds in plants is rare (Robinson, 1980).

Deviations of sulphur-containing compounds from the relationship described in eqn (4) depend on the convention used in combustion calorimetry. Two conventions exist for dealing with sulphur-containing compounds. Sulphur dioxide gas is generally taken as the standard combustion product of sulphur because it is the product formed in combustion at normal atmospheric pressure (Barker, Mott & Thomas, 1955). In reality, most of the sulphur is converted to dilute sulphuric acid under the conditions present in a calorimeter (i.e. pressurization with oxygen gas; Cox & Pilcher, 1970). Heats of combustion are generally corrected for this extra oxidation by measuring the amounts of sulphur- and nitrogen-containing acids formed and applying 'acid corrections'. Acid corrections subtract the heat associated with this extra oxidation from the measured heat release (Barker *et al.*, 1955; Lieth, 1975). When dilute sulphuric acid is taken as the standard combustion product of sulphur, and GE'_o is calculated with $m = +6$ (the oxidation value of sulphur in sulphuric acid), the relationship between ΔH_C and GE'_o conforms more closely to eqn (4) than when SO_2 is taken as the standard combustion product and GE'_o is calculated with $m = +4$ (Fig. 1). This discrepancy results from the fact that the acid correction presented by Barker *et al.* (1955) is higher than that expected on the basis of eqn (4) by approximately 88 kJ/mol sulphur. (Equation (4) predicts approximately 108 kJ of heat release per mol electron transferred to an oxygen-containing bond.) In practice, the sulphur content of biomass is generally so low that these considerations have little effect on the estimation of GE'. For greater accuracy, however, GE'_o should be calculated with $m = +6$, the oxidation state of sulphur in sulphuric acid:

$$GE'_o = \left[\frac{c}{6} + \frac{h - 2x + 6s}{24}\right]\frac{180.15}{MW}. \qquad (5)$$

The ratio, 180.15 (the molecular mass of glucose) over MW (the molecular mass of the compound) converts the units in eqn (1) to g glucose/g.

Combining eqns (1), (3), (4) and (5) yields an equation for predicting tissue construction cost from heat of combustion, ash content, organic nitrogen content and organic sulphur content. The GE'_o of organic material is predicted from its heat of combustion with a rearrangement of eqn (4):

$$GE'_o = 0.06968 \times \Delta H_C - 0.065. \qquad (6)$$

Since plant tissues always contain minerals, a value of GE_o' corrected for the ash content of the sample can be obtained as follows:

$$GE_o' = (0.06968 \times \Delta H_C - 0.065)(1 - A), \quad (7)$$

where ΔH_C is the ash-free heat of combustion of the sample in kJ/g and A is the ash fraction of the sample (g ash/g dry weight). The first term of eqn (7) predicts GE_o' for the organic fraction of the tissue (eqn (6)) and the last term puts GE_o' on a total dry weight basis.

Combining eqn (5) with eqn (1), corrects for the incorporation and possible reduction of the nitrogen and sulphur substrates:

$$GE' = GE_o' + \left[\frac{kN}{14.0067} + \frac{(m-6)S}{32.06}\right]\frac{180.15}{24}, \quad (8)$$

where N is the organic nitrogen content of the tissue in g/g dw, 14.0067 is the atomic mass of nitrogen, S is the organic sulphur content of the tissue in g/g dw, 32.06 is the atomic mass of sulphur and 180.15 converts moles of glucose to grams of glucose. Since the most common sulphur substrate is sulphate ($m = +6$), eqn (8) will usually reduce to:

$$GE' = GE_o' + \frac{kN}{14.0067} \times \frac{180.15}{24}, \quad (9)$$

or

$$GE' = (0.06968 \times \Delta H_C - 0.065)(1 - A) + \frac{kN}{14.0067} \times \frac{180.15}{24}. \quad (10)$$

No measurement of organic sulphur content is then required.

As discussed above, an efficiency factor is required to obtain the total construction cost (C) from GE' (eqn (3)). The full expression for estimating construction cost from heat of combustion, nitrogen content and ash content is:

$$C = \left\{(0.06968 \times \Delta H_C - 0.065)(1 - A) + \frac{kN}{14.0067} \times \frac{180.15}{24}\right\}\frac{1}{E_G}. \quad (11)$$

The deviation of growth efficiencey (E_G) from 100% represents the fraction of cost which provides reductant that is not incorporated into biomass. This cost includes the reductant which generates ATP through oxidative phosphorylation. The amount of ATP required for growth processes varies depending on the ATP requirements of the biosynthetic pathways and the ATP-consuming processes subsumed under 'growth'. The carbon or glucose cost of ATP varies depending on the pathway by which ATP is generated in a tissue. The ATP cost associated directly with a compound's biosynthetic pathway may be estimated through biochemical pathway analysis, if certain assumptions are made regarding the cost of ATP generation. Defining E_G as GE/C, where C, the construction cost, is calculated from biochemical pathway analysis (Penning de Vries *et al.*, 1974), subjects the value of E_G to all of the assumptions inherent in the biochemical pathway analysis approach. To predict the construction cost of plant tissue from its GE, an average estimate of E_G must be used.

McDermitt & Loomis (1981) calculated an average growth efficiency of 0.884 for crop seeds encompassing a range of chemical compositions. The compositional data they used were the protein, lipid and carbohydrate contents of seeds published by Sinclair & de Wit (1975). In order to evaluate the generality of this value for use with plant tissue of more complex composition, we calculated the efficiencies of many biologically important compounds and the overall efficiencies for four tissues. glucose equivalents, pathway costs and biosynthetic efficiencies for representative biomolecules are presented in Appendices I and II. The construction costs calculated for these compounds include only those costs directly calculable from analysis of the biosynthetic pathways and do not include estimates of any other growth-related costs. We define the resulting efficiency (GE/C) as the biosynthetic efficiency (E_B).

The biosynthetic efficiencies for the compounds examined covered a wide range (0.231–1.000). Substances synthesized with S-adenosylmethionine had particularly low efficiencies because of the high cost of forming the methyl group donor. Additionally, incorporation of O_2 into organic compounds resulted in low efficiencies. However, constraints on the variation in chemical composition of plant tissues should restrict the range of biosynthetic efficiencies encountered in actual plant samples.

Published chemical compositions and calculated biosynthetic efficiencies for rice seed, wood, maize seedlings, and *Diplacus* leaf are shown in Table 1. Rice seed is almost all carbohydrate (Sinclair & de Wit, 1975) and has the highest overall efficiency at 0.94. A highly resinous leaf of *Diplacus aurantiacus* has the lowest overall efficiency of the tissues examined, at 0.85. In the extreme, this range of efficiencies could only be slightly broader. For example, if rice seed carbohydrate were entirely sugar (not true) its overall E_B would be 0.95. If the resin content of the *Diplacus* leaf is actually 30%, as reported by Lincoln (1980), and some of its nitrogen were in glycine-betaine, an osmoticum with a low biosynthetic efficiency, rather than in protein, its overall E_B would be 0.84. (We assumed a glycine-betaine concentration of 7%, the highest reported by Storey, Ahmad & Wyn-Jones, 1977, for plant tissue.) Construction costs of plant tissues (excluding transport costs) can therefore be estimated to within 6% of the true value by using an efficiency of 0.89 in eqn (11). The estimation is probably closer than

Table 1. Per cent composition of plant organs and calculated overall efficiency of ash-free biomass

Compound type	Average biosynthetic efficiency (E_B)	Per cent composition of: Rice seed*	Wood†	Young maize plant‡	*Diplacus* leaf§
Organic acid	0.946	—	—	5	1.5
Carbohydrate	0.946	88	65–80	56.5	25.07
(cellulose)	0.947	—	(40–50)**	(22.6)	(10.11)
(hemicellulose)	0.874–0.939	—	(20–30)	(25.43)	(5.02)
(non-structural)	0.955	—	—	(8.48)	(9.94)
Nucleic acid	0.788–0.878	—	—	0.69	—
Amino acid	0.903–0.939	—	—	2.3	—
Protein	0.826–0.875	8	—	20.01	14.85
Lipid	0.923	2	—	2.5	5.45
Lignin	0.726–0.766¶	—	20–35	8	15.73
Phenols	0.922	—	—	—	6.57
Diplacol	0.841	—	—	—	20
Unrecovered	—	—	—	—	6.54
Ash or minerals	—	2	—	5	4.29
Overall ash-free efficiency	—	*0.94*	*0.85–0.90*	*0.89–0.91*	*0.85–0.88*

*Sinclair & de Wit (1975).
†Poulton (1981).
‡Penning de Vries *et al.* (1974).
§Merino *et al.* (1984); compositional data for middle-aged leaves; uncertainty in overall efficiency due to incomplete recovery.
¶Lignin assumed to be equal parts coniferyl alcohol and sinapyl alcohol derivatives except in the case of maize. Grass lignin contains higher amounts of coumaryl alcohol derivatives (Poulton, 1981), so maize lignin was assumed to have equal parts of derivatives of all three alcohols.
**Parethetical values included in value for 'carbohydrate'.

$\pm 6\%$ for most plant tissues because the extreme E_B values calculated above characterize fairly unusual plant tissues. This value is close to that determined by McDermitt & Loomis (1981).

Including the costs of growth-related processes, such as substrate transport, in the calculation of construction cost lowers the value of the efficiency factor. Penning de Vries *et al.* (1974) included costs of transporting monomers across membranes in their calculations of *PV*. They used an estimate of 1 mol ATP per mol glucose required in biosynthesis. If their estimate of membrane transport costs is incorporated into calculations of construction cost, E_G is lowered from 0.89 (the midrange E_B found here) to 0.87.

Comparison of Methods for estimating construction costs

The leaf construction costs of the chaparral shrubs, *Lepechinia calycina, Diplacus aurantiacus*, and *Heteromeles arbutifolia*, have been estimated previously, using analysis of growth respiration (Merino, Field & Mooney, 1982) and biochemical pathway analysis (Merino *et al.*, 1984). The same leaf samples that were used for pathway analysis were subjected to elemental analysis, combustion calorimetry, and organic nitrogen determination (Table 2). From these data, we performed two more cost estimates: one based on the *GE* estimated from heat of combustion and nitrogen content (eqn (11)) and one based on the *GE* calculated from elemental analysis (eqn (3)). Nitrate nitrogen was less than 0.01% of the tissue dry weight in almost all cases. Therefore, Kjedahl nitrogen was taken as a measurement of organic nitrogen in eqn (11). A value of 0.89 was used as an estimate of growth efficiency (E_G) in both equations.

Agreement between the two methods that base their estimates on a tissue's *GE* (i.e. elemental analysis and heat of combustion) was generally good (Table 3). Most cost values obtained using heat of combustion were slightly higher than those calculated from elemental composition. The greatest discrepancy occurred in the sample for which recovery during elemental analysis was poorest (*Lepechinia*, age class IV).

There was agreement between pathway analysis and methods based on *GE* in the relative costs of the leaves of the deciduous species, *D. aurantiacus* and *L. calycina*. However, the absolute values for the pathway costs were uniformly 20–22% higher than the cost estimated from bomb calorimetry and organic nitrogen analysis. This difference is greater than can be accounted for by the uncertainty in assigning a biosynthetic efficiency to the tissue. The agreement between the present estimates and the pathway costs for *H. arbutifolia* is

Table 2. Heat of combustion and elemental composition of chaparral shrub leaves. Five leaf age classes are represented

	ΔH_C (ash-free) (kJ/g)	Kjeldahl *N* (%)	Per cent composition: C	H	N	O	Ash	Per cent recovery
L. calcina								
I	22.44	2.65	48.66	6.65	2·76	35.83	6.51	100.41
II	21.48	2.92	48.46	5.99	3.11	35.64	6.88	100.08
III	—	—	45.00	6.00	2.41	—	11.30	—
IV	20.51	2.71	44.08	5.40	2.55	35.94	8.51	96.48
V	20.36	2.39	43.72	5.85	2.30	36.50	10.29	98.66
D. aurantiacus								
I	23.67	1.93	51.24	6.93	1.70	33.95	3.65	97.47
II	22.68	1.90	51.06	6.34	1.99	35.45	4.26	99.10
III	22.04	1.91	49.20	6.88	1.59	35.88	4.29	97.84
IV	21.47	1.85	48.35	6.89	1.63	36.54	5.49	98.90
V	20.84	1.57	46.26	6.39	1.32	37.41	6.33	97.71
H. arbutifolia								
I	20.28	2.33	46.48	6.43	2.03	38.05	4.90	97.89
II	20.52	1.84	46.45	6.60	1.49	38.10	4.80	97.44
III	20.64	1.51	46.39	6.64	1.37	38.65	5.65	98.70
IV	20.49	1.13	46.34	6.84	1.01	39.39	5.15	98.73
V	20.44	0.85	47.23	6.84	0.73	39.22	4.35	98.37

I, youngest; V, oldest.

better. However, in performing the pathway analysis for this species, Merino *et al.* (1984) could account for only 70% of the mass of their leaf samples. Difficulties in obtaining complete and accurate recovery of all biochemical components in a tissue limit the accurancy with which the construction cost of a tissue may be determined from its biochemical composition. The poor recovery of biochemical components in *Heteromeles* leaves (Merino *et al.*, 1984) renders the agreement between these cost

Table 3. Construction cost estimates using different methods. Units are g glu/gdw. The figures for the first three methods are calculated with nitrate as the nitrogen source

	Heat of combustion, nitrogen and ash*	Elemental analysis*	Biochemical analysis†	Gas exchange and growth analysis‡
L. calcina				
I	1.69	1.63	2.00	
II	1.61	1.58	1.90	
III	—	—	1.79	1.73 (1.50§–1.48¶)
IV	1.51	1.39	1.78	
V	1.46	1.40	1.74	
D. aurantiacus				
I	1.79	1.71	2.14	
II	1.70	1.65	2.06	
III	1.65	1.63	2.02	1.83 (1.48§–1.63¶)
IV	1.59	1.60	1.92	
V	1.52	1.48	1.86	
H. arbutifolia				
I	1.53	1.50	1.62	
II	1.53	1.50	1.65	
III	1.52	1.49	1.56	1.72 (1.44¶)
IV	1.50	1.49	1.53	
V	1.49	1.51	1.60	

*The $\pm 6\%$ uncertainty in these figures, due to the use of an average E_B in the cost calculations (eqns (3) and (10)), amounts to approximately ± 0.10 g glucose/g.)

†Merino *et al.* (1984).

‡Merino *et al.* (1982). Values in parentheses reflect corrections to respiration for diurnal fluctuations in temperature. Calculations use gas exchange and growth data taken by Merino *et al.* (1982) in March § and May ¶. Respiration is corrected for temperature, using temperature data for typical days in March and May, 1980, and assuming that respiration responds to temperature with a Q_{10} of 2 throughout the day.

estimates and those obtained with other methods largely fortuitous. Merino *et al.* (1984) could account for much more of the mass of *Diplacus* and *Lepechinia* leaves. However, fractions of relatively expensive components may have been overestimated. As an example, Merino *et al.* (1984) used a standard method of estimating protein content by multiplying the tissue's nitrogen content by 6.25. Some authors have suggested that this method overestimates protein content in plant tissues and that a factor of 5.3 is more realistic (Milton & Dintzis, 1981). We speculate that uncertainty in the biochemical composition of the tissues examined, due to the multiple sources of error accompanying multiple biochemical analyses, may account for much of the poor agreement between biochemical pathway analysis and other methods of estimating construction cost.

The construction cost estimates obtained by bomb calorimetry and organic nitrogen determination agreed more closely with those calculated from estimates of growth and growth respiration. In making these comparisons, we have refined the cost calculations of Merino *et al.* (1982) to include the effect of diurnal temperature fluctuations on calculated growth respiration. Merino *et al.* (1982) made all respiration measurements at 20 °C, a realistic daytime temperature. We recalculated leaf construction costs using an approximated daily course of temperature, assuming that temperature affected dark respiration with a Q_{10} of 2 and assuming that light had no effect on the rate of respiratory processes (results in parentheses, Table 3). With these adjustments, estimates of construction cost derived from gas exchange and growth analysis agreed fairly well with estimates derived from heat of combustion and organic nitrogen content.

It could be argued that gas exchange and growth analysis should yield lower estimates of construction cost than either heat of combustion or elemental analysis. Some of the leaf biomolecules, such as some amino acids, may be imported from other parts of the plant during certain stages of leaf development. The respiration associated with their formation is evolved elsewhere in the plant. Thus, part of the construction cost associated with these molecules is reflected in a leaf's heat of combustion but not in its respiration.

Conceptual problems, as well as technical problems, attend the quantification of respiration in the light when analysing costs through gas exchange and growth analysis. Some evidence suggests that light reduces the rates of processes comprising dark respiration (e.g. Piesker & Apel, 1980; Brooks & Farquhar, 1985). Energy and reductant derived directly from light-driven electron transport, however, may contribute to biosynthetic processes in photosynthetic tissue. It could be argued that this energy and reductant should be included in estimates of 'respiration' when quantifying growth costs. Without knowing the extent to which these opposing processes offset each other in the light, it is difficult to predict how cost estimates derived from gas exchange and growth analysis should compare to estimates based on tissue composition and heat of combustion. Therefore, although the agreement between gas exchange/growth analysis and heat of combustion in estimating construction cost is encouraging, it is difficult to interpret.

Conclusions

Construction costs of plant tissues may be estimated fairly easily from measurements of heat of combustion, ash content and organic nitrogen content. This method, which is based on prediction of a tissue's *GE*, yields very similar construction cost estimates to those derived from elemental composition. It should estimate total construction cost, as determined from biochemical pathway analysis, to within $\pm 6\%$. When construction cost includes only those costs directly calculable from biosynthetic pathways, 0.89 is a good estimate for E_G, the efficiency factor converting *GE* to total construction cost. Including a rough estimate of membrane transport cost in construction cost (i.e. 1 mol ATP per mol glucose required; Penning de Vries *et al.*, 1974) reduces E_G to 0.87.

Estimates of total construction cost compared fairly poorly with published values obtained from biochemical pathway analysis (although the two methods generally yielded the same relative rankings in cost for the tissues examined). This discrepancy was most likely due to the difficulty in obtaining complete and accurate recovery of organic compounds in analysing the biochemical composition of the tissue. Agreement was better with cost estimates obtained from gas exchange and growth analysis.

The proposed method is less time-consuming than biochemical pathway analysis and less costly than elemental analysis. In cases where it is difficult to obtain full and accurate recovery of biochemical fractions for biochemical pathway analysis, heat of combustion and organic nitrogen content may provide a more accurate estimate of total construction cost. Only three determinations are required; heat of combustion, organic nitrogen content and ash content. These measurements may be obtained using commonly available equipment.

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Appendix I

Table AI

Glucose Equivalents, construction costs and biosynthetic efficiencies of some non-nitrogenous plant constituents are given below. Glucose Equivalents (*GE*) were calculated from the elemental compositions of the compounds (eqn (1)). Pathway costs were calculated using biochemical pathways presented in Dagley & Nicholson (1970), Lehninger (1970), Robinson (1980), Goodwin & Mercer (1983) and Luckner (1984). Values for alternate pathways were averaged. Biosynthetic efficiencies were calculated as GE'/Cost

Compound	Molecular mass (g/mol)	GE' (g glu/g)	Cost (g glu/g)	Biosynthetic efficiency (E_B)
Organic acids				
pyruvate	88	0.852	0.852	1.000
malate	134	0.672	0.690	0.973
citrate	192	0.703	0.716	0.982
oxaloacetic acid	132	0.568	0.587	0.968
fumarate	116	0.776	0.798	0.973
succinate	118	0.890	0.869	1.024
oxalate	90	0.167	0.378	0.441
Carbohydrates				
glucose	180	1.000	1.000	1.000
fructose	180	1.000	1.028	0.973
mannose	180	1.000	1.028	0.973
galactose	180	1.000	1.056	0.947
lactose	342	1.053	1.082	0.973
cellulose	(162)§	1.111	1.173	0.947
hemicellulose*	—	1.132	1.296	0.874
hemicellulose†	—	1.132	1.205	0.939
sucrose	342	1.053	1.096	0.960
starch	(162)§	1.111	1.173	0.947
ribose-5-P	230	0.652	0.671	0.973
erythrose-4-P	200	0.600	0.642	0.935
pinitol	194	1.160	1.302	0.890
Fatty acids and triglycerides				
caprylic acid	144	2.290	2.412	0.950
capric acid	172	2.440	2.576	0.947
lauric acid	200	2.548	2.694	0.946
myristic acid	228	2.629	2.783	0.945
palmitic acid	256	2.693	2.852	0.944
stearic acid	285	2.744	2.908	0.944
oleic acid	283	2.711	2.984	0.908
linoleic acid	280	2.677	3.060	0.875
linolenic acid	278	2.642	3.138	0.842
glyceryl tripalmitate	807	2.696	2.852	0.946
Other non-nitrogenous compounds				
rubber	(68)§	3.085	3.318	0.930
diplacol (resin)	440	1.943	2.310	0.841
Lignin				
coumaryl alcohol radical	149	2.063	2.431	0.849
coniferly alcohol radical	179	1.885	2.488	0.758‡
sinapyl alcohol radical	209	1.758	2.528	0.695‡
Monoterpenes				
camphor	152	2.663	2.868	0.928
pinene	136	3.085	3.431	0.899
Tannins (condensed)				
procyanidin subunit	288	1.471	1.591	0.924
prodelphinidin subunit	304	1.431	1.558	0.919

* Hemicellulose with residue composition reported by Bauer *et al.* (1973). Myo-inositol pathway used.

† As above but with pathways employing dehydrogenases.

‡ S-adenosylmethionine involved in biosynthetic pathway.

§ The molecular mass of the monomer in the polymer.

Appendix II

Table AII

Glucose Equivalents, construction costs and biosynthetic efficiencies of some nitrogen-containing plant constituents are given below. Glucose Equivalents (GE'), pathway costs and biosynthetic efficiencies were calculated as in Appendix I. The nitrogen substrate forms used for the various calculations are indicated

Compound	Molecular mass (g/mol)	GE' (g glu/g)		Cost (g glu/g)		Biosynthetic efficiency (E_B)	
		NH_4^+	NO_3^-	NH_4^+	NO_3^-	NH_4^+	NO_3^-
Amino acids							
alanine	89	1.011	1.685	1.072	1.746	0.943	0.965
arginine	174	0.948	2.327	1.230	2.609	0.770	0.892
aspartate	133	0.677	1.128	0.736	1.187	0.919	0.950
asparagine	132	0.682	1.591	0.855	1.764	0.797	0.902
glutamate	147	0.918	1.326	0.972	1.380	0.945	0.961
glutamine	146	0.925	1.746	1.013	1.835	0.913	0.952
glycine	75	0.600	1.400	0.689	1.489	0.870	0.940
isoleucine	131	1.717	2.174	1.863	2.321	0.921	0.937
leucine	131	1.717	2.174	1.784	2.242	0.962	0.970
lysine	146	1.438	2.259	1.460	2.282	0.985	0.990
ornithine	132	1.250	2.158	1.392	2.300	0.898	0.938
phenylalanine	165	1.818	2.181	1.994	2.358	0.911	0.925
proline	115	1.434	1.956	1.574	2.095	0.911	0.933
serine	105	0.714	1.286	0.842	1.413	0.848	0.910
threonine	119	1.008	1.512	1.120	1.625	0.900	0.930
tryptophan	204	1.691	2.279	1.943	2.530	0.870	0.901
tyrosine	181	1.574	1.906	1.733	2.065	0.908	0.923
valine	117	1.538	2.050	1.588	2.100	0.969	0.976
Protein							
zein*	—	1.453	2.030	1.741	2.318	0.835	0.876
zein†	—	1.457	2.178	1.762	2.484	0.827	0.877
hordein*	—	1.384	1.967	1.675	2.259	0.827	0.871
hordein†	—	1.388	2.158	1.697	2.467	0.818	0.875
Nucleic acid							
AMP	347	0.649	1.513	0.877	1.741	0.740	0.869
GMP	363	0.579	1.405	0.817	1.644	0.708	0.855
UMP	324	0.695	1.065	0.801	1.172	0.867	0.909
CMP	323	0.697	1.254	0.819	1.376	0.851	0.911
dAMP residue	313	0.767	1.726	1.053	2.012	0.728	0.858
dGMP residue	329	0.684	1.596	0.979	1.891	0.699	0.844
dTMP residue	304	0.938	1.332	1.058	1.453	0.886	0.917
dCMP residue	289	0.831	1.454	1.003	1.626	0.828	0.894
Other nitrogen-containing compounds							
protoporthyrin	563	2.001	2.428	2.324	2.751	0.861	0.883
Cyanogenic glucosides							
dhurrin	311	1.350	1.543	1.678	1.871	0.805	0.825
prunasin	295	1.474	1.678	1.821	2.024	0.810	0.829
Alkaloids							
nicotine	162	2.221	2.961	2.686	3.247	0.827	0.864‡
caffeine	194	1.005	2.242	1.661	2.898	0.607	0.774‡
N storage and transport							
allantoin	158	0.190	1.709	0.820	2.339	0.231	0.731
N-acetyl arginine	216	1.041	2.152	1.268	2.379	0.821	0.905
N-acetyl ornithine	174	1.293	1.982	1.400	2.090	0.923	0.948
sarcosine	89	1.011	1.685	1.303	1.977	0.777	0.853‡
betaine	117	1.538	2.050	2.087	2.600	0.737	0.789‡

*Amino acid composition for zein from Wilson (1983), for hordein from Shewry & Miflin (1983); all glu, gln, asp and asn assumed to be glu and asp, respectively.

†All glu, gln, asp and asn assumed to be gln and asn, respectively.

‡S-adenosylmethionine involved in biosynthetic pathway.

References

Barker, J.E., Mott, R.A. & Thomas, W.C. (1955) Studies in Bomb calorimetry. IV. Corrections. *Fuel*, **34**, 303–316.

Bauer, W.D., Talmadge, K.W., Deegstra, K. & Albersheim, P. (1973) The structure of plant cell walls. II. The hemicellulose of the walls of suspension-cultured sycamore cells. *Plant Physiology*, **51**, 174–187.

Bloom, A.J., Chapin, F.S. III & Mooney, H.A. (1985) Resource limitation in plants—an economic analogy. *Annual Review of Ecology and Systematics*, **16**, 363–392.

Brooks, A. & Farquhar, G.D. (1985) Effect of temperature on the CO_2/O_2 specificity of ribulose-1,5-bisphosphate carboxylase/oxygenase and the rate of respiration in the light. *Planta*, **165**, 397–406.

Cataldo, D.A., Haroon, M., Schrader, L.E. & Youngs, V.L. (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Communications in Soil Science and Plant Analysis*, **6**, 71–80.

Cox, J.D. & Pilcher, G. (1970) *Thermochemistry of Organic and Organometallic Compounds*. Academic Press, London.

Dagley, S. & Nicholson, D.E. (1970) *An Introduction to Metabolic Pathways*. Blackwell Scientific Publications, Oxford.

Goodwin, T.W. & Mercer, E.I. (1983) *Introduction to Plant Biochemistry*, 2nd edn. Pergamon Press, Oxford.

Isaac, R.A. & Johnson, W.C. (1976) Determination of total nitrogen in plant tissue, using a block digestor. *Journal of the Association of Official Analytical Chemists*, **59**, 98–100.

Kharasch, M.S. (1929) Heat of combustion of organic compounds. *Journal of Research of the National Bureau of Standards*, **2**, 359–430.

Kharasch, M.S. & Sher, B. (1925) The electronic conception of valence and heats of combustion of organic compounds. *Journal of Physical Chemistry*, **29**, 625–658.

Lambers, H., Szaniawski, R.K. & de Visser, R. (1983) Respiration for growth, maintenance and ion uptake. An evaluation of concepts, methods, values and their significance. *Physiologia Plantarum*, **58**, 556–563.

Lehninger, A.L. (1970) *Biochemistry*. Worth Publishers, New York.

Leith, H. (1975) Measurement of caloric values, In *Primary Productivity of The Biosphere* (eds H. Leith and R. Whittaker), pp. 119–129. Springer Verlag, New York.

Lincoln, D.E. (1980) Leaf resin flavonoids of *Diplacus aurantiacus*. *Biochemical Systematics and Ecology*, **8**, 397–400.

Luckner, M. (1984) *Secondary Metabolism in Microorganisms, Plants, and Animals*, 2nd edn. Springer-Verlag, Berlin.

McDermitt, D.K. & Loomis, R.S. (1981) Elemental composition of biomass and its relation to energy content, growth efficiency, and growth yield. *Annals of Botany*, **48**, 275–290.

Merino, J., Field, F. & Mooney, H.A. (1982) Construction and maintenance costs of mediterranean-climate evergreen and deciduous leaves, I. Growth and CO_2 exchange analysis. *Oecologia (Berlin)*, **53**, 208–213.

Merino, J., Field, C. & Mooney, H. A. (1984) Construction and maintenance costs of mediterranean-climate evergreen and deciduous leaves II.—Biochemical pathway analysis *Acta Oecologica/Oecologia Plantarum*, **5**, 211–223.

Milton, K. & Dintzis, F.R. (1981) Nitrogen-to-protein conversion factors for tropical plant samples. *Biotropica*, **13**, 177–181.

Mooney, H.A. (1972) The carbon balance of plants. *Annual Review of Ecology and Systematics*, **3**, 315–146.

Mooney, H.A. & Gulmon, S.L. (1982) Constraints on leaf structure and function in reference to herbivory. *BioScience*, **32**, 198–206.

Orians, G. & Solbrig, O. (1977) A cost-income model of leaves and roots with special reference to arid and semiarid areas. *American Naturalist*, **111**, 677–690.

Penning de Vries, F.W.T., Brunsting, A.H.M. & van Laar, H.H. (1974) Products, requirements and efficiency of biosynthesis: a quantitative approach, *Journal of Theoretical Biology*, **45**, 339–377.

Piesker, M. & Apel, P. (1980) Dark respiration and the effect of oxygen on CO_2 compensation concentration in wheat leaves. *Zeitschrift fur Pflanzenphysiologie*, **100**, 389–395.

Pirt, S.J. (1975) *Principles of Microbe and Cell Cultivation*. Blackwell Scientific Publications, Oxford.

Poulton, J.E. (1981) Transmethylation and demethylation reactions in the metabolism of secondary plant products, In *The Biochemistry of Plants: A Comprehensive Treatise; Vol. 7: Secondary Plant Products*. (ed. E.E. Conn), pp. 668–724. Academic Press, New York.

Robinson, T. (1980) *The Organic Constituents of Higher Plants*, 4th edn. Cordus Press, North Amherst, Massachusetts.

Shewry, P.R. & Miflin, B.J. (1983) Characterization and synthesis of barley seed proteins, In *Seed Proteins* (eds W. Gottschalk and H.P. Muller), pp. 143–206. Martinus Nijhoff/Dr W. Junk Publishers, The Hague.

Sinclair, T.R. & de Wit, C.T. (1975) Photosynthate and nitrogen requirements for seed production by various crops. *Science*, **189**, 565–567.

Storey, R., Ahmad, N. & Wyn-Jones, R.G. (1977) Taxonomic and ecological aspects of the distribution of glycine betaine and related compounds in plants. *Oecologia (Berlin)*, **27**, 319–332.

Thornley, J.H.M. (1976) *Mathematical Models in Plant Physiology*. Academic Press, London and New York.

Weast, R.C. (ed.) (1975) *Handbook of Chemistry and Physics*, 56th edn. CRC Press, Cleveland, Ohio.

Wilson, C.M. (1983) Seed protein fractions of maize, sorghum and related cereals. In *See Proteins*, (eds W. Gottschalk and H.P. Muller), pp. 271–308. Martinus Nijhoff/Dr W. Junk, The Hague.