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Seasonal changes in chemical composition and construction costs of grapevine tissues

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Summary

Modelling of the whole-vine carbon balance requires accurate estimates of tissue construction costs, *i.e.* the amount of glucose involved in the synthesis of a unit of biomass. In order to quantify construction costs during the vine's growth cycle, chemical compositions of leaves, stems, fruits, fine roots and trunk of 10-year-old grapevines (cv. Merlot) were determined in two seasons. Tissue construction costs were estimated using (i) an approach based on the quantification of the amount of glucose required for the synthesis of major chemical components of vine organs by the most probable metabolic pathways (coded as CC_p) and (ii) a simpler technique in which costs were derived from tissue ash, carbon and nitrogen concentrations (coded as CC_w). Both methods were well-correlated in all grapevine tissues despite CC_p values were higher than CC_w estimates. Grapevine leaves had higher concentrations of compounds with a high proportion of C and N atoms (proteins, lipids and phenolics) and higher CC_w values throughout the season than other tissues. Small variation in CC_w values however were observed seasonally in vegetative tissues despite their chemical composition varied considerably with plant development. Significant changes in CC_w appeared in berry tissues between fruit set and maturity, reflecting a proportional increase in concentration of inexpensive metabolites (soluble sugars and organic acids).

K e y w o r d s : C:N ratio, chemical composition, construction cost, ontogeny, *Vitis vinifera*.

Introduction

Modelling of the whole-plant carbon balance is a useful research tool for understanding the processes involved in grapevine growth and yield and for developing viticultural management strategies (Guttierez *et al*. 1985, Wermelinger *et al*. 1991, Vivin *et al*. 2002). In such process-based models, part of the daily photoassimilated carbon is converted into an increased dry matter through a parameter *CC*, named 'construction cost' (for a complete list of parameters used in different methods of estimating the construction cost of biomass see Gary *et al*. 1995), which represents the amount of glucose used to provide the carbon skeletons, reducing power (NADH or equivalent) and chemical energy (ATP or equivalent) that are involved in the synthesis of a unit of biomass (Penning de Vries *et al*. 1974, Williams *et al*. 1987). Although it is widely accepted that *CC* is dependent on chemical composition of the synthesized plant material and will change with time if the composition of the tissue changes with ontogeny (Walton *et al*. 1990 a, Gary *et al*. 1998 b) or environmental conditions (Griffin *et al*. 1996, Poorter *et al*. 1997), plant modellers used single values of *CC* to calibrate their models. This simplification is questionable since any error in the estimation of *CC* has direct consequences on the calculation of the dry matter production from the daily carbon acquisition. Obviously, using seasonal values of construction costs for each class of tissues may enable a more accurate simulation of experimental data; but to our knowledge such reference information is not available for grapevine tissues.

Several methods have been developed for estimating the costs of plant growth (reviews by Walton *et al*. 1990 b, Griffin 1994, Gary *et al*. 1995). Some of these methods are easier to use than others, but there are also trade-offs in that each method is subject to some uncertainty due to the many assumptions and approximations that must be made (Wullschleger *et al*. 1997). The main method used in the present study to estimate tissue construction costs (coded CC_p) was based on the approach by Penning de Vries *et al*. (1974), revised by Poorter (1994), which consisted of quantifying the amount of glucose required for the synthesis of the major chemical components of the plant organs by the most probable metabolic pathways. These CC_p values were compared with a simpler technique in which estimates of growth costs (coded CC_w) were derived from tissue ash, carbon and nitrogen concentrations, and from assumptions on the energetic costs of nitrogen assimilation and carbohydrate translocation (Vertregt and Penning de Vries 1987, modified by Wullschleger *et al*. 1997).

Estimating the cost of producing new plant tissues from chemical compositions may seem crude in regard to the complexity of the biochemical processes involved, but constitutes crucial information to fully develop and parametrize source-sink relationhip-based models simulating the daily carbon supply and partitioning among vegetative and reproductive plant organs of individual grapevines throughout the growing cycle (Vivin *et al*. 2002); even if, as emphasized by Gary *et al*. (1998 b), it should be noted that the estimations of *CC* are based on analyses of tissues that are results of the integration of growth during a period of time, whereas models likely need at each time step a conversion factor for the increment of organic biomass. The objectives

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of this study were to obtain a complete picture of chemical compositions for grapevine tissues (leaves, shoots, fruits, fine roots and trunk) in order to quantify for, and detect possible differences in, its construction costs during the plant's growth cycle.

Material and Methods

Plant material and preparation of samples: In 1998 and 1999 data were collected on 10-year-old grapevines (*Vitis vinifera* L. cv. Merlot) grafted on Fercal and growing in the INRA Couhins experimental vineyard, near Bordeaux, France (for specific site information see RODRIGUEZ-LOVELLE *et al*. 2000, CASTELAN 2001). In both years, 5 annual shoots - one per vine - were sampled at 5 sampling dates from bud burst to grape maturity; they were separated into 5 tissues types: main stems, laterals, leaves on main stems, leaves on laterals, and fruits; fruits represent inflorescences (flowers + peduncles) before fruit set and whole bunches (berries + rachis) after fruit set. Additionally, samples of the trunk (rootstock and scion) and of fine roots were also taken periodically but only in 1999. All samples were dried at 80 °C for 48 h, weighed, and ground before analysis.

Determination of tissue chemical compositions: Tissue carbon and nitrogen concentrations (mg g^{-1} dry mass) were determined by combusting 3 mg aliquots in an elemental analyser (NA 2100, Carlo Erba, Milan, Italy). In order to estimate the chemical composition and, subsequently, construction costs, as defined by PENNING DE VRIES *et al*. (1974), tissue material was categorized in 6 different classes of constituents computed as follows. First, the amount of the total nitrogenous compounds, the 'protein fraction' (PRO), *i.e*. amino acids, proteins and nucleic acids were estimated by mutiplying total N by 6.25 (POORTER 1994). Nitrogen was included as total N (organic and mineral N, *i.e*. mainly nitrate-N); only the values for reduced, organic nitrogen should have been considered for calculating construction costs but the underestimation of this cost by including the values for mineral N is small (GARY *et al*. 1998 a). Ash (ASH) was determined by ashing 100 mg aliquots for 6 h in a muffle furnace at 550 °C and weighing the remaining residue. Subsequently, ash alkalinity was determined by NaOH titration after dissolution of the ashes in 0.1 N HCl. During the combustion process, NO_3^- and organic acids disappear, leaving an oxide, which reacts with CO_2 after cooling to form CO_2^{3-}. Therefore, the total mineral concentration (MIN) was calculated by substracting from the ash content a value (in g g^{-1}) equal to 30 times the ash alkalinity (in meq g^{-1}) to correct for the carbonate formed (POORTER and VILLAR 1997). The total organic acid concentration (OA) was estimated by multiplying ash alkalinity (in meq g^{-1}) by 62.1 g eq^{-1} which represents an average molecular weight per equivalent of all organic acids present in a vegetative tissue (POORTER and BERGKOTTE 1992). In berry tissue, the latter value was arbitrarily fixed to 70 g eq^{-1} to correct for the higher molecular weights per equivalent of tartaric and malic acids, which together may represent 90 % of the total organic acids pool (ILAND and COOMBE 1988, DIAKOU *et al*. 1997). It is also assumed that from fruit set to maturity, the ash alkalinity measured in berries only represent half of the total organic acids pool, due to partial salt formation, of the organic acids at the berry pH (USSEGLIO-TOMASSET 1995); however possible variations in the salt formation process during berry development were not taken into account for the estimation of OA concentration. In addition, a third aliquot (200 mg) was extracted with chloroform, methanol and water in a ratio of 5:12:3 (v/v/v) ; the residue left after drying off the chloroform phase, which largely contains phospholipids and galactolipids as well as some sterols, was determined gravimetrically and termed lipids (LIP). Finally, two classes of constituents representing total soluble and insoluble carbohydrates (SUG) and soluble phenolics (PHE) were estimated by difference assuming a 100 % recovery of the compounds present in a tissue, using the following two equations:

$$SUG + PHE = 1000 - (PRO + LIP + OA + MIN) \quad (1)$$

$$0.42\,SUG + 0.64\,PHE = C - (0.53\,PRO - 0.68\,LIP - 0.36\,OA) \quad (2)$$

where the constants are the theoretical mean carbon content of carbohydrates (0.42), phenolics (0.64), proteins (0.53), lipids (0.68) and organic acids (0.36) while C represents the mean carbon content of the sample. The concentrations of these compounds are expressed as mg g^{-1} dry mass.

Construction costs: The cost of synthesizing plant tissue was first calculated according to PENNING DE VRIES (1974, revised by POORTER 1994), by multiplying the concentrations of each of the 6 classes of chemical compounds, obtained from equation 1 and 2, by the respective specific glucose requirement coefficients shown in Tab. 1. The protein class was calculated by assuming that nitrogen is only assimilated as NO_3^- although grapevine roots take up N as NH_4^+ as well (ROUBELAKIS-ANGELAKIS and KLIEWER 1991). The sum of the 6 products (concentration of compound class x specific construction cost), expressed in g glucose g^{-1} dry mass, gave the value of total construction costs, coded CC_p. The total amount of glucose required to construct one gram of dry mass was also estimated by a second, simple method resulting in a value coded CC_w. Here, the concentrations (g g^{-1} dry mass) of tissue ash (ASH), carbon (C) and nitrogen (N) enter into equation 3, based on

Table 1

Mean specific construction cost (g glucose g^{-1} dry weight) for major chemical fractions. The values for lipids, total carbohydrates, phenolics, and organic acids as reported by POORTER and VILLAR (1997). For nitrogenous compounds (amino acids, proteins, and nucleic acids), the value is based on the assumption that all nitrogen is assimilated exclusively from NO_3^- (POORTER 1994)

Chemical fraction	Cost (g glucose g^{-1})
Nitrogenous compounds	2.48
Phenolics	2.60
Lipids	3.03
Carbohydrates	1.17
Organic Acids	0.91
Minerals	0

the assumptions made for the energetic costs of nitrogen assimilation and carbohydrate translocation (VERTREGT and PENNING DE VRIES 1987, modified by WULLSCHLEGER *et al*. 1997):

$$CC_w = (5.39\,C + 0.80\,ASH + 5.64\,f_{N,h}\,N - 1.191)\,(1 + r_T) \quad (3)$$

For this equation, $f_{N,h}$, the fraction of nitrogen used in growth that is assimilated heterotrophically (unitless, 0-1), was set at 0.5 for leaves (*i.e*. half of nitrate is reduced heterotrophically) and at 1.0 for trunk, stems, fruit and roots (WULLSCHLEGER *et al*. 1992). The value of r_T, the added cost of translocating photosynthates from sources to sinks, was set for all tissues at 5.3 % (VERTREGT and PENNING DE VRIES 1987). Note that only an apparent construction cost was estimated from both methods at the time of harvest of each plant; the composition of a tissue is the result of the integration of growth during a period of time and can partly be the result of the remobilization of some compounds in other organs (VALANTIN *et al*. 1999).

Statistical analysis: All statistical analyses were performed on SYSTAT 5.1 software. Means are given with standard error; values were significantly different if $P \leq 0.05$. Time of sampling was expressed both as day of the year (DOY) and as cumulative degree-days above 10 °C (calculated from January 1 of each year).

Table 2

Mean carbon, nitrogen, and ash concentrations (mg g^{-1}), standard error of the mean (n=2) for various grape tissues sampled throughout 1998. DOY = day of the year. Cumulative degree-days (Ddays, base 10 °C) were calculated from January 1, 1998

Tissue	DOY	Ddays	C	N	Ash
	139	282	437 (1)	30.6 (1.7)	60 (4)
Main	156	414	442 (3)	33.4 (0.9)	57 (28)
Leaves	166	483	441 (2)	28.9 (0.6)	68 (9)
	180	614	442 (4)	25.6 (0.4)	82 (5)
	271	1547	438 (6)	13.2 (0.7)	124 (11)
Lateral	139	282	458 (1)	39.8 (2.6)	55 (12)
Leaves	156	414	456 (2)	40.1 (0.9)	56 (7)
	166	483	451 (0)	34.5 (1.2)	52 (9)
	139	282	421 (17)	11.1 (0.7)	63 (18)
Main	156	414	412 (11)	10.9 (0.1)	63 (8)
Stems	166	483	418 (7)	9.4 (1.5)	63 (18)
	180	614	430 (8)	5.7 (0.3)	52 (9)
	271	1547	456 (3)	4.0 (0.3)	29 (4)
	156	414	442 (-)	32.7 (-)	62 (-)
Lateral	166	483	434 (2)	27.7 (2.4)	62 (15)
Stems	180	614	440 (5)	25.7 (0.4)	70 (7)
	271	1547	437 (1)	13.7 (0.8)	107 (11)
	139	282	442 (1)	28.8 (1.7)	58 (3)
	156	414	443 (3)	26.8 (0.7)	66 (6)
Fruit	166	483	445 (2)	25.1 (2.2)	67 (9)
	180	614	439 (8)	12.7 (1.1)	49 (1)
	271	1547	406 (0)	2.6 (0.3)	19 (1)

Results and Discussion

Chemical composition: As reported in the literature (WILLIAMS 1987, WERMELINGER and KOBLET 1990, CONRADIE 1992, KELLER *et al*. 1995), C and N concentrations of grapevine tissues varied with time, reflecting changes in the chemical composition of organs during plant development. In most of the growing season, nitrogen concentrations of the leaves greatly exceeded that of the stems (Tabs 2 and 3), and lateral leaves and stems had significantly higher N concentrations than main leaves and stems in 1998

Table 3

Mean carbon, nitrogen, and ash concentrations (mg g^{-1}), standard error of the mean (n=5) for various grape tissues sampled throughout 1999. (*) Values for trunk are the means (n = 2) of samples from scion and rootstock. For details see Tab. 2

Tissue	DOY	Ddays	C	N	Ash
	132	244	464 (2)	34.4 (0.7)	50 (3)
Main	145	433	490 (2)	34.9 (1.3)	-
Leaves	187	720	461 (2)	23.7 (1.4)	77 (4)
	221	1133	496 (2)	24.0 (0.5)	-
	249	1448	493 (2)	21.7 (0.6)	84 (2)
	145	433	480 (3)	34.8 (1.6)	-
Lateral	187	720	457 (3)	25.5 (0.8)	66 (2)
Leaves	221	1133	487 (2)	26.1 (0.4)	-
	249	1448	483 (1)	25.0 (0.7)	80 (6)
	132	244	426 (3)	15.1 (0.3)	61 (4)
Main	145	433	465 (3)	11.1 (0.5)	-
Stems	187	720	458 (3)	4.4 (0.1)	37 (1)
	221	1133	471 (5)	3.5 (0.1)	-
	249	1448	478 (1)	3.7 (0.2)	46 (1)
	145	433	441 (4)	17.4 (1.1)	-
Lateral	187	720	431 (5)	6.8 (0.3)	72 (6)
Stems	221	1133	458 (3)	5.6 (0.3)	-
	249	1448	442 (4)	5.0 (0.1)	95 (5)
	132	244	456 (2)	29.5 (0.7)	51 (3)
	145	433	480 (3)	24.2 (1.2)	-
Fruit	187	720	458 (1)	11.3 (0.3)	38 (3)
	221	1133	484 (4)	6.7 (0.4)	-
	249	1448	425 (2)	2.8 (0.1)	21 (1)
	159	450	453 (4)	11.3 (2.7)	-
	190	753	452 (2)	7.3 (3.1)	40 (8)
Fine	221	1133	460 (3)	7.1 (1.5)	-
Roots	251	1469	455 (9)	7.1 (2.5)	48 (5)
	278	1688	467 (6)	5.1 (0.5)	-
	309	1819	476 (3)	6.4 (0.9)	44 (3)
	96	92	460 (3)	3.7 (0.7)	13 (5)
Trunk (*)	218	1099	460 (1)	3.1 (0.5)	9 (5)
	342	1824	477 (3)	2.4 (0.5)	12 (3)

and to a lesser extend in 1999. Among the annual tissues, the N-concentrations in fine roots were similar throughout the sampling periods (on average 7.4 mg g^{-1}), while they were at high levels during early growth of leaves and stems (>29 mg g^{-1} in leaves and >10 mg g^{-1} in stems); they sharply decreased after bloom (around 400 degree-days), possibly due to the onset of N retranslocation to permanent woody organs. This seasonal pattern confirms that grapevine stems and leaves have a high N turnover (*e.g.* 40 % of the N may be recycled in senescent leaves according to WERMELINGER 1991), and that they seem to act as intermediate N reservoirs between roots and fruits (CONRADIE 1992). Furthermore, N concentrations were high and stable in inflorescences (on average >24 mg g^{-1}), but also dropped rapidly after bloom to reach a minimum value of 3 mg g^{-1} in mature berries suggesting that N import did not keep up with fruit growth. The seasonal trends observed in fruit tissue were similar in 1998 and 1999 (Tabs 2 and 3). Unlike N, total carbon concentrations were stable with time in most of the vegetative parts (Tabs 2 and 3); only main stems and the trunk accumulated more carbon per unit dry mass at the end of the growing season. Carbon concentrations in fruit tissue were also stable until fruit set (on average 443 mg g^{-1} in 1998 and 456 mg g^{-1} in 1999), but between fruit set and maturity decreased by about 10 % in both years. Furthermore, all grapevine tissues had significantly higher C contents in 1999 than in 1998. Finally, the C:N ratios increased dramatically in each growing season in all above-ground grapevine parts, mainly in fruit tissue where mean values were 10 times higher at maturity than at flowering in inflorescences. With mean values ranging from 11 to 33, leaves had the lowest C:N ratio among all grapevine organs (Fig. 1); generally expanding leaves had a lower C:N ratio than fine roots because of their higher N concentrations while roots had greater C losses (WERMELINGER 1991).

Chemical composition varied between organs and during the vegetation period. At each sampling date, leaves of the main shoots contained relatively more proteins, lipids and minerals per unit dry mass than stems, grapes, roots, and trunks, but had significantly less proteins and organic acids per unit dry mass than leaves on laterals (Fig. 2, Tab. 4). In 1999 more lipids and phenolics, and less carbohydrates and minerals per unit dry mass were found in main vegetative tissues compared to 1998. Furthermore, in both main leaves and stems, the concentrations of proteins and total carbohydrates decreased over time whereas those of the phenolic fractions increased strongly (Fig. 2). The concentrations of lipids, organic acids and minerals increased over time only in main leaves but not in main stems. At flowering, in reproductive tissues the major compounds were proteins and total carbohydrates, representing both more than 80 % of the inflorescence dry mass (Fig. 2). Later, the concentration of total carbohydrates increased dramatically in berries partly diluting fractions of others compounds - *e.g.* organic acids (RUFFNER 1982) and N-containing compounds; the concentration of the latter decreased by a factor of 10 from flowering to fruit maturity. Finally, in 1999 fruit tissues had also significantly higher amounts of lipids than in 1998, but, unlike vegetative main tissues, no significant year effect was observed for the total content of carbohydrates (Fig. 2).

Construction costs: Biochemical pathway analysis often gives higher estimates than other analytical techniques (WILLIAMS *et al.* 1987, GRIFFIN 1994, POORTER 1994); a general tendency indicated that construction costs (CC_w) were lower than those calculated as CC_p mainly for leaves and to some extent for other tissues (Fig. 3). Despite these quantitative differences, construction costs estimated by the two methods were closely correlated in all grapevine tissues ($r^2 = 0.87$, $P < 0.001$ in 1998 and $r^2 = 0.96$, $P < 0.001$ in 1999). The method adapted from PENNING DE VRIES *et al.* (1974) provides, of course, most information about factors causing changes in growth costs over time; but the time consuming, detailed chemical analysis and the potential errors, given the crudeness of the methods and the number of

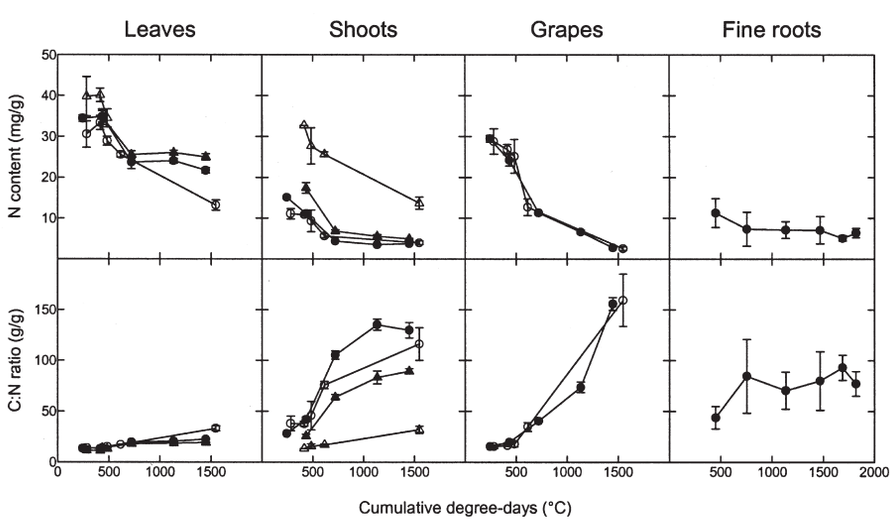

Fig. 1: Seasonal changes of mean N concentration and C:N ratio for various tissues of cv. Merlot sampled throughout 1998 (open symbols, n=2) and 1999 (closed symbols, n=5). Circles are for main, triangles for lateral tissues. Cumulative degree-days were calculated from January 1 of each year. Bars represent standard errors.

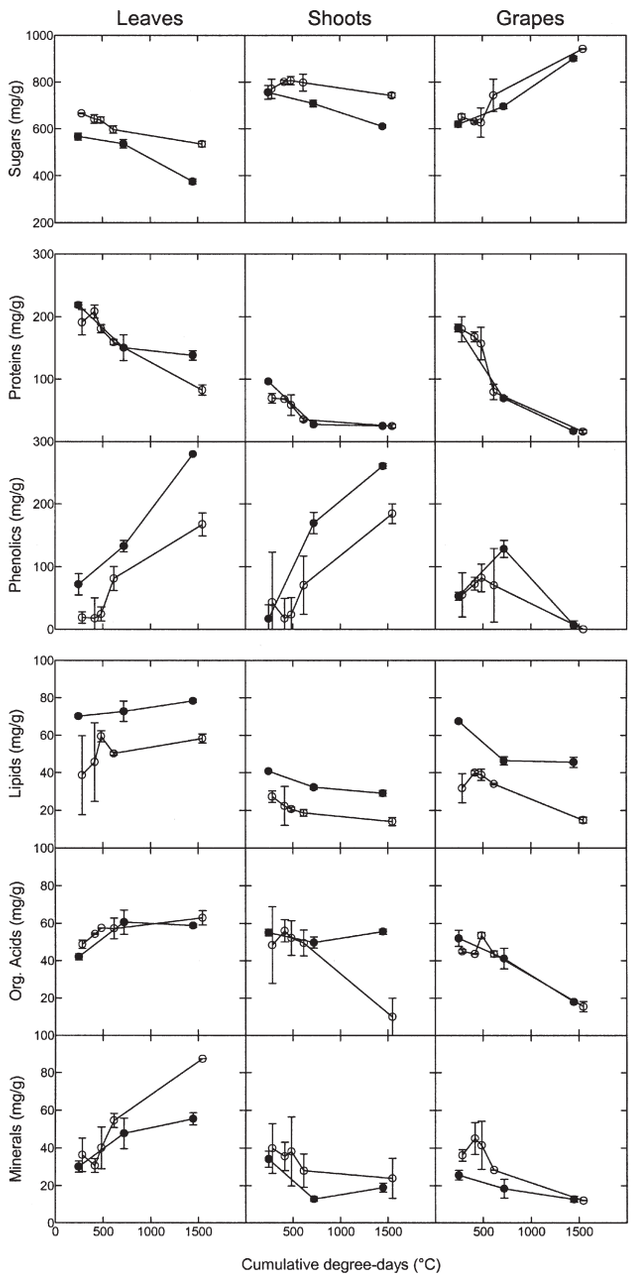

Fig. 2: Seasonal changes of mean chemical composition for main leaves, main stems and fruit tissues. For details see Fig. 1.

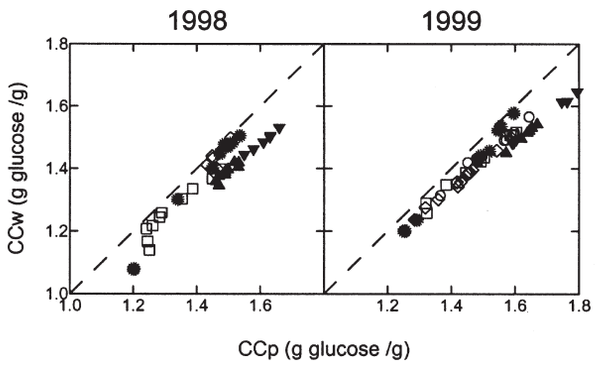

Fig. 3: Relationships between construction costs calculated as CC_w and CC_p in various tissues sampled throughout 1998 or 1999. For definition of CC_w and CC_p see text. ▲, main leaves; ▼, lateral leaves; ❑, main stems; ◇, lateral stems; ✹, fruit; ❍, fine roots.

replicates required, are rather disadvantageous (WALTON *et al*1990 b, POORTER 1994). The revised short-cut method of VERTREGT and PENNING DE VRIES (1987) which originally required information on only carbon and ash concentrations to estimate CC_p, therefore appears to be a more simple alternative approach to give estimates on costs of grapevine growth. Nevertheless the reliability of assumptions regarding heterotrophic nitrogen assimilation ($f_{N,h}$) and added translocation costs (r_T) as proposed by WULLSCHLEGER *et al* (1997), still needs to be tested for the present grapevine tissues.

Grapevine leaves had higher amounts of C and N than other tissues, due to their higher concentrations of compounds with a high proportion of both atoms (proteins, lipids and phenolics). Since the production of these compounds is comparatively 'expensive', leaf tissues as a whole had higher apparent specific construction costs throughout the season compared with other tissues (Fig. 4). The mean annual costs of main leaves synthesis calculated as CC_w (1.43 ± 0.01 and 1.56 ± 0.02 g glucose g^{-1} dry mass in 1998 and 1999, respectively) were appreciably higher than those calculated for kiwi leaves (WALTON and FOLKE 1995) but not dissimilar with those calculated for apple leaves (1.44 g glucose g^{-1} dry mass, WALTON *et al*. 1999).

Small seasonal variations in the apparent amount of glucose needed to construct 1 g of biomass were observed in all vegetative grapevine tissues (Fig. 4), although their chemical composition varied considerably with plant development; a common feature reported previously on several herbaceous and woody species (POORTER and BERGHOTTE 1992, POORTER and VILLAR 1997, VILLAR and MERINO 2001, MARTINEZ *et al.* 2002) and mostly explained by positive correlation between 'expensive' and 'cheap' compounds (*e.g.* lipids and mineral/organic acids) or negative correlation between various 'expensive' ones (*e.g.* proteins and lipids/phenolics) (Fig 5). Indeed, main stems cause significantly higher specific costs of synthesis at the last sampling dates, likely due to lignification of the shoots when berries ripen (PRATT 1974). Similar ontogenetic increases in costs of synthesis were measured in stems, and to a lesser extent, in leaves of kiwifruits (WALTON and FOWKE 1995), but not in leaves of tomato (GARY *et al*. 1998 b) and chaparral shrub (MERINO *et al*1984). In the two latter experiments, decreases in *CC* during development were explained by an accumulation of minerals and especially by a shift from the synthesis of costly compounds (lipids) to the synthesis of 'cheap' structural compounds (cellulose and hemicellulose).

The results show that the costs of synthesis in fruit tissues significantly change during their development, inflorescences having higher apparent construction costs than mature berries (Fig 4). The decline in CC_w observed when berries ripen, is likely to reflect the proportinal decline in N-containing compounds (proteins, amino acids, nitrate) and lipid concentrations accompanied with a proportional increase in the 'inexpensive' metabolites concentrations (soluble sugars) (Figs 4 and 5). The end-of-season costs for syntheses in berries calculated from elemental analysis (1.10 and 1.12 g glucose g^{-1} dry weight in 1998 and 1999, respectively) were similar to those estimated for kiwifruits (1.21 g gluc-

Table 4

Mean chemical concentrations (mg g^{-1}), standard error of the mean (n = 2 to 5) for various grape tissues sampled throughout 1998 and 1999. For details see Tab. 2 resp. Material and Methods

Tissue	Year	Ddays	PRO	LIP	MIN	OA	SUG	PHE
Lateral	1998	282	249 (16)	50 (0)	36 (2)	38 (12)	559 (38)	68 (7)
Leaves		414	251 (6)	54 (3)	39 (4)	35 (2)	561 (11)	60 (2)
		483	216 (7)	41 (1)	32 (7)	41 (0)	613 (22)	58 (10)
	1999	720	160 (5)	73 (3)	34 (2)	67 (1)	570 (4)	92 (7)
		1448	163 (3)	73 (2)	50 (7)	61 (3)	427 (16)	231 (12)
Lateral	1998	414	204 (-)	56 (-)	37 (-)	50 (-)	637 (-)	15 (-)
Stems		483	173 (15)	49 (4)	35 (10)	56 (1)	682 (27)	5 (5)
		614	160 (2)	46 (0)	40 (5)	61 (1)	644 (7)	48 (14)
		1547	86 (5)	57 (1)	71 (6)	74 (3)	592 (19)	121 (6)
	1999	720	43 (2)	32 (1)	41 (5)	63 (3)	736 (24)	83 (26)
		1448	31 (1)	32 (2)	58 (6)	75 (3)	649 (21)	154 (18)
Fine	1999	753	46 (20)	24 (4)	18 (6)	45 (3)	727 (9)	140 (21)
Roots		1469	44 (16)	22 (1)	24 (2)	50 (5)	689 (32)	171 (41)
		1819	40 (6)	22 (1)	21 (2)	47 (3)	606 (22)	264 (12)
Trunk	1999	92	24 (4)	19 (0)	7 (4)	14 (3)	773 (5)	163 (5)
		1099	20 (3)	21 (0)	4 (4)	11 (2)	785 (12)	160 (3)
		1824	15 (3)	21 (1)	5 (1)	14 (4)	703 (21)	242 (13)

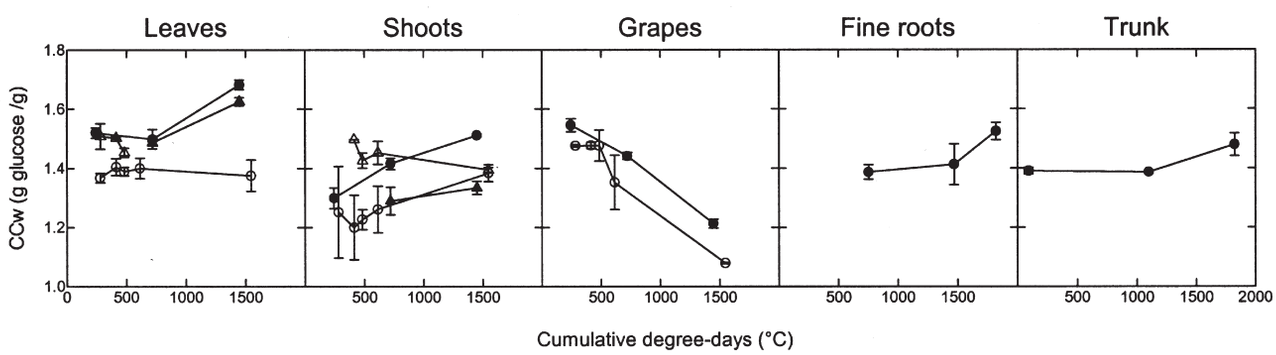

Fig. 4: Seasonal changes of mean construction costs (CC_w) for various tissues. For details see Fig. 1.

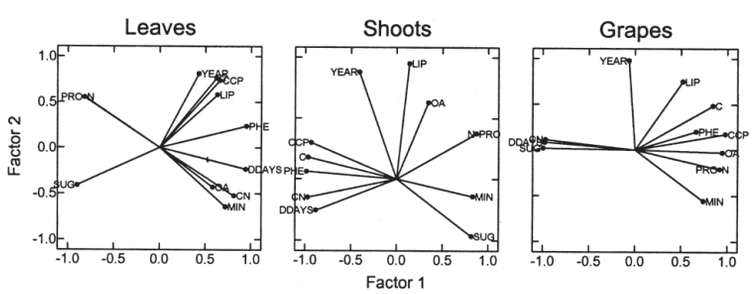

Fig. 5: Principal component analysis of data on the chemical composition for various tissues sampled throughout 1998 and 1999. The two factors explained 87-91% of the total variation. For explanation of abbreviations see Material and Methods.

ose g^{-1}; WALTON *et al.* 1990 a), tomato (1.15 g glucose g^{-1}; GARY *et al.* 1998 b), and apple (1.16 g glucose g^{-1}; WALTON *etal* . 1999). They were lower however than those for cantaloupe (1.31 g glucose g^{-1}, VALANTIN *et al.* 1999) where *CC* were larger at harvest due to higher concentration of lipids in seeds (about 40 % of the seed dry mass). WALTON *et al.* (1990 a) carried out similar experiments on kiwi fruit and showed that *CC* decreased when organic acids accumu-

lated and then increased during seed formation and the synthesis of their lipid reserves. Grape seeds contain relative high concentrations of lipids (5-20 % of the seed dry mass) and phenolics depending on the variety and the stage of development (Miele *et al*. 1993); however, they are not a large fraction of the whole grape dry mass. In the present study, preliminary results on potted Cabernet Sauvignon fruiting cuttings revealed that CC_p calculated from chemical analyses were higher at fruit maturity in the berry seeds (1.97 g glucose g^{-1}) than in the berry pulp (1.24 g glucose g^{-1}) and the grape peduncle (1.43 g glucose g^{-1}) due to higher levels of N-containing compounds, lipids and phenolic compounds in the seeds (Castelan 2001). Further analyses are still necessary to address a complete seasonal picture of the relative contribution of each berry tissue (skin, pulp and seeds) to the whole grape contruction costs in vineyard.

Conclusions

In this study the chemical compositions of various tissues of *Vitis vinifera* cv. Merlot were determined and herewith the mass-specific construction costs. This approach, to our knowledge, is original for grapevine. Grapevine leaves had higher construction costs than other tissues due to their higher relative contribution of 'expensive' compounds such as lipids, soluble phenolics, or proteins. Although their chemical composition varied considerably with plant development, small variation in the apparent amount of glucose necessary to construct 1 g of biomass was observed in vegetative tissues throughout the growing cycle; this is probably due to the pattern of covariation between the various classes of constituents. On the other hand, significant developmental changes in CC_w clearly appeared in reproductive tissues between flowering and fruit maturity. In the grapevine modelling perspective, the present results stress the fact that mass-based construction costs are tissue specific and at least for berry tissues might fluctuate throughout the growing season. Further analyses are still needed to explore the sensibility of *CC* values to various environmental conditions or grapevine varieties.

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