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Plant physiology

Heat of combustion, degree of reduction and carbon content: 3 interrelated methods of estimating the construction cost of plant tissues

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Summary — During the last decade, degree of reduction (calculated from elemental composition), heat of combustion and carbon content have been proposed as 3 ways of estimating the construction cost of plant tissues. There is a fairly good agreement among these 3 methods, as they are all based on the link between the energy content of biomass and its reduction level. This relationship is not absolute and the regression coefficient between heat of combustion and degree of reduction may vary according to the chemical composition of biomass. The relation between degree of reduction and carbon content is implicitly based on the consequences in terms of molecular weight of the replacement of 1 oxygen atom by 2 hydrogen atoms in the process of reduction of photoassimilates (carbohydrates) into the various biomass compounds. Different trends can be observed on woody and non-woody tissues, which get richer in energy by increasing their content in lignin and lipids, respectively. Finally, the biosynthetic efficiency is also affected by the chemical composition of biomass. Therefore, while these 3 methods provide efficient and fairly simple tools for the estimation of the construction cost of biomass, they should not be used without considering what the dominant compounds of the plant tissues under study are and without choosing proper parameters in consequence.

construction cost / growth yield / heat of combustion / elemental analysis / carbon content / energy content / *Juglans regia* L = walnut

Résumé — Chaleur de combustion, degré de réduction et teneur en carbone : 3 méthodes voisines d'estimation du coût de croissance des tissus végétaux. *Au cours des dernières années, 3 méthodes nouvelles d'estimation du coût de synthèse de la biomasse ont été proposées, à partir de son degré de réduction (calculé grâce à sa composition élémentaire), de sa chaleur de combustion ou bien de sa teneur en carbone. Ces 3 méthodes donnent des résultats comparables car elles reposent toutes sur une relation générale entre le contenu énergétique de la biomasse et son niveau de réduction. On n'observe pourtant pas de corrélation parfaite entre ces résultats car le coefficient de régression entre chaleur de combustion et degré de réduction varie avec la composition chimique des tissus végétaux analysés. La relation entre degré de réduction et teneur en carbone est implicitement basée sur les conséquences pondérales du remplacement d'un atome d'oxygène par 2 atomes d'hydrogène au cours de la réduction des assimilats en divers constituants de la biomasse. De fait, on observe des comportements différents pour les tissus*

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ligneux et non-ligneux, qui deviennent riches en énergie par accumulation respectivement de lipides et de lignine. Enfin, l'efficience biosynthétique est également sensible à la composition chimique de la biomasse. Par conséquent, si les 3 méthodes présentées dans cet article constituent un ensemble cohérent d'outils opérationnels et relativement simples d'estimation du coût de synthèse de la biomasse, elles ne doivent pas être utilisées sans connaître quels sont les composants dominants du matériel végétal étudié et sans en tirer des conséquences pour le choix des paramètres des relations proposées.

efficience de croissance / chaleur de combustion / analyse élémentaire / teneur en carbone / contenu énergétique /* Juglans regia *L = noyer

INTRODUCTION

In the process of plant tissue construction, photoassimilates (carbohydrates such as glucose) provide carbon skeletons for the synthesis of a whole range of organic compounds. The reduction level, *ie* the number of electrons per mole of these compounds, varies (Roels, 1983). It is sometimes lower (in the case of organic acids) and more often higher (*eg*, in the case of amino acids or lipids) than that of carbohydrates. Therefore, some extra amount of this substrate generally has to be oxidized into CO_2 to provide electrons for the reduction of carbon, sulfur, and nitrogen if necessary, during the biosynthesis of new tissues. In addition, a further supply of carbohydrate is necessary to produce chemical energy to ensure the transport of substrates to the sites of biosynthesis and the polymerization of monomers. This energy is provided by the total oxidation of substrate by molecular oxygen into CO_2 and H_2O. The ratio of the weight of tissue produced to the total weight of substrate consumed is the growth yield, Y_G (Thornley, 1970), or production value, *PV* (Penning de Vries *et al,* 1974; a list of parameters used in this field by various authors is included in table I). The construction cost, *C* (Williams *et al,* 1987), is the inverse of the growth yield (excluding cost of translocation) and is expressed as the sum of 3 terms, namely, the amount of carbohydrate (glucose) used to provide carbon skeletons (g_c), the reducing power (g_r) and the chemical energy (g_o) per amount of biomass formed (McDermitt and Loomis, 1981):

$$C = g_c + g_r + g_o \qquad [1]$$

Chemical energy used for the maintenance of living tissues is not considered in the calculation of the cost of construction.

Different techniques have led to estimations of growth yield (see Lambers *et al,* 1983, for a detailed discussion). One is based on correlations between night respiration and either daily photosynthesis (McCree, 1970) or daily growth (Szaniawski and Kielkiewicz, 1982); the growth yield is directly calculated from the slope of such linear relationships.

A second approach involves an analysis of the biochemical pathways leading to the synthesis of all biomass compounds and the calculation of the weight of glucose required for the production of carbon skeletons, for the supply of reducing power and of chemical energy and for the translocations contributing to the formation of a unit weight of biomass (Penning de Vries *et al,* 1974). The total content in carbohydrates, nitrogenous compounds, lipids, organic acids and lignin of the organic fraction of biomass are sufficient for the computation of growth yield, if assumptions are made on the composition of these 5 classes of compounds.

A third approach involves a separate estimate of the sum $g_B = g_c + g_r$ named 'glucose equivalent' which appears in the expression of the construction cost (equation [1]). During the combustion of plant tissue, the energy available in the carbon skeletons and associated with their reduction is released; dividing this heat value (ΔH_c, in kJ g^{-1}) by the heat of combustion of glucose (15.65 kJ g^{-1}) leads to an approximation of the glucose equivalent:

$$g_B = \Delta H_c / 15.65 \qquad [2]$$

In fact, the heat of combustion is related to the enthalpy of oxidation of all the main elements in CO_2, H_2O, N_2 (or NO_3^-) and SO_2 (or SO_4^{2-}) in a calorimeter. While CO_2 and H_2O are the sources of carbon and hydrogen in plants, nitrogen and sulfur can be used in reduced (NH_4^+, H_2S) or oxidized (NO_3^-, SO_4^{2-}) forms. Thus, the heat of combustion allows an estimation of g_B, provided a correction is made for the contribution of the nitrogen source (sulfur content is generally low enough to be ignored) in the degree of reduction of the plant tissue (Williams *et al,* 1987).

Moreover, the glucose equivalent and the heat of combustion can be predicted using the calculation of the generalized degree of reduction, γ, provided the elemental composition of biomass is determined. This concept is commonly used in microbiology (Roels, 1983; Sandler and Orbey, 1991) and represents the number of available electrons per mole product:

$$\gamma = 4c + h - 2x + \mathrm{k}n \qquad [3]$$

where c, h, x and n indicate the number of atoms of carbon, hydrogen, oxygen and nitrogen, respectively, per mole product; they are multiplied by the valences of these atoms in their ground state (CO_2, H_2O). For nitrogen, k = 0, k = +5 or k = –3 if N_2 (after combustion), NO_3^- or NH_4^+ (in the process of plant tissue construction) are taken as the reference level. For plant bio-

Table I. Parameters used in different methods of estimating the construction cost of biomass.

Parameter	*Symbol*	*Definition*	*Unit*	*Reference*
Growth yield	Y_G	Biomass formed from 1 g substrate (glucose)	g g^{-1}	(1)
Production value	*PV*	Product (or biomass) formed from 1 g glucose, including the supply of carbon skeletons, reductant, ATP and translocation, same as Y_G at the whole plant level	g g^{-1}	(2)
Glucose equivalent	*GE*	Glucose required to provide carbon and reductant for 1 mole product	mol mol^{-1}	(3)
Glucose value	*GV*	Product (or biomass) formed from 1 g glucose, including only the supply of carbon and reductant	g g^{-1}	(3)
Growth efficiency	E_G	*PV/GV*, fraction of substrate electrons retained in the product	mol mol^{-1}	(3)
	g_c	Glucose required to provide carbon for 1 g product	g g^{-1}	(3)
	g_r	Glucose required to provide reductant for 1 g product	g g^{-1}	(3)
Glucose equivalent	g_B	$g_B = g_c + g_r = 1/GV$	g g^{-1}	(3)
	g_o	Glucose required to provide ATP for 1 g product	g g^{-1}	(3)
Generalized degree of reduction	γ	Number of electrons per mole product	mol mol^{-1}	(4)
Inverse of production value	*PVI*	Glucose required to supply carbon, reductant, ATP and translocation for 1 g biomass	g g^{-1}	(5)
Inverse of glucose value	*GVI*	Glucose required to supply carbon and reductant for 1 g biomass, same as g_B	g g^{-1}	(5)
Construction cost	*C*	Same as *PVI*, excluding cost of translocation	g g^{-1}	(6)
Glucose equivalent	*GE'*	$1/GV$; glucose required to provide carbon and reductant for 1 g product	g g^{-1}	(6)
Glucose equivalent	GE'_o	*GE* with the oxidation number of the nitrogen and sulfur combustion products	g g^{-1}	(6)
Biosynthetic efficiency	E_B	*GE'/C*, same as E_G, excluding cost of translocation	mol mol^{-1}	(6)

(1) Thornley, 1970; (2) Penning de Vries *et al*, 1974; (3) McDermitt and Loomis, 1981; (4) Roels, 1983; (5) Vertregt and Penning de Vries, 1987; (6) Williams *et al*, 1987.

mass, γ, c, h, x and n are usually expressed in moles per gram organic biomass; with such units we will write these variables γ', c', h', x' and n', respectively. Considering that the oxidation of one mole of glucose provides 24 moles of electrons, $GE = \gamma/24$ is the amount of glucose containing the same number of electrons as one mole product (McDermitt and Loomis, 1981), an expression of the glucose equivalent in moles glucose per mole product. Then:

$$g_B = \gamma' \,(180.15/24) \qquad [4]$$

where 180.15 g mol^{-1} is the molar mass of glucose. Combining equation [2] and [4] leads to the relationship:

$$\Delta H_c = 117.5\, \gamma' \qquad [5]$$

γ' calculated here with $n' = 0$.

Finally, there is a positive correlation between the glucose equivalent and the carbon content of the organic fraction of a plant tissue (Vertregt and Penning de Vries, 1987). The contribution of carbon to the total mass of a molecule should indeed increase with the replacement of C = O bonds by C-H bonds during the reduction of this molecule. A few experimental data were used to verify this relation and its predictive quality was discussed for low carbon contents (Lafitte and Loomis, 1988; Walton *et al,* 1990). The same kind of correlation, between heat of combustion and carbon content, is used in fuel engineering, but different correlations were found for charcoals and for biomass materials (Graboski and Bain, 1981).

There is no way to estimate directly g_o, but the ratio

$$E_B = (g_c + g_r) \,/\, (g_c + g_r + g_o) = g_B/C$$

named 'biosynthetic efficiency', presents a low variability among plant tissues: 0.89 ± 0.05 (mean ± sd) on rice seed, wood, maize seedlings and *Diplacus* leaves (Williams *et al,* 1987). McDermitt and Loomis (1981) defined $E_G = g_B \times PV$ as 'growth efficiency', a parameter that also varies little: 0.88 ± 0.02 on seeds or caryopses of 13 crop species (McDermitt and Loomis, 1981); and 0.84 ± 0.01 on shoots of grain sorghum at 3 developmental stages and 2 levels of nitrogen supply (Lafitte and Loomis, 1988). When adding an estimated transport cost (one mole ATP per mole glucose required) to their construction cost, Williams *et al* (1987) transformed E_B into E_G around 0.87.

This paper examines the relationships between heat of combustion, degree of reduction and carbon content of biomass, with different data sets presenting a large range of energy contents. It aims at providing guidelines for a proper use of these recent methods of estimating the construction cost of plant tissues in ecology and crop physiology studies. Correlations between heat of combustion and degree of reduction, or glucose equivalent, were calculated from tables of organic compounds by various authors (McDermitt and Loomis, 1981; Roels, 1983; Williams *et al,* 1987; Sandler and Orbey, 1991); they are verified and discussed here in relation to measurements on plant tissues. The same data sets made it possible to discuss the relation between degree of reduction and carbon content. The composition of biomass affects this correlation and this explains discrepancies among various authors working on plant tissues having different major compounds (Vertregt and Penning de Vries, 1987; Lafitte and Loomis, 1988; Walton *et al,* 1990).

MATERIALS AND METHODS

Three previously published sets of data were used, combining measurements of heat of combustion and elemental composition on various plant tissues. A fourth is original and contains heat of combustion and carbon content measurements (table II) of different parts of walnut (*Juglans regia* L) seedlings during germination. Proximal analyses were also available for part of set 1 (Merino *et al,* 1984) and for set 3 (Walton and de Jong, 1990), while only lipid and carbohydrate content was measured in set 4. All data were expressed per gram organic biomass, and the mineral fraction was estimated to be 67% of the ash content (Vertregt and Penning de Vries, 1987).

In the fourth set of data, heat of combustion was measured with an adiabatic calorimeter at constant volume. Combustion gases passed through a mixture of soda lime and sodium hydroxide; the weight change of this mixture during combustion represented the mass of CO_2 that was converted into mass of carbon. After correction by the amount of CO_2 remaining in the calorimeter at the end of the experiment, the carbon content of the sample was calculated. After combustion, the ashes were weighed to determine the ash content (Frossard *et al,* 1992). The lipid content of the kernel was evaluated by NMR, using crude walnut oil as the reference. Soluble carbohydrates were extracted in boiling ethanol (80% v/v) and assayed by the anthrone method (Halhoul and Kleinberg, 1972). Starch was assayed from the ethanol-extracted residue, as previously reported (Frossard and Friaud, 1989). Soluble carbohydrates and starch were both expressed in grams of glucose.

Table II. Characteristics of the different data sets.

Data set	*Author(s)*	*Species*	*Plant tissue*	*Sample number*
1	Williams, 1986	Various	Various	29
1	Williams *et al,* 1987	Chaparral shrubs	Leaves	14
2	Jenkins, 1989	Field and energy crops	Straw, wood	18
2	Jenkins, 1989	Forest and fruit trees	Wood	23
2	Jenkins, 1989	Food and fiber wastes	Various	20
3	Walton *et al,* 1990	Kiwifruit	Berries	12
4	Original data	Walnut seedlings	Kernel	5
4	Original data	Walnut seedlings	All plant organs	8

RESULTS AND DISCUSSION

Heat of combustion and degree of reduction

The explanation of the heat of combustion of a compound by its degree of reduction can be roughly verified on the organic fraction of plant biomass through a multiple regression of heat of combustion (ΔH_c, in kJ g^{-1}) *versus* carbon, hydrogen, oxygen and nitrogen contents (c', h', x' and n', respectively, in mol g^{-1}). Backwards multiple regression enabled us to eliminate n' as a significant explicative variable, as expected from the final reduction state of nitrogen after combustion (N_2):

$$\Delta H_c = 109.2\ (4\ c' + 0.83\ h' - 1.21\ x') \qquad [6]$$

(data sets 1, 2 and 3; $df = 114$, $r^2 = 0.998$, SE of the estimate = 0.508)

When the multiplicative factor of c' is set to 4 (valence of carbon), the multiplicative factors of h' and x' are fairly far from the valences of these elements even if the signs are correct.

Plant tissues do not offer a range of combinations of carbon, hydrogen and oxygen contents large enough to find multiplicative factors closer to the valences of these elements, as Sandler and Orbey (1991) did from a table of 96 organic compounds. The multiplicative factor of the expression within brackets (109.2 kJ mol^{-1} electron) is not far from published values of the heat of combustion associated with the displacement of one mole of electrons (see below).

Using the practical definition of the generalized degree of reduction $\gamma' = 4c' + h' - 2x'$, the data points for the heat of combustion are fairly well correlated with γ' (fig 1):

$$\Delta H_c = 115.4\ \gamma' \qquad [7]$$

(data sets 1, 2 and 3; $df = 117$, $r^2 = 0.857$, SE of the estimate = 0.909)

The origin was set to zero in equation [6] and [7] as expected from theoretical calculations (equation [5]). The slope of equation [7] is comparable to other estimations made from tables of organic compounds: 108–118 kJ mol^{-1} electron (recalculated from McDermitt and Loomis, 1981), 111 kJ mol^{-1} (Patel and Erickson, 1981), 115 kJ mol^{-1} (Roels, 1983), 108 kJ mol^{-1} (recalculated from Williams *et al,* 1987), 104 kJ mol^{-1} (Sandler and Orbey, 1991). These are not far from the theoretical value of 109 kJ mol^{-1} proposed by Kharasch and Sher (1925). Only microbiologists have previously checked this relation on biomass (bacteria or fungi); they found similar slopes of 112 kJ mol^{-1} (Minkevich and Eroshin, 1973; Ho and Payne, 1979).

However, these slight discrepancies among authors can be questioned. On figure 1, scattering is about 8% around mean values and it is bounded by 2 theoretical lines representing the same correlation recalculated from McDermitt and Loomis (1981) for carbohydrates (upper line) and for other compounds (lower line). Data set 3 is on the upper line; these samples were rich in carbohydrates (Walton and de Jong, 1990). Samples with the highest degree of reduction are rich in lipids (set 1; Merino *et al,* 1984) and lie close to the lower line. The difference between the 2 lines fits with the range of corrections (about 1.2 kJ g^{-1}) some authors (Kharasch and Sher, 1925; Sandler and Orbey, 1991) suggested to account for the discrepancies they observed between measured and simulated (*ie* calculated from a fixed energy content per mole of electrons displaced) heats of combustion. There is a theoretical reason for such a variability. The degree of reduction, or glucose

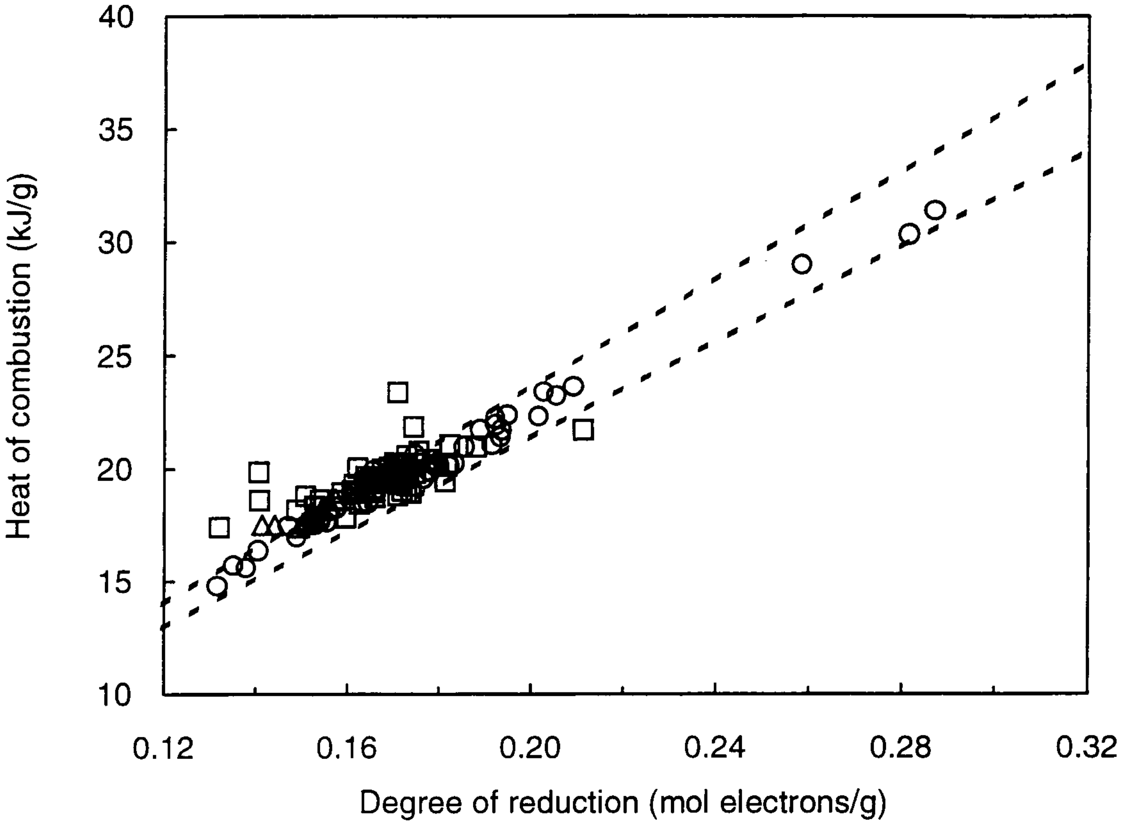

Fig 1. Heat of combustion and degree of reduction of various plant tissues (data sets 1: O, 2: □and 3: Δ). The 2 broken lines were recalculated (degree of reduction rather than glucose equivalent, expressed per gram rather than per mole product) from McDermitt and Loomis (1981) and represent the relationships for carbohydrates (upperline) and other organic compounds (lower line).

equivalent, provides information only on stoichiometry, whereas energy content can also be affected by the structure of the molecule(s). This is why the slope of equation [5], which is based on stoichiometry, fits with the higher slope calculated by McDermitt and Loomis (1981).

These results show (i) that there is a theoretical limit to the precision of prediction of heat of combustion from elemental analysis, and (ii) that the accuracy of measurement of these variables is generally good enough for them to fit into the theoretical range of errors; 72% of the experimental data lie between the 2 theoretical boundaries. Errors of measurement could explain the position of the remaining 28% samples, but they could not be identified. Microbiologists stressed the importance of a careful preparation of the dry biomass samples (Cordier *et al,* 1987; Gurakan *et al,* 1990).

Heat of combustion and carbon content

From all 4 sets of data, the correlation between heat of combustion and carbon content is verified (fig 2):

$$\Delta H_c = 720.4\ c' - 9.34 \qquad [8]$$

($df = 129$, $r^2 = 0.915$, SE of the estimate = 1.059)

Data set 2 seems to present a particular behaviour. For data sets 1, 3 and 4:

$$\Delta H_c = 754.3\ c' - 10.47 \qquad [9]$$

($df = 68$, $r^2 = 0.967$, SE of the estimate = 0.716).

While for data set 2:

$$\Delta H_c = 322.4\ c' + 6.457 \qquad [10]$$

($df = 59$, $r^2 = 0.489$, SE of the estimate = 0.501).

The origin was not set to zero in equations [8] to [10], as ΔH_c can be equal to zero with positive c' (for CO_2). Vertregt and Penning de Vries (1987) calculated a higher value for the slope of a similar relation, 870 kJ mol^{-1} (after multiplication of their glucose equivalents by the heat of combustion of glucose, namely 15.65 kJ g^{-1}). This slope did not enable Lambers and Rychter (1989) and Walton *et al* (1990) to compute construction costs comparable with those acquired through other methods. Going back to Vertregt and Penning de Vries' data, Walton *et al* (1990)

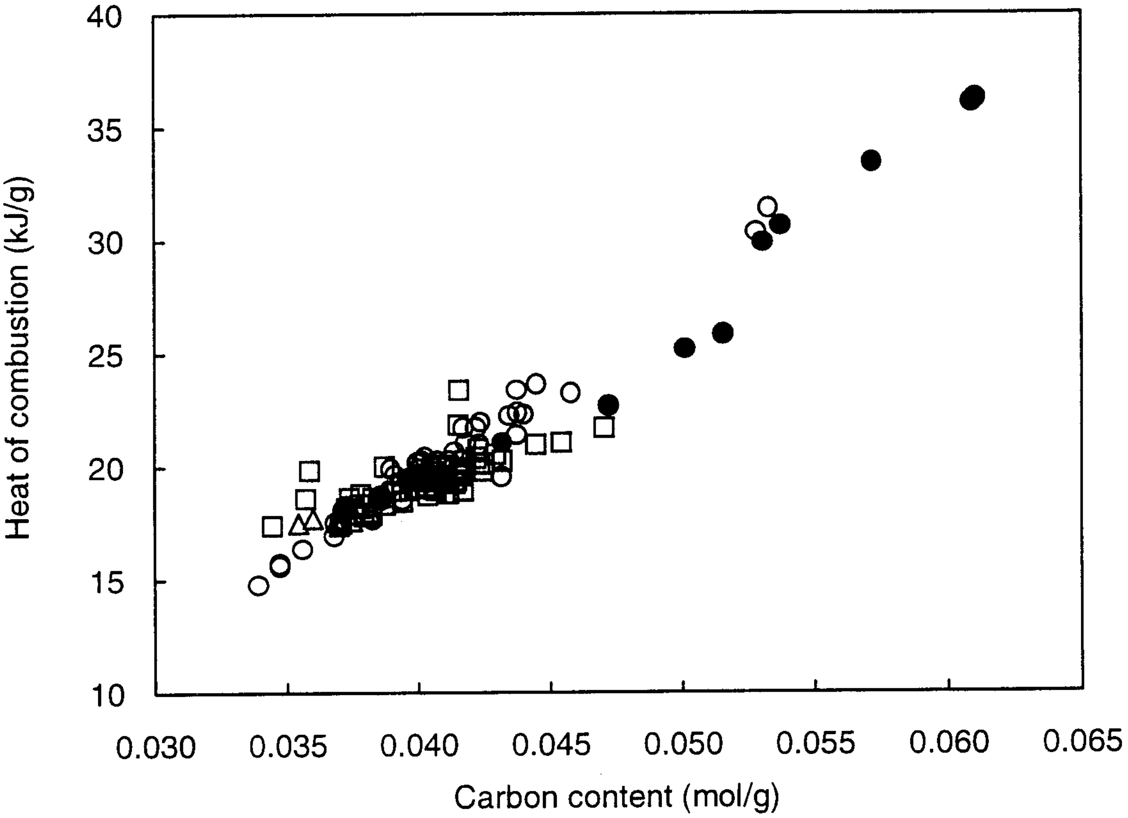

Fig 2. Heat of combustion and carbon content of various plant tissues (data sets 1: O, 2: □, 3: and 4: ●).

found a lower slope for the range of low carbon contents they observed on their own samples: 648 kJ mol^{-1}. In the field of fuel engineering, Tillman's equation (reported by Graboski and Bain, 1981) is used with a slope of 524 kJ mol^{-1}. In microbiology, Ho and Payne (1979) proposed a model that, after analysis, is equivalent to the same linear relationship between heat of combustion and carbon content with a slope of 539 kJ mol^{-1}.

It might be surprising that the heat of combustion is linearly correlated with both the carbon content and the degree of reduction. This can be explained considering that living matter is constituted of varying proportions of C=O (γ = 2 e^- C-mol^{-1} and c' = 1/28 mol C g^{-1}) and C-H (γ = 5 e^- C-mol^{-1} and c' = 1/13 mol C g^{-1}) bonds, indicating a rough proportionally between γ and c'. This simple rule theoretically allows us to replace the degree of reduction, calculated from the analysis of 3 elements (C, H and O) by the content in only 1 of these elements, carbon. However, when a range of organic compounds with various degrees of reduction is considered, it can be observed that a higher degree of reduction can be reached either with a lower oxygen content or with a higher hydrogen content (fig 3). For example, although the heat of combustion is lower for organic acids than for carbohydrates, their carbon content is not very different; the same comparison can be made between lipids and lignin (fig 4). In contrast, there is a large difference in oxygen content between carbohydrates and organic acids on the one hand, and lipids and lignin on the other. This difference explains the larger carbon content of the latter.

In plant tissues, which are mixtures of these various compounds, differences in the way the degree of reduction is found are buffered, but not completely. The variability of heats of combustion of tissues having the highest carbon content, reaches 20% around a mean value (fig 2). Tissues belonging to data sets 1 and 4 present high levels of lipids (Merino *et al*, 1984, and table III), while those belonging to set 2 are woody tissues with high levels of lignin (Graboski and Bain, 1981; Jenkins, 1989). Consequently, the relationship between heat of combustion and carbon content is not independent of the chemical composition of biomass. When working on a specific plant material, we suggest calibrating equation [8].

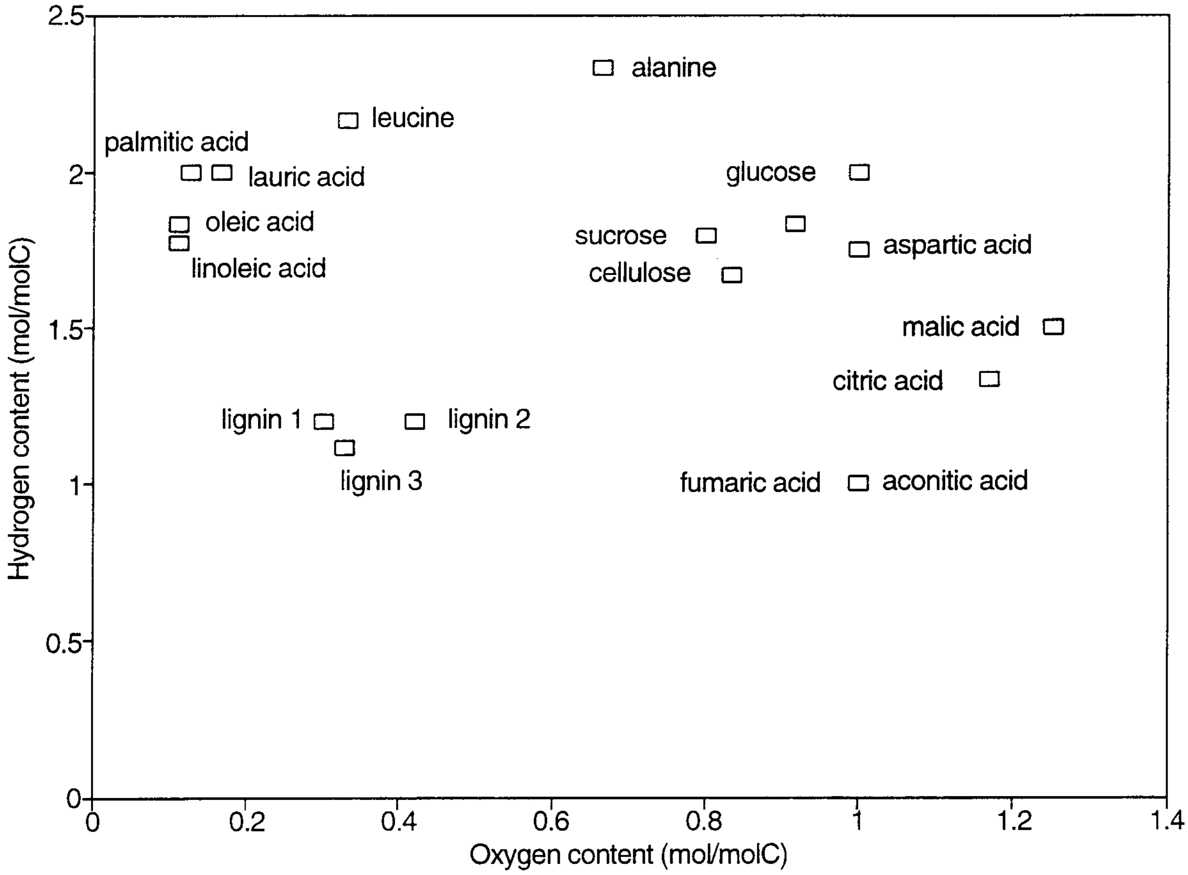

Fig 3. Hydrogen *vs* oxygen content of some common plant carbohydrates (glucose, sucrose and cellulose), amino acids (alanine, aspartic acid, glutamic acid and leucine), lipids (lauric acid, linoleic acid, oleic acid and palmitic acid), organic acids (aconitic acid, citric acid, fumaric acid and malic acid) and lignin. The elemental composition and heat of combustion of lignin varies with the source; 3 values were taken from Domalski *et al* (1987).

Table III. Heat of combustion and chemical composition of data set 4. Lipids were analysed only on kernels.

Harvest number	*Organ*	*Heat of combustion (kJ g^{-1} d.wt)*	*Carbon content (g g^{-1} d.wt)*	*Lipid content (g g^{-1} d.wt)*	*Carbohydrate content (g g^{-1} d.wt)*	*Ash content (g g^{-1} d.wt)*
1	Kernel	33.3	0.674	0.71	0.05	0.119
2	Kernel	33.0	0.670	0.70	0.05	0.124
3	Kernel	30.8	0.633	0.60	0.11	0.115
4	Kernel	28.4	0.600	0.47	0.17	0.104
5	Kernel	27.3	0.582	0.44	0.19	0.128
2–5	Tap root	17.3	0.423		0.22	0.165
2–3	Tap root	17.0	0.420		0.27	0.137
5	Tap root	17.0	0.419		0.26	0.090
2–5	Tap root	16.9	0.424		0.19	0.376
2–4	Stem	17.7	0.436		0.22	0.153
5	Stem	17.7	0.437		0.19	0.235
3–4	Leaves	19.0	0.455		0.14	0.364
5	Leaves	18.9	0.452		0.14	0.403

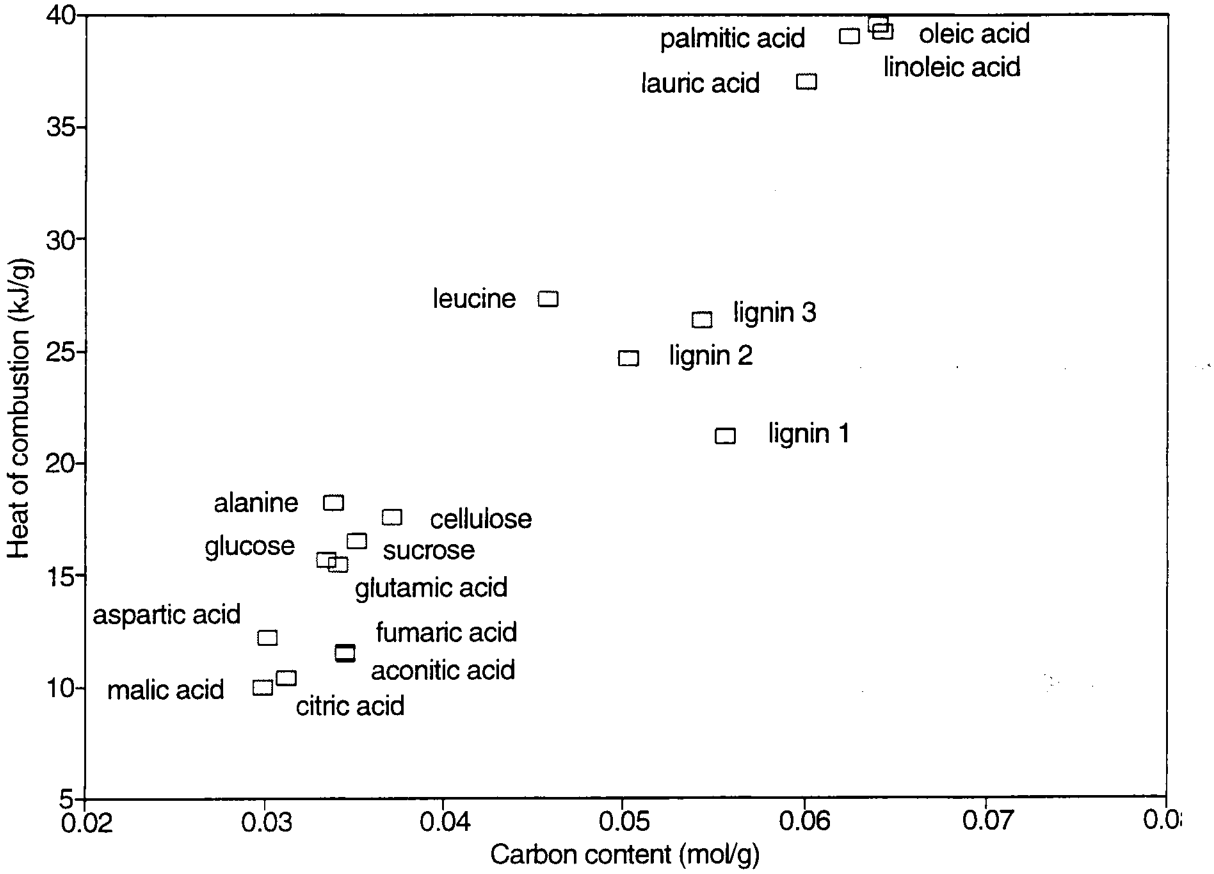

Fig 4. Heat of combustion (Kharasch, 1929) and carbon content of the same plant compounds as in figure 3.

Estimation of the construction cost of plant tissues

The heat of combustion, which can be measured or estimated from the degree of reduction (equation [5]), or the carbon content (equation [8]) can be used for the estimation of the construction cost. When the nitrogen source for growth is nitrate, the cost of reducing nitrate should be considered, as suggested by Williams *et al* (1987). This cost, C_N (in grams glucose per gram biomass), is calculated as a function of the nitrogen content of biomass:

$$C_N = kn'(180.15/24) \qquad [11]$$

where k is the degree of reduction of the nitrogen source, namely k = 5 for nitrate, 180.15 g mol^{-1} is the molar mass of glucose and 24 represents the number of moles of electrons provided by the oxidation of 1 mole glucose. The glucose equivalent, *ie* the total amount of glucose required to provide carbon skeletons, g_c, and reducing power, g_r, is then given by:

$$g_B = g_c + g_r = 0.0639\ \Delta H_c + 7.506\ k\ n' \qquad [12]$$

which is equation [2] corrected by equation [11]. Finally, the construction cost is calculated by using the biosynthetic efficiency:

$$C = g_B/E_B \qquad [13]$$

If E_B is replaced by E_G in equation [13], then $1/Y_G$ is calculated instead of C and transport cost is taken into account. Equations [5] or [8] and/or [12] together with equation [13] allow the calculation of the construction cost from any of the 3 methods (degree of reduction, carbon content and heat of combustion) compared in this paper. Note that all values are expressed here per gram organic biomass. There is no constant in equation [12], and so they can also be expressed per gram dry weight (organic biomass + ashes).

These 3 methods of estimating the construction cost of plant tissues were compared by Lafitte and Loomis (1988) and Walton *et al* (1990). The discrepancies they observed are of the same magnitude as the variability around the expressions [7] and [8] which we attributed to the chemical composition of the analysed tissues. Williams *et al* (1987) proposed a single mean linear relationship between heat of combustion and degree of reduction. For tissues rich in carbohydrates (kiwifruit berries in Walton *et al,* 1990), the use of this single relationship leads to an overestimation of the construction cost. For tissues richer in other compounds (Lafitte and Loomis, 1988), the same relationship may lead to an underestimation of the construction cost. Likewise, Walton *et al* (1990) suggested that the relationship between the inverse of glucose value (equivalent to g_B) and the carbon content published by Vertregt and Penning de Vries (1987) overestimated the construction cost of tissues rich in organic acids (Walton and de Jong, 1990). Note that biosynthetic efficiency itself is not independent of the composition of biomass; it is high for carbohydrates, organic acids (up to 0.95) and low for protein and lignin (down to 0.73; Williams *et al,* 1987). All these errors could be corrected by considering the chemical composition of the tissues, but it would greatly reduce the simplicity of the 3 methods examined in this paper.

Estimating the cost of producing new plant tissues from variables such as heat of combustion or elemental composition may seem crude in regard to the complexity of the biochemical processes involved. The major uncertainty lies in the estimation of the amount of substrate electrons that are not retained in the product. Biosynthetic efficiency (E_B) was calculated from theoretical values of the construction cost, on the basis of the biochemical pathways used to synthesize the various tissue compounds (McDermitt and Loomis, 1981; Williams *et al,* 1987). The respiratory coefficient (P/O ratio) was set to 3, but evidence for the existence of a wasteful respiratory pathway has been presented (Lambers and Rychter, 1989). However a change in the P/O ratio from 3 to 2 should not affect the construction cost more than by 5% (Penning de Vries *et al,* 1974). Transport cost is also estimated with a poor precision but again the growth yield is not very sensitive to it. Furthermore, the methods discussed in this paper have been designed and should be used for studies at the organ or whole plant level and over long time periods, when possible variations in the efficiency of energy production and use are likely to be buffered.

CONCLUSION

The heat of combustion, degree of reduction and carbon content are well correlated in plant tissues, and each of these variables may be used for the calculation of the construction cost of biomass. Heat of combustion is certainly the most direct way to estimate the construction cost, after division by the heat of combustion of glucose, correction for the possible cost of reduction of nitrogen and division by the biosynthetic efficiency. Going through a correlation between heat of combustion and glucose equivalent (Williams *et al,* 1987) and using the elemental composition for the calculation of the glucose equivalent (McDermitt and Loomis, 1981) may lead to errors theoretically limited to 8%. Carbon content measurements are fairly simple and precise but they should be combined with a correction for the cost of reducing nitrogen. The chemical composition of biomass affects (i) the relationship between heat of combustion and degree of reduction; (ii) the relationship between carbon content and heat of combustion or construction cost; and (iii) the biosynthetic efficiency. In this paper, taking into account this chemical composition helped understand discrepancies between various authors. Before using any method, preliminary studies on the composition of the plant tissues under analysis or calibration of relationships like equation [8] would enable the choice of proper parameters.

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