



The respiratory energy requirements involved in nocturnal carbohydrate export from starch-storing mature source leaves and their contribution to leaf dark respiration

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Abstract

The present study explores the potential contribution of the energy requirements associated with nocturnal carbohydrate export to (1) the fraction of dark respiration correlating with leaf nitrogen concentration and (2) the dark respiration of mature source leaves. To this end, we determined the nocturnal carbohydrate-export rates from leaves with an optimal nitrogen supply, and the correlation between the nitrogen concentration and the dark respiration of leaves. The specific energy costs of carbohydrate export from starch-storing source leaves were determined both experimentally and theoretically. The present estimate of the specific energy cost involved in carbohydrate export as obtained by linear regression ($0.70 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$), agrees well with both literature data obtained by different methods (0.47 to $1.26 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$) and the theoretically calculated range for starch-storing species (0.40 to $1.20 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$). The conversion of starch in the chloroplast to sucrose in the cytosol is a major energy-requiring process. Maximally 42 to 107% of the slope of the relationship between respiration rate and organic nitrogen concentration of primary bean leaves, may be ascribed to the energy costs associated with nocturnal export of carbohydrates. Total energy costs associated with export were derived from the product of the specific costs of carbo-

hydrate export and the export rates, either measured on full-grown (primary) leaves of potato and bean or derived from the literature. These export costs account, on average, for 29% of the dark respiration rate in starch-storing species. We conclude that nocturnal carbohydrate export is a major energy-requiring process in starch-storing species.

Key words: Carbohydrate export, leaf dark respiration, nitrogen concentration, respiratory costs, specific energy cost.

Introduction

The rate of dark respiration of leaves is often positively correlated to their nitrogen concentration (De Visser *et al.*, 1992; Makino and Osmond, 1991; Pons *et al.*, 1989; Van der Werf *et al.*, 1994). Lambers *et al.* (1989) hypothesized that this relationship is associated with energy costs for (i) export of carbohydrates and (ii) protein turnover. In starch-storing species, nocturnal carbohydrate export involves the transformation of starch in the chloroplast to sucrose in the cytosol and the subsequent phloem loading of this sucrose. Phloem loading of sucrose is an active process (Gardner and Peel, 1972; Geiger, 1975, and references therein). Protein turnover involves the synthesis of new proteins not resulting in an increase

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of the protein concentration. De Visser *et al.* (1992) calculated that protein turnover can account for the relationship between dark respiration and nitrogen concentration of leaves. However, there are reasons to hypothesize that, in addition to protein turnover, the energy cost involved in the export of carbohydrate also contributes to this relationship. Firstly, the costs of protein turnover may vary, in proportion to the degradation constants, by a factor of 20 (Bouma *et al.*, 1994; De Visser *et al.*, 1992). Secondly, photosynthesis and thus carbohydrate availability increase with the nitrogen concentration of the leaf, due to a higher photosynthetic capacity (Evans, 1983, 1989; Makino and Osmond, 1991; Nátr, 1975). If the energy costs involved in the export of carbohydrate do not affect the relationship between dark respiration and nitrogen concentration of leaves, these costs might still contribute significantly to the overall dark respiration of leaves. For example, the positive correlation between the rate of leaf dark respiration and the level of irradiation during the preceding photoperiod (Irving and Silsbury, 1988; Mullen and Koller, 1988) has been ascribed to the energy costs for the export of carbohydrates (Irving and Silsbury, 1988).

The aim of the present study is to assess the potential contribution of the energy costs of nocturnal carbohydrate export from mature source leaves to the fraction of dark respiration correlating with leaf nitrogen concentration and to the overall dark respiration rate of leaves grown with an optimal nitrogen supply. To this end, we determined (1) the relationships between nitrogen concentration of leaves and both leaf dark respiration and photosynthesis, (2) nocturnal carbohydrate-export rates from leaves with an optimal nitrogen supply, and (3) the specific costs of carbohydrate export.

Materials and methods

Plant material and growth conditions

Experiments were carried out with youngest full-grown leaves of potato (*Solanum tuberosum* L. cv. Alcmaria) and primary

leaves of bean (*Phaseolus vulgaris* L. cv. Berna). Potato plants were propagated by *in vitro* culture (Bouma *et al.*, 1992) and bean plants were grown from seeds (Bouma *et al.*, 1994). Growth conditions and objectives of the individual experiments are summarized in Table 2. Plants were grown in a nutrient solution containing macro-nutrients at 1/2 strength according to Hoagland and Snyder (1933) and micro-nutrients at 1/2 strength according to Lewis and Powers (1941) with iron as Fe(III)-EDTA.

The correlation of photosynthesis and dark respiration with leaf-nitrogen concentration (α)

Table 1 gives a list of the abbreviations used in the text with their corresponding units. The effect of the leaf organic nitrogen concentration on dark respiration (α , nmol CO₂ (mmol organic nitrogen)⁻¹ s⁻¹) was described by a linear model:

$$R_d = \alpha \times N_{\text{org}} + R_r \quad (1)$$

where R_d is the dark respiration (nmol CO₂ m⁻² s⁻¹), N_{org} is organic nitrogen concentration (mmol organic nitrogen m⁻²) and R_r is a constant rest component of respiration (nmol CO₂ m⁻² s⁻¹). The relationship between photosynthesis and nitrogen concentration was described by a similar equation. Respiration and photosynthesis rates were measured under growth conditions (Table 2), on primary leaves of 17–20-(experiments b1 and b2) and 21–24-(experiment b3) d-old bean plants. A range of leaf-nitrogen concentrations was obtained by daily supply of nitrogen at different relative nitrate addition rates (RAR) to aerated N-free nutrient solution ($RAR=0.05, 0.085, 0.115, \text{ and } 0.15 \text{ d}^{-1}$; after Ingestad, 1979). In this solution, calcium and potassium were available at half the normal strength, as CaCl₂ and KH₂PO₄ or KCl, respectively. Nitrate was added as Ca(NO₃)₂ and KNO₃ in the ratio present in the standard nutrient solution.

Experimental estimation of the specific energy costs for carbohydrate export (E_e)

The specific costs for carbohydrate export (E_e ; mol CO₂ [mol sucrose]⁻¹) were estimated by analysing the linear regression of dark respiration rate (R_d ; nmol CO₂ m⁻² s⁻¹) on the nocturnal carbohydrate export rate (C_e ; nmol sucrose m⁻² s⁻¹):

$$R_d = E_e \times C_e + R_r \quad (2)$$

where R_r is a constant rest component of respiration (nmol CO₂ m⁻² s⁻¹). Respiration was measured on full-grown potato leaves with different carbohydrate export rates, assuming

Table 1. List of abbreviations

If indicated in the text that the values are expressed on a dry weight basis, the m⁻² should be substituted by (g dw)⁻¹ in the units given below.

Abbreviation	Description	Unit
α	Slope of the relationship of respiration versus organic nitrogen concentration	nmol CO ₂ (mmol N _{org}) ⁻¹ s ⁻¹
a_e	Fraction of α explained by carbohydrate export only	—
C_e	(Observed) carbohydrate export rate	nmol sucrose m ⁻² s ⁻¹
E_e	Specific energy costs for carbohydrate export	mol CO ₂ (mol sucrose) ⁻¹ or, mol ATP (mol sucrose) ⁻¹
N_{org}	Organic nitrogen concentration	mmol m ⁻²
ΔN_{org}	Range of organic nitrogen concentrations underlying α	mmol m ⁻²
P	Photosynthesis rate	nmol CO ₂ m ⁻² s ⁻¹
RAR	Relative addition rate	d ⁻¹
R_d	(Observed) dark respiration rate	nmol CO ₂ m ⁻² s ⁻¹
R_e	Dark respiration rate associated with carbohydrate export	nmol CO ₂ m ⁻² s ⁻¹
R_r	Constant rest component of respiration	nmol CO ₂ m ⁻² s ⁻¹

Table 2. The parameters derived and growth conditions in the present experiments

For potato, the youngest full-grown (YFG) leaves were used. The abbreviations used for the different light types are: d=natural daylight in the greenhouse, F & I=fluorescent light (Philips TLD36W/54) and incandescent light (Pope 60 W) in a ratio of 12:1, H=HPI light (Philips HPI/T 400 W). The relative humidity was 70–75%.

Parameters	Experiment	Species	Leaf age (d)	Light			Temperature (°C)
				Period (h)	PPFD ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Type	
α	b1 and b2	Bean	17–20	16	500	H	21
α	b3	Bean	21–24	16	500	H	21
E_e, C_e	P1	Potato	YFG	14 or 9		d & H	20
C_e	P0	Potato	YFG	12 or 18	240	F & I	18
C_e	b4	Bean	14–17	16	240	F & I	21
				16	500	H	21
C_e	b5	Bean	17–20	16	500	H	23

constant costs for other processes. Carbohydrate export was varied by changing the root temperature (i.e. changing the sink activity; cf. Verkleij and Challa [1988] and references therein). In a preliminary experiment (P0; Table 2), root temperature and leaf respiration rate were correlated, but E_e could not be estimated as the variation in carbohydrate concentration between potato leaves of a single plant was larger than the decrease due to carbohydrate export. In the next experiment (P1), nocturnal export rates were derived as the decrease in specific dry weight of samples of the youngest full-grown leaves on plants with root temperatures changed from 7.5 to 20 °C and vice versa, after subtracting the carbohydrates respired. In these leaves, net export of amino acids was assumed to be negligible. Nocturnal respiration rates of these leaves were determined simultaneously, at a constant leaf temperature. Leaf discs were sampled during the dark period from the leaf fraction outside the respiration cuvette.

Theoretical estimation of the specific energy cost for carbohydrate export (E_e)

The specific cost for carbohydrate export (E_e) is calculated from the fraction of the carbohydrates exported that originates from starch (f_{st}), the specific energy costs of the conversion of starch in the chloroplast to sucrose in the cytosol (E_{st} ; mol CO₂ [mol sucrose]⁻¹) and the specific costs of sucrose loading into the phloem (E_{pl} mol CO₂ [mol sucrose]⁻¹).

$$E_e = f_{st} \times (E_{st} + E_{pl}) + (1 - f_{st}) \times E_{pl} \quad (3)$$

In starch-storing species, the nocturnal carbohydrate export originates for 70–100% from starch (i.e. $f_{st}=0.7$ to 1.0; e.g. data in Caspar *et al.*, 1985; Fondy and Geiger, 1982; Grange, 1985; Kalt-Torres *et al.*, 1987). The remaining carbohydrates may originate from, for example, sucrose in the cytosol. Carbon moves to the cytosol from the chloroplast as (1) triose-phosphate, (2) hexose, or as (3) the disaccharide maltose (Beck, 1985; Servaites *et al.*, 1989; Stütt, 1990). All pathways from starch in the chloroplast to sucrose in the cytosol require 3 ATP per sucrose, except when maltose is degraded by maltose phosphorylase (i.e. $E_{st}=2$ ATP per sucrose; Beck, 1985). These values are based on the assumption that hexoses and maltose cross the chloroplast envelope passively, down their concentration gradient resulting from the continuous biochemical conversion. In the present species, phloem loading is expected to occur apoplastically (Van Bel, 1992, 1993, and personal communication). The specific costs associated with apoplastic phloem loading (E_{pl}) are 1 ATP per sucrose in plasma membrane

vesicles (Slone and Buckhout, 1991). Thus, using equation 3, E_e is found to range from 2.4 to 4 mol ATP (mol sucrose)⁻¹.

Measurements of carbohydrate export (C_e) and simultaneous respiration rates (R_d)

Nocturnal carbohydrate export rates (C_e ; nmol sucrose m⁻² s⁻¹) were determined on bean leaves, which accumulate significant amounts of starch (Plaut *et al.*, 1987). Our treatments to manipulate carbohydrate export rates were insufficient to obtain a range suitable for estimating E_e .

In experiment b4, dark respiration was monitored during three successive parts (2.67 h) of the normal dark period (8 h), using different plants for each period (Table 2). At the end of each period, the leaf used for the respiration measurements and the control leaf of each plant were harvested. Export rates during the three dark periods were derived from the decrease in carbohydrate concentration between succeeding periods, after subtraction of the carbohydrates respired.

In experiment b5, the shoot was pruned just above the primary leaves, and a metal cooler (4 °C) was placed around the stem directly under the primary leaves, at the start of the light period. The leaf temperature was kept constant. The light period preceding the respiration measurements was 2 or 16 h. At the onset of the dark period, one leaf per plant was harvested, and stem coolers were removed. Respiration was followed on the remaining leaf, which was harvested 2 h later. Dark export rates were derived from the differences in carbohydrate concentration between the leaves, after subtracting the carbohydrates respired.

Calculations on the contribution of the energy costs of carbohydrate export to α and R_d

The fraction α_e of the effect of leaf organic nitrogen concentration on dark respiration (α ; see eq. 1) that may be explained by the energy costs involved in carbohydrate export was calculated from the specific costs for export (E_e ; mol CO₂ [mol sucrose]⁻¹) and the decrease in carbohydrate export rate (ΔC_e ; nmol sucrose m⁻² s⁻¹) at decreasing organic nitrogen concentration (ΔN_{org} ; mmol organic nitrogen m⁺²):

$$\alpha_e = (E_e \times \Delta C_e / \Delta N_{org}) / \alpha \quad (4)$$

The upper limit of α_e was calculated by estimating ΔC_e as the carbohydrate export rate at optimal nitrogen supply (C_e ; nmol sucrose m⁻² s⁻¹), i.e. assuming a zero export rate at the lowest organic nitrogen concentration.

The respiration involved in carbohydrate export (R_e ; $\text{nmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) was obtained from the specific costs for carbohydrate export (E_e ; $\text{mol ATP} [\text{mol sucrose}]^{-1}$), and the carbohydrate-export rates (C_e ; $\text{nmol sucrose m}^{-2} \text{ s}^{-1}$) determined on the youngest full-grown leaves of potato (experiments P0 and P1) and primary leaves of bean (experiments b4 and b5) with an optimal nitrogen supply:

$$R_e = E_e \times C_e \quad (5)$$

Analysis of leaf development

In bean, expansion of individual primary leaves was monitored non-destructively, with a video camera (SONY AVC 3250 CE, Japan) and monitor (SONY PVM 90 CE, Japan) connected to an integrator (TFDL nr 720314, Wageningen, the Netherlands), to establish the full-grown stage of the leaf. For potato, the youngest full-grown leaves were selected, using older leaves as a reference.

Dark respiration and photosynthesis measurements

Dark respiration and photosynthesis were measured on leaves of intact plants by infra-red gas analysis (IRGA; Analytical Development Co., Type 225-2B-SS, Hoddesdon, UK) as described by Louwse and Van Oorschot (1969) with computerized control and continuous data processing. To enable reliable determination of nocturnal CO_2 -production, flow rates were low ($\geq 0.12 \text{ m}^3 \text{ h}^{-1}$ or $0.033 \times 10^{43} \text{ m}^3 \text{ s}^{-1}$) and leaf area was large ($25\text{--}64 \text{ cm}^2$). During these dark respiration measurements, leaf temperature was closely regulated by the air temperature, as there was no increment due to light interception. The enclosure of the leaf in the respiration cuvette had no effect on the concentration of total reducing soluble carbohydrates and starch in the leaf (data not shown).

Chemical analyses

Plant material was dried at a temperature of 70°C , in an oven with forced ventilation. Total soluble carbohydrates were extracted by 10 min boiling in demineralized water, which does not affect cell walls. Reducing monosaccharides were determined in the extract by titration with sodium thiosulphate (AOAC, 1984). The total soluble carbohydrates were obtained by hydrolysing the extract by boiling in $0.05 \text{ M H}_2\text{SO}_4$ (i.e. $0.25 \text{ ml } 5 \text{ M H}_2\text{SO}_4$ in 25 ml extract), for 30 min. The resulting reducing monosaccharides were determined by titration (AOAC, 1984). To determine starch, the plant material was extracted twice with 40% ethanol (15 min, 20°C) to remove low molecular weight carbohydrates. The residue was boiled for 1 h at 125°C at 0.13 MPa . Starch was enzymatically hydrolysed for 1 h at 60°C by amyloglucosidase (EC 3.2.1.3; Merck, Darmstadt BRD) containing negligible sugar contamination. Subsequently, the glucose formed was determined by titration (AOAC, 1984). Starch concentration was expressed in $\text{mg CH}_2\text{O (g dw)}^{-1}$.

The organic nitrogen concentration (N_{org}) of leaves of various ages was determined as the difference between the total nitrogen and NO_3^- -nitrogen. Total nitrogen and NO_3^- -nitrogen were determined by CHN analysis on a Heraeus CHN-rapid (Hanau, Germany) and a TRAACS 800 continuous flow system (Bran and Luebbe, Germany), respectively.

Statistics

The statistical package GENSTAT 5 (Payne, 1987) was used for the linear regression analysis.

Results

The correlation of photosynthesis and dark respiration (α) with leaf-nitrogen concentration

Photosynthesis correlated strongly with the organic nitrogen concentration of the primary leaves of bean (Fig. 1). The slope of the regression lines ($0.11 \mu\text{mol CO}_2 (\text{mmol organic N})^{-1} \text{ s}^{-1}$) was similar for both the 17–20-d-old and the 21–24-d-old leaves, whereas the intercepts were -0.64 and $-1.74 \mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$, respectively. A correlation of photosynthesis and organic nitrogen concentration has generally been found for several species, whereas a relationship between respiration and organic nitrogen concentration is less often studied (references in the Introduction). The regression of respiration on organic nitrogen concentration for 17–20-d-old and 21–24-d-old primary leaves (eq. 1), also yielded a strong correlation (Fig. 2), both on a leaf area and a leaf dry weight basis (Table 3).

Measurements of carbohydrate export (C_e) and simultaneous respiration rates (R_d)

For both potato and bean, the carbohydrate concentration in the leaf decreased mainly due to export, whereas the effect of respiration was at least a factor of 2 lower (Table 4). The strong decrease in carbohydrate concentration in experiment b4 can at least be partly ascribed to growth, as up to day 17 the primary leaves of bean continued to expand (data not shown). For this reason, in experiment b4 the export rate may have been overestimated. In all other experiments the leaves did not grow.

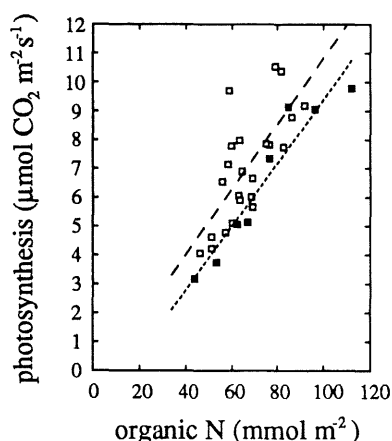


Fig. 1. Relationship between photosynthesis rate and organic nitrogen concentration (N_{org}) for 17–20 (\square) and 21–24 (\blacksquare)-d-old primary leaves of bean. The lines indicate the regression equations: $P = 0.11 \times N_{\text{org}} - 0.64$ ($r = 0.72$; $\text{df} = 21$) and $P = 0.11 \times N_{\text{org}} - 1.74$ ($r = 0.96$; $\text{df} = 6$). Each symbol represents an independent measurement on an individual plant at growth conditions (Table 2). Different nitrogen concentrations were obtained by nitrogen supply at different relative addition rates.

Table 3. The fraction of α (i.e. the effect of the leaf organic nitrogen concentrations on the dark respiration) that can be explained by carbohydrate export rates (α_e), for a range of 50–100 mmol organic N m⁻² in bean leaves

To obtain the upper limit of α_e , we used specific cost for carbohydrate export (E_e) of 1.2 mol CO₂ per mol sucrose. All regressions were significant (r from above to below was 0.73, 0.82, 0.62, and 0.53; df is 30 and 14 for 17–20 [exp. b1 plus b2] and 21–24 [exp. b3]-d-old leaves, respectively).

Age (d)	Regression of leaf respiration versus organic nitrogen (cf. eq. 1)			α_e (cf. eq. 4) upper limit
	Basis	α (nmol CO ₂ [mmol organic N] ⁻¹ s ⁻¹)	R_r (nmol CO ₂ [m ² or g dw] ⁻¹ s ⁻¹)	
17–20	m ²	8.8	331	0.54
	g dw	11.3	3.6	0.42
21–24	m ²	5.3	655	0.89
	g dw	4.4	13.2	1.07

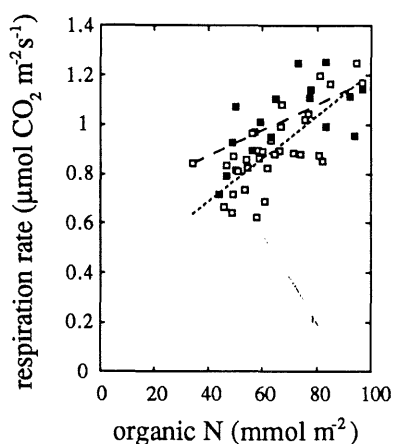


Fig. 2. Relationship between respiration rate and organic nitrogen concentration for 17–20 (□) and 21–24 (■)-d-old primary leaves of bean. The lines indicate the regression equations: $R_d = 8.8 \times N_{org} + 331$ ($r = 0.73$; $df = 30$) and $R_d = 5.3 \times N_{org} + 655$ ($r = 0.62$; $df = 14$) with the slope (α) and the intercept (R_r ; all units cf. eq. 1). Each symbol represents an independent determination on an individual plant at growth conditions (Table 2). Different nitrogen concentrations were obtained by nitrogen supply at different relative addition rates.

Specific energy costs for carbohydrate export (E_e); experimental and theoretical estimates

Linear regression of the respiration rate on carbohydrate export rate (eq. 2) yielded a value of 0.7 ± 0.2 mol CO₂ (mol sucrose)⁻¹ for the specific cost of carbohydrate export (Fig. 3 derived from experiment P1). The theoretical estimate of these specific costs (E_e) ranges from 2.4 to 4 mol ATP per mol sucrose (calculation in Materials and methods). Regarding the values generally observed for the ATP/O₂-ratio (6 to 4; e.g. Table 2 in Lambers *et al.*, 1983) and the RQ (1 to 1.2, but most often close to 1; Yemm, 1965) of full-grown leaves, this equals a range of E_e from 0.4 to 1.2 mol CO₂ per mol sucrose. Thus, although the correlation in Fig. 3 is not strong, the value of E_e agreed well with theoretical estimates.

Calculations of the contribution of the energy costs of carbohydrate export to α : upper limits

The effect of leaf organic-nitrogen concentration on leaf dark respiration is characterized by the regression coef-

Table 4. The percentage dark respiration explained by the cost of carbohydrate export for potato (P0 and P1) and bean (b4 and b5)

R_e was calculated according to equation 5, using the theoretically derived range of the specific cost for carbohydrate export of 0.4 to 1.2 mol CO₂ per mol sucrose. To express the rate of respiration and carbohydrate export measured in the preliminary potato experiment (P0) on a leaf area basis, we used a specific leaf area of 47 m² (kg dw)⁻¹ (Bouma *et al.*, 1992). The decrease in carbohydrate concentration due to export was calculated as the difference between the total decrease and respiratory losses. The export rate calculated in experiment b4 (*) might have been overestimated, as leaves were still expanding.

Experiment	Treatment	Dark respiration		Decrease in carbohydrate concentration		
		Total (R_d) (nmol CO ₂ m ⁻² s ⁻¹)	Export (R_e) (%)	Total (nmol sucrose m ⁻² s ⁻¹)	Export (%)	Respiration (%)
P0		379	7–20	96	67	33
P1	20→20	676	17–51	343	84	16
	20→7.5	688	12–37	272	79	21
	7.5→20	526	9–26	158	72	28
	7.5→7.5	602	8–23	168	70	30
b4	HL	1156	18–54*	616	84*	16
	LL	719	18–55*	392	85*	15
b5	2 h light	1072	8–23	296	70	30
	16 h light	1022	7–22	272	69	31
Average		760	12–35	290	76	24

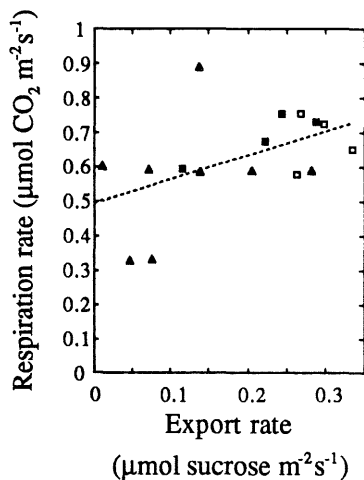


Fig. 3. Relationship between respiration rate and carbohydrate export for full-grown leaves of potato. Roots were transferred from 20 to 20°C (□), 20 to 7.5°C (■), 7.5 to 20°C (△), and 7.5 to 7.5°C (▲). The dashed line indicates the regression equation: $R_d = 0.70 \times C_e + 0.49$ ($r = 0.498$; $df = 14$). Each symbol represents an independent determination on an individual plant.

ficient α (see eq. 1). The fraction a_e of α that can be explained by respiratory energy costs associated with carbohydrate export was calculated using equation 4, as described in Materials and methods. The decrease in nocturnal carbohydrate export rate (ΔC_e) was taken to be equal to the export rate observed in experiment b5 (i.e. $197 \text{ nmol sucrose m}^{-2} \text{ s}^{-1}$; after Table 4), for a decrease of the organic nitrogen concentration (ΔN_{org}) with approximately 50 mmol m^{-2} (data values in Figs 1 and 2). Table 3 shows the upper limits of a_e , based on the highest estimate of the specific costs of carbohydrate export ($E_e = 1.2 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$). When using the experimentally derived estimate of E_e ($0.7 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$), the value for a_e ranges from 0.24 to 0.31 and 0.52 to 0.63, for 17–20-d-old and 21–24-d-old leaves, respectively. We conclude that carbohydrate export may account for a significant part of the effect of leaf organic nitrogen concentration on dark respiration (a), in the present tissue (a_e in Table 3).

Calculations of the contribution of the energy costs of carbohydrate export to R_d : upper and lower limits

Combining the calculated range of the specific costs for carbohydrate export ($E_e = 0.4\text{--}1.2 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$) with the observed rates of carbohydrate export (C_e ; cf. eq. 5), leads to the conclusion that export costs account for approximately 7–51% of the dark respiration rates (R_e in Table 4). When using the experimentally derived estimate of E_e ($0.7 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$), the average value of this fraction is 20%.

Thus, carbohydrate export can account for a significant part of R_d .

Discussion

Specific energy costs of carbohydrate export (E_e)

Estimates of the specific cost of carbohydrate export (E_e) are required to determine the contribution of the energy costs of nocturnal carbohydrate export to leaf dark respiration rate (R_d). The estimate of E_e obtained by linear regression ($0.7 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$; cf. eq. 2) was within the theoretically calculated range ($0.4\text{--}1.2 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$). Table 5 compares the present estimate of E_e to the values in the literature. These literature values were obtained by (1) linear regression of respiration rate versus carbohydrate export rate, (2) dividing the export rate by the total respiration rate or (3) dividing the export rate by the respiration rate corrected for maintenance respiration as estimated by the dark-decay method. Method 2 yields an upper limit of the specific costs of export, as all respiration is ascribed to carbohydrate export. Method 3 lacks power, as it is largely unknown which processes underlie the respiration determined by the dark-decay approach. In the present experiment (P1; see Table 2) we used Method 1. The sometimes observed asynchronous time-course of the respiration and export rates during the dark period (Grange, 1985), might be seen as a possible objection against Method 1 (Hendrix and Grange, 1991). Apparently, the activities of the various energy-requiring processes in the leaf do not always have the same time pattern during the dark period. Even starch degradation and phloem loading do not necessarily occur synchronously. However, as long as the overall nocturnal energy demand of all processes other than carbohydrate export remains constant between treatments, it is valid to use average export and respiration rates. In our opinion, this is best achieved when the environmental conditions of all source leaves studied are similar (i.e. previous illumination period and light intensity, relative air humidity, temperature and CO_2 -concentration). Therefore, we varied the export (and respiration) rates of potato leaves by manipulating root temperatures. The average export and respiration rates of Grange (1985) agree with present experimental observations and the fitted regression line.

Most experimental estimates of the specific costs of carbohydrate export agree well with the theoretical values, despite the different methods used (Table 5). The high values for tomato (Ho and Thornley, 1978) and soybean cultivar Wells II (after Mullen and Koller, 1988) are difficult to explain. In many cases the energy costs for carbohydrate export would exceed the actual respiration rates when multiplying these high specific costs for carbohydrate export with the observed export rates shown in

Table 5. Theoretical and experimental estimates of the specific costs for carbohydrate export in starch-storing species

The literature values were expressed on an ATP-basis using a combination of the P/O₂-ratio and RQ of either 4 and 1.2 or 6 and 1, respectively; these combinations give the maximum range. The specific cost of export based on Grange (1987) was derived by regression, using his unpublished respiration data of plants, 0, 4 and 8 d after transfer to high or low irradiance (personal communication, R.I. Grange). Hendrix and Grange (1991) calculated E_e from the values after 4 h in the dark, as presented by Fondy and Geiger (1982).

Specific costs for carbohydrate export (E _e)		Species	References
(mol CO ₂ [mol sucrose] ⁻¹)	(mol ATP [mol sucrose] ⁻¹)		
E _e = theoretically calculated 0.4–1.2	2.4–4.0	Species with apoplastic loading	Present paper
E _e = slope regression			
0.70	2.3–4.2	Potato	Present paper
0.79	2.6–4.7	Pepper	After Grange (1987)
1.20	4.0–7.2	Cotton	Hendrix and Grange (1991)
0.96	3.2–5.8	Barley	Hitz (1982)
2.64	8.8–15.8	Tomato	Ho and Thornley (1978)
0.47	1.6–2.8	Soybean cv. Amsoy 71	After Mullen and Koller (1988)
3.84	12.8–23.1	Soybean cv. Wells II	After Mullen and Koller (1988)
E _e = respiration rate/export rate			
1.00	3.3–6.0	Sugar beet	After Fondy and Geiger (1982), as derived by Hendrix and Grange (1991)
0.60–1.26	2.0–7.6	Field bean	Calculation method 1 Irving and Silsbury (1988)
E _e = (respiration rate – dark decay respiration)/export rate			
0.60–0.77	2.0–4.6	Field bean	Calculation Method 2 in Irving and Silsbury (1988)

Table 6. The reasons for these deviating results remain obscure, but may be additional energy-requiring processes, correlating with carbohydrate export. The good agreement between the theoretical range and most of the

experimental data, indicate that the present theoretical range of the specific energy costs of carbohydrate export is a sound basis for calculating the contribution of the energy costs of carbohydrate export to α and R_d.

Table 6. The percentage dark respiration (R_d) explained by costs for carbohydrate export, for data derived from the literature on starch-storing species

The costs for carbohydrate export (R_e) were calculated using equation 5, with C_e derived from the literature and a range of 0.4 to 1.2 mol CO₂ per mol sucrose for E_e.

Dark respiration for export (R _e)		Species	Data recalculated after the references given
(nmol CO ₂ m ⁻² s ⁻¹)	(% of R _d)		
98	—	<i>Arabidopsis</i>	Caspar <i>et al.</i> (1985), Fig. 1; Table 3
177	23–69	Sugar beet	Fondy and Geiger (1982), Fig. 1a
71–174	12–78	Pepper	Grange (1985), Fig. 4; Table 2
8–180	1–38	Cotton	Hendrix and Grange (1991), Fig. 6
171–343	7–60	Cotton	Hendrix and Huber (1986), Fig. 1
310–388	32–96	Field bean	Irving and Silsbury (1988), Fig. 1
257	11–33	Maize	Kalt-Torres <i>et al.</i> (1987), Figs 1 and 2
83–500	10–116	Soybean cv. Amsoy 71	Mullen and Koller (1988), Figs 5 and 7
93–208	12–40	Soybean cv. Wells II	Mullen and Koller (1988), Figs 5, 7
90	12–35	Cucumber	Verkleij and Baan Hofman-Eijer (1988), Fig. 2
23–46	3–17	Cucumber	Verkleij and Challa (1988), Fig. 1, Table 1
126–224	12–55		Average literature data
45–201	11–32	Potato	Present experiments, Table 4
131–365	13–39	Bean	Present experiments, Table 4

Calculations of the contribution of the energy costs of carbohydrate export to α : upper limits

The correlation between the dark respiration rate and the nitrogen concentration of (mature) leaves was hypothesized to be due to energy costs for (1) export of carbohydrates and (2) protein turnover (Lambers *et al.*, 1989). Assuming an average degradation constant of $1.39 \times 10^{-6} \text{ s}^{-1}$, De Visser *et al.* (1992) showed that protein turnover often accounts for a major part of the effect of the leaf organic nitrogen concentration on the dark respiration (α). If the costs of protein turnover are lower, for example, due to lower degradation constants, the energy costs associated with carbohydrate export may easily account for the remaining part of α (upper limits a_e in Table 3). This is especially the case in 21–24-d-old leaves (a_e up to 1). For 17–20-d-old leaves a_e was approximately 50% lower, due to the higher value of α : $8.8 \text{ nmol O}_2 [\text{g dw}]^{-1}$ compared to 5.3 for 21–24-d-old leaves (Table 3). We can not account for this high value of α , but note that it is also high compared to most observations in (full-grown) leaves of other starch-storing species (up to 7.3 nmol CO_2 or $\text{O}_2 [\text{mmol organic N}]^{-1} \text{ s}^{-1}$; De Visser *et al.*, 1992, and references therein; Irving and Silsbury, 1988; Makino and Osmond, 1991; Pons *et al.*, 1989); only the value obtained by Irving and Silsbury (1987) is as high as that for the 17–20-d-old bean leaves.

Calculations on the contribution of the energy costs of carbohydrate export to R_d : upper and lower limits

The present export rates (C_e) and respiratory costs involved in export (R_e) agree with literature data, although the present export rates are in the lower part of the range (Table 6). Combining the present estimates of the specific costs for carbohydrate export ($E_e = 0.4\text{--}1.2 \text{ mol CO}_2 [\text{mol sucrose}]^{-1}$) with literature data (cf. eq. 5), shows that, on average, carbohydrate export explains 12–55% of leaf dark respiration (R_e in Table 6). Some of these high values in the literature, might be partly due to carbohydrate use for leaf growth, which is a relatively expensive process (Poorter *et al.*, 1991). In conclusion, carbohydrate export is an important energy-requiring process in leaves of starch-storing species, at least in the dark. Together with the costs of protein turnover (17–35% of the leaves' dark respiration as calculated by Bouma *et al.* [1994]; the range of 5–76% of leaf dark respiration (De Visser *et al.* [1992] may include some costs for export), it may account for the major part of dark respiration in full-grown leaves.

Are there energy costs of other processes contributing to α and R_d ?

The sum of the average range of costs associated with carbohydrate (12–55% of R_d ; Table 6) plus protein turnover (17–35% of R_d ; Bouma *et al.*, 1994) does not exclude

the possibility that other energy-dependent processes account for part of the dark respiration of leaves (R_d). For example, maintaining ion gradients was shown to be important in roots (Bouma and De Visser, 1993). There is also no conclusive evidence to exclude processes other than carbohydrate export and protein turnover, which may contribute to the effect of leaf organic nitrogen concentration on dark respiration (α). One of these processes may be nitrate reduction. For a plant with a relative growth rate of $0.25 \text{ g g}^{-1} \text{ d}^{-1}$ and an organic nitrogen concentration of $60 \text{ mg N (g dw)}^{-1}$, a reduction rate of $12.4 \text{ nmol NO}_3^- (\text{g dw})^{-1} \text{ s}^{-1}$ is required to produce the new biomass. The relative importance of nitrate reduction in the shoot increases with increasing nitrate supply (Gojon *et al.*, 1994). Assuming that leaves reduce only the nitrate for their own demand, and using a specific leaf area as observed for potato ($47 \text{ m}^2 [\text{kg dw}]^{-1}$; Bouma *et al.*, 1992), the reduction rate is $264 \text{ nmol NO}_3^- \text{ m}^{-2} \text{ s}^{-1}$. Since the reduction of NO_3^- to NH_3 gives $2 \text{ mol CO}_2 [\text{mol NO}_3^- \text{ reduced}]^{-1}$, the calculated nitrate reduction rate gives a CO_2 production rate of $528 \text{ nmol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$. In general, and in the present experiments, its contribution is expected to be much lower than calculated here, for the following reasons: (1) nitrate reduction in the leaves occurs predominantly in the light (Beevers and Hageman, 1969; Rufty *et al.*, 1984), (2) up to 1 CO_2 per nitrate reduced in the shoot may be exported to the root via the malate shuttle (Ben Zioni *et al.*, 1970, 1971; Kirkby and Knight, 1977) and (3) nitrate reduction rate in full-grown leaves is much lower than in growing leaves (Beevers and Hageman, 1969). Moreover, the relative growth rate and organic nitrogen concentration assumed in present calculations were relatively high compared to that of potato (Bouma *et al.*, 1992), so that an upper limit was obtained.

Conclusions

Dark respiration of full-grown leaves correlates with the nitrogen concentration of these leaves. In starch-storing species, nocturnal carbohydrate export may account for a significant part (up to 100%) of the slope of this regression, and on average for 29% of total dark respiration. Thus, in starch-storing species, energy costs associated with nocturnal carbohydrate export are quantitatively important.

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