

Effect of water stress on partitioning of ^{14}C -labelled photosynthates in *Vitis vinifera*

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Abstract. The influence of fruits on export and distribution of photosynthates was studied in *Vitis vinifera* L. cv. Tempranillo using ^{14}C labelling. Also, the influence of water stress was analysed in fruiting and fruitless plants of Tempranillo and fruiting plants of cv. Alfonso Lavallée. In fruitless plants of Tempranillo, reserve organs (trunk, roots and lower shoot) represented 80% of total plant dry matter (DM), and imported up to 90% of the total ^{14}C exported from the fed leaf. Therefore, the distribution pattern of photosynthates in these plants reflected mainly the sink size. However, the presence of fruits in Tempranillo strongly stimulated ^{14}C export and changed the distribution pattern of assimilates. Fruits imported up to 70–80% of the total ^{14}C exported, while representing only 25% of the total plant DM. Therefore, the strength of fruits as carbon sinks was independent of sink size, and it is discussed on the basis of a water potential gradient theory.

Water stress caused a significant reduction of leaf water potential, photosynthesis and stomatal conductance, but caused only a slight, non-significant, decrease of carbohydrate export from the fed leaves, and did not affect the distribution pattern of ^{14}C except in some minor fractions in Tempranillo. The ^{14}C distribution into different fruit components was also unaffected. In contrast, in Alfonso Lavallée water stress resulted in a highly significant reduction of export, and an altered photosynthate distribution pattern. These differences could be due to the lower water potential attained in stressed Alfonso Lavallée plants.

Keywords: carbon export, carbon partitioning, ^{14}C -photoassimilates, *Vitis vinifera*, water stress.

Introduction

Soil water deficit is the main environmental constraint for plant production under Mediterranean conditions. The constraints of water stress on plant productivity have long been recognised in agriculture. Grapevine (*Vitis vinifera*) is a common crop in Mediterranean areas, with its most active growth being in spring and summer. Despite its adaptation to these climatic conditions, a combination of soil water deficit with frequent environmental constraints such as steep leaf-to-air water vapour gradients, high light intensity and high temperature cause a progressive reduction of grapevine stomatal conductance, due to increases in xylem content of abscisic acid and/or decreases in xylem conductivity (Lovisoló *et al.* 2002; Schultz 2003; Souza *et al.* 2003). As a consequence, important reductions in photosynthesis,

plant growth and grape yield are present (Kliewer *et al.* 1983; Bota *et al.* 2001; Flexas *et al.* 2002; Maroco *et al.* 2002; Medrano *et al.* 2003). Water stress can severely affect not only yield but also grape composition and, consequently, wine quality (Medrano *et al.* 2003). Carbon assimilation, photoassimilate exportation out of the producing leaves, and photoassimilate transport and partitioning within the plant are likely to be the physiological processes linking photosynthesis to crop yield and fruit quality.

While the effects of water stress on photosynthesis have long been studied in grapevines (Chaves *et al.* 1987; Downton *et al.* 1987; Schultz 1996; Flexas *et al.* 1998, 1999a, b, 2002; Escalona *et al.* 1999), much less is known about the effects of water stress on assimilate transport and partitioning. In general, the transport and partitioning of

Abbreviations used: A_N , net CO_2 assimilation rate; DM, dry matter; ET, evapotranspiration; g_s , stomatal conductance; RWC, relative water content; Ψ , leaf water potential.

photoassimilates within the plant is strongly dependent on the assimilate production in the source, the rates of loading and unloading of the sieve-tube elements, the velocity of the phloem flow, and the sink incorporation rate (Begg and Turner 1976). It has been thought traditionally that sinks are in some form of competition with each other for the available photosynthates. Therefore, partitioning among sinks is primarily regulated by the sinks themselves and their ability to import the photo-assimilates (Evans 1975; Gifford and Evans 1981; Ho 1988; Patrick 1990, 1997). The degree of competition between sinks depends upon the stage of plant development and other factors like cultural practices and environmental factors (Wardlaw 1976).

In grapevine, the distribution of the photosynthetic products to the different parts of the plant during the developmental stages has been extensively studied (Hale and Weaver 1962; Koblet 1969; Quinlan and Weaver 1970; Koblet and Perret 1979; Hunter and Visser 1988*a, b*; Motomura 1990, 1993). Also the effects of different cultural practices on carbon supply to different parts of the plant, especially to the fruit, have been tested in grapevine: shoot pinching, girdling of shoot (Motomura 1993), canopy manipulation (Candolfi-Vasconcelos *et al.* 1994; Iacono *et al.* 1995), and crop load (Edson *et al.* 1993, 1995*a, b*; Iacono *et al.* 1995; Foyer *et al.* 1995; Miller and Howell 1998). Currently, the mechanisms underlying source–sink interactions observed at the whole-plant level are still under study in grapevine through either measurements or modelling (Minchin *et al.* 1993; Bindi *et al.* 1997; Quereix *et al.* 2001). However, the effects of environmental factors on carbon partitioning have been less studied. Sepúlveda *et al.* (1986) demonstrated that high temperature causes changes in the distribution patterns of ¹⁴C-labelled photosynthates. More recently, Vanden Heuvel *et al.* (2002) showed an important effect of leaf light environment on the photoassimilate partitioning in chardonnay grapevines. Regarding the effects of water stress only, Quick *et al.* (1992) showed that sucrose levels in leaves of cv. Rosaki remained high under water stress with an important reduction of sugar export out of the leaves. However, it remains unclear whether or not reductions in soil water availability could cause changes in the pattern of distribution of assimilated carbon between different plant parts in grapevine. In other C₃ plants, translocation of assimilates from leaves to other plant organs through the phloem has been shown to be reduced by water stress (Huber *et al.* 1984; Deng *et al.* 1989), but not in all cases (Gallagher *et al.* 1976). Also, plant water status appears to strongly affect assimilate translocation in *Phaseolus* (Lang and Thorpe 1986). In *Lupinus albus* L., water stress seems to stimulate sink capacity of certain organs like seeds and pods (Withers and Forde 1979; Chaves *et al.* 2002). Labelling studies allow direct quantification of carbon fluxes and carbon partitioning among plant parts and also partitioning among different compounds inside the berries. In this study the main

objective was to evaluate the effect of water stress on export and partitioning of ¹⁴C-labelled photosynthates in vines with and without fruit.

Materials and methods

Plant material and treatments

Experiments were carried out with potted grapevine plants (*Vitis vinifera* L. cv. Tempranillo) in their first and second years of growth (2002 and 2003, respectively). In 2002 rooted cuttings grafted to R-110 rootstock were planted in 5-L pots containing a 2 : 1 ratio of organic soil to perlite (v : v) with soil solution pH of approximately 6.5 (within the optimum range for this rootstock). None of these plants developed fruit clusters. In 2003, plants growing under the same conditions developed fruit clusters. In both experiments (with and without fruit), nine plants were grown outdoors and irrigated daily with a drip system. Nutrient solution (100% Hoagland's solution, Hoagland and Arnon 1950) was applied twice a week until the start of treatments. Drought treatments started in the second week of July. In 2003, the fruit clusters were at veraison stage. To set the different treatments, all plants were irrigated daily with different irrigation regimes. Control plants were irrigated with a dose equal to 100% of real pot evapotranspiration (ET) of the previous day, estimated by weighting pots daily. Moderate and severe drought treatments were set with an irrigation dose of 50% and 33% of ET, respectively. By day seven, the moderately water stressed plants transpired 63% of the control and the soil water content was 66% of the control. At the same time the severely water stressed plants transpired 35% of the control and the soil water content was approximately 55% of the control. Drought treatments were sustained for 2 weeks before the onset of ¹⁴C-labelling.

Additionally, in 2002, a second experiment was carried on with a different cultivar (Alfonso Lavallée). Plants were grafted to SO4 rootstock and grown outdoors in 7-L pots containing substrate conditions similar to cv. Tempranillo (see above). Both were at similar pH to prevent the reported pH effects on source–sink ratio (Bavaresco and Poni 2003). There were only two treatments, control and severe drought, with the same irrigation routine as described above. However, after a week of treatment transpiration was down to 26% of the control, although the water content of the pot was still 70% of the control. Labelling with ¹⁴C in this experiment also began 2 weeks after the onset of the drought treatment.

Plant water status

Pre-dawn leaf water potential (Ψ) was determined with a Scholander chamber (Soilmoisture Equipment Corp., Santa Barbara, CA).

The leaf relative water content (RWC) was measured around mid-morning just before the start of labelling. RWC was determined as follows:

$$\text{RWC} = (\text{fresh weight} - \text{dry weight}) / (\text{turgid weight} - \text{dry weight}).$$

Leaf turgid weight was determined by keeping the leaves in distilled water in darkness at 4°C to minimise respiration losses, until they reached a constant weight (full turgor, typically after 12 h). Their dry weight was obtained after 48 h at 70°C in an oven.

Gas-exchange measurements

Net CO₂ assimilation rate (A_N) and stomatal conductance (g_s) were measured on 3–5 mature leaves per plant (9–15 measurements per treatment) just before labelling under saturating light (Li-6400, Li-Cor Inc., Lincoln, NE).

¹⁴C labelling and measurement

Each experiment was performed in three consecutive days. One plant per treatment was labelled daily. Leaf feeding with ¹⁴CO₂ began at mid-morning (1000–1100 hours) on sunny days under saturating light

intensity using a closed gas system composed of a leaf chamber, peristaltic pump, large Erlenmeyer flask (approximately 2 L) and gas-flow indicator. The leaf chamber consisted of two parts: a top part with 21-cm² exposing area with a refrigerated water circulation system to control leaf temperature and a bottom part that allowed gas to circulate. The total volume of the system was approximately 2.2 L. One fully expanded leaf per plant (in experiments with fruitless plants normally the seventh or eighth leaf; in experiments with fruiting plants the closest leaf to the cluster with a photosynthetic activity was similar to average) was enclosed in the chamber and fed with 1.85 MBq of ¹⁴C. Labelled ¹⁴CO₂ was generated inside a large Erlenmeyer flask 30 min before start of labelling by adding 50% (v/v) lactic acid to a 0.1 M ¹⁴C-labelled sodium carbonate solution. The CO₂ concentration in the system was estimated between 1000 (start) and, at least 500 (end) μmol CO₂ mol⁻¹. Gas circulation in the system started immediately after the leaf was enclosed and sealed in leaf chamber. Gas-flow rate during the labelling was maintained at 0.75 L min⁻¹. Leaves were exposed to natural sunlight for 40 min, with temperature controlled at 25–26°C. After ¹⁴CO₂ feeding, the chamber was flushed with non-labelled air for 5 min and residual ¹⁴CO₂ was trapped with a 0.5 M NaOH solution. After the chamber was removed, the plants were allowed to return to their normal metabolism and translocation of the labelled photosynthates during a 24-h period. Since a kinetic analysis of ¹⁴C transport was not made, the results should be viewed with some care since it is not clear whether a steady state was reached in each compartment. Nevertheless, a period of 24 h has been usually reported as sufficient, and represents a compromise between achieving a steady state and preventing misleading results due to losses of recently labelled carbohydrates by different respiration rates among different organs (Quinlan and Weaver 1970; Hunter and Visser 1988a, b; Motomura 1993; Vanden Heuvel *et al.* 2002).

After 24 h, Tempranillo plants were harvested and divided into 11 (plants without fruit) or 12 (plants with fruit) different parts. For this, the main shoot (i.e. that which contained the fed leaf) was subdivided into the following: upper part, lower part, upper leaves, and lower leaves. The other parts into which plants were divided were: main tip, lateral tips, fed leaf, cluster (when present), lateral shoots, lateral leaves, trunk, and root. For cv. Alfonso Lavallée, plants were divided in the following fractions: fed leaf, main shoot, leaves of main shoot, main cluster, lateral shoots, lateral leaves, lateral clusters, trunk and root.

These fractions were oven-dried and the dry weight was determined. Dry material was ground using a mechanical Culatti Micro Hammer mill (Culatti AG, Zürich, Switzerland). ¹⁴C was determined as described by Amato (1983) with minor modifications. Samples of dry material (15–30 mg) were weighed into digestion tubes. Aliquots (6 mL) of the digestion mixture were quickly added to the bottom of each tube (Voroney *et al.* 1991). A glass support was placed into the digestion tube with a small glass vial containing 1 mL of ethanolamine, and the tube was immediately stopped with a subaseal. Duplicate samples of each tissue were analysed. The tubes were placed in a heating block for 1 h at 130°C, then cooled and held overnight at room temperature. Vials containing ethanolamine were removed and placed into 20-mL plastic vials. Scintillation solution (11 mL, Ultima Gold, Canberra Packard, Groningen, The Netherlands) were added to the vial, which was capped tightly and shaken. The ¹⁴C activity was determined using a liquid scintillation spectrometer (Canberra Packard) after 3–5 h. ¹⁴C exported from fed leaves was expressed as percentage, calculated as follows:

$$\%^{14}\text{C exported} = 100 \times \left(\frac{^{14}\text{C recovered from fed leaf}}{^{14}\text{C recovered}} \right)$$

Partitioning into the fractions was expressed as percentage of distribution, calculated as follows:

$$\% \text{ distribution of } ^{14}\text{C into the fraction } i = 100 \times \left(\frac{^{14}\text{C recovered in fraction } i}{^{14}\text{C recovered excluding fed leaf}} \right)$$

Analysis of ¹⁴C partitioning among main fractions of metabolites

Ground dry material from clusters was first extracted in ethanol : water (80 : 20, v : v) at 80°C and then extracted successively with 10 mL of ethanol : water (60 : 40, v : v), followed by ethanol : water (40 : 60, (v : v) at 25°C. The extracts were pooled and dried by a rotary vacuum dryer at 40–50°C and the precipitate was dissolved in 16.5 mL of chloroform : methanol : water (12 : 11 : 10) and divided into aqueous and hydrophobic fractions. Water-soluble compounds were separated into neutral, acidic and basic fractions by ion exchange chromatography. The neutral fraction (soluble carbohydrates) was obtained by passing the extract through successive cationic (Dowex-50 in the H⁺-form; from SUPELCO, Bellefonte, PA) and anionic (Dowex-1 in the HCOO⁻-form; SUPELCO) columns. The basic fraction (amino acids) was eluted from the cation-exchange resin with 3 N NH₄OH in water. The acidic fraction (organic acids) was eluted from anion-exchange resin with 4 N HCOOH. The cation-exchange column was regenerated with 2 N HCl. All fractions were dried under vacuum. Neutral and acidic fractions were dissolved in water. The basic fraction was solubilised in 10% (v : v) isopropanol. Aliquots of each water-soluble fraction as well as hydrophobic fraction, containing ethanol-extractable lipids, were counted for radioactivity.

Total leaf area

To assess any possible interference of plant leaf area variation among plants with differing treatments, total plant leaf area was calculated. Leaf area was measured with a portable area meter (ΔT Area meter; Delta-T Devices, Hoddesdon, UK). A very strong correlation ($r^2=0.99$ in 2002 and $r^2=0.96$ in 2003) was obtained between the length of the main vein of the leaf and leaf area using several leaves of the same cultivar. Total leaf area of each plant was calculated after measuring the length of the main vein in all leaves and applying the correlation formula.

Statistics

One-way ANOVA was applied to assess the differences between treatments. Differences among means were established using a Duncan's test ($P<0.05$). The data were analysed using the SPSS 10.0 programme for Windows (SAS Institute, Cary, NC).

Results

Changes in water relations and photosynthetic parameters during progressive drought in Tempranillo plants

The depletion of soil water content induced a progressive water stress. In fruitless plants, pre-dawn leaf water potential (Ψ) was significantly lower in leaves under severe water stress than in leaves of control plants (Table 1). Nevertheless, under moderate water stress, although Ψ was lower, it was not significantly different from the control ($P<0.05$) (Table 1). In plants with fruit, Ψ was significantly different among treatments (Table 1). Leaf RWC of moderately and severely water-stressed plants was not significantly different from that of control plants ($P<0.05$), regardless of the presence of fruit (Table 1).

Water stress induced significant reductions in leaf gas-exchange parameters (Table 2). These changes were similar regardless of fruit presence. The reduction of g_s was approximately 50% under moderate and approximately 75%

Table 1. Pre-dawn leaf water potential (Ψ) and leaf relative water content (RWC) in mature leaves of Tempranillo plants with and without fruitData are the mean \pm SE of 4–9 replicates. Within each column, values with different superscripted letters are significantly different according to Duncan's test ($P=0.05$)

Treatment	Fruiting status	RWC (%)	Ψ (MPa)
Control	With fruit	88.04 \pm 0.66 ^a	-0.28 \pm 0.01 ^a
	Without fruit	90.04 \pm 1.05 ^a	-0.25 \pm 0.02 ^a
Moderate drought	With fruit	87.04 \pm 1.89 ^a	-0.44 \pm 0.03 ^b
	Without fruit	88.14 \pm 2.40 ^a	-0.28 \pm 0.03 ^a
Severe drought	With fruit	87.42 \pm 3.06 ^a	-0.58 \pm 0.03 ^c
	Without fruit	89.33 \pm 0.82 ^a	-0.46 \pm 0.06 ^b

Table 2. Net photosynthesis (A_N) and stomatal conductance (g_s) in mature leaves of Tempranillo plants with and without fruitData are the mean \pm SE of 9–15 replicates. Within each column, values with different superscripted letters are significantly different according to Duncan's test ($P=0.05$)

Treatment	Fruiting status	A_N ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	g_s ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
Control	With fruit	12.81 \pm 0.90 ^a	239.91 \pm 0.02 ^a
	Without fruit	12.04 \pm 0.45 ^a	184.83 \pm 0.01 ^b
Moderate drought	With fruit	8.19 \pm 0.87 ^b	127.13 \pm 0.02 ^c
	Without fruit	9.00 \pm 0.79 ^b	98.16 \pm 0.01 ^c
Severe drought	With fruit	4.12 \pm 0.60 ^c	58.22 \pm 0.01 ^d
	Without fruit	5.90 \pm 0.46 ^c	47.24 \pm 0.01 ^d

under severe water stress (Table 2). Reductions in A_N were lower than in g_s , but also significantly lower in water-stressed plants, regardless of fruit presence.

Under labelling conditions, the total carbon assimilated was not significantly affected by a moderate water stress, but it was strongly reduced under severe water stress in both varieties (Table 3). Under labelling conditions, assimilation may be not strongly limited by stomatal closure, since the elevated CO_2 concentration used may partially overcome stomatal limitations. Therefore, these results suggest that photosynthesis was only limited by stomatal closure in moderately stressed plants, while reductions in ^{14}C assimilation under severe stress suggests that some non-stomatal limitations were present. This was expected, based on the values of g_s in each treatment, and according to the photosynthesis

regulation pattern shown by Flexas *et al.* (2002) in grapevines.

Dry matter partitioning and source–sink ratio in Tempranillo plants

In fruitless plants the largest percentages of DM were measured in the storage organs (trunk and roots) (Fig. 1A), accounting for up to 60% of total DM. In fruiting plants, berries represented a substantial percentage (around 25%) of the DM at the cost of trunk and root DM (Fig. 1B). No effects of water stress on DM partitioning in Tempranillo plants were observed (Fig. 1).

The ratio between leaf area and berry DM in control plants was similar to that previously observed for Tempranillo plants in field experiments (Escalona *et al.* 2003). The application of water deficit did not suppose significant

Table 3. Total ^{14}C assimilated (KBq plant^{-1}) in Tempranillo and Alfonso Lavallée plants under different water stress treatmentsData are the mean \pm SE of three plants. Within each column, values with different superscripted letters are significantly different according to Duncan's test ($P=0.05$)

Treatment	Tempranillo		Alfonso Lavallée
	without fruit	with fruit	with fruit
Control	316.91 \pm 75.88 ^a	189.40 \pm 30.85 ^a	529.37 \pm 126.58 ^a
Moderate drought	288.67 \pm 70.97 ^{a,b}	236.58 \pm 24.29 ^a	–
Severe drought	108.66 \pm 29.97 ^b	119.15 \pm 20.80 ^b	87.64 \pm 13.43 ^b

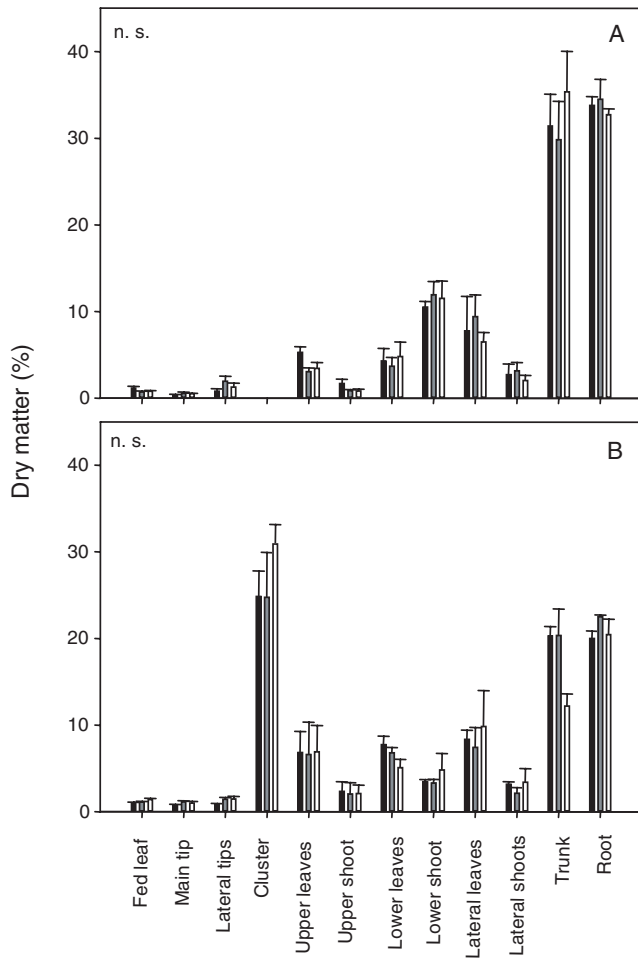


Fig. 1. Percentage of dry matter partitioning of the vine organs in three treatments: control, well watered (black bars), moderately water-stressed (grey bars) and severely water-stressed (white bars) Tempranillo plants without fruit (A) and with fruit (B). Data are means \pm SE of three plants.

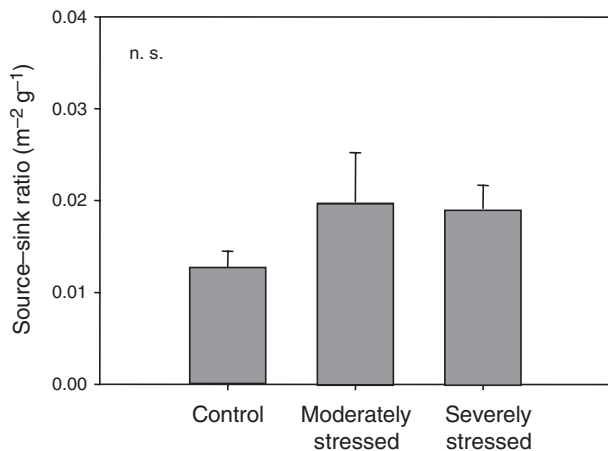


Fig. 2. Source-sink ratio, expressed as m^2 of leaf area per g of fruit dry weight, in Tempranillo plants in three treatments: control, well watered moderately water-stressed, and severely water-stressed. Data are means \pm SE of three plants.

differences between treatments on source-sink ratios (Fig. 2).

Export of ^{14}C -assimilates from the individual Tempranillo leaves

Tempranillo plants, both with and without fruit, showed some reduction of carbon export with progressive water stress (Fig. 3). However, these differences were not significant.

In control plants, the percentage of ^{14}C exported from the fed leaf was significantly greater ($P < 0.001$) in plants with fruit (59%) than in fruitless plants (38%). These differences were reduced, although still significant, as water stress intensified. In fruitless plants subjected to moderate and severe water stress, the percentages of ^{14}C exported were 28% and 24%, respectively. For the same treatments, the presence of fruit increased those percentages to 53% and 48%, respectively (Fig. 3).

Distribution of ^{14}C -assimilates exported to different fractions in Tempranillo plants

In fruitless plants, most of the ^{14}C was recovered from the shoots, trunk and roots (Fig. 4A), which reflects the important sink activity of these storage organs. In these plants, any clear effect of water stress was observed in the general pattern of distribution of the total exported ^{14}C to different plant fractions (Fig. 4A). In contrast, for plants with fruit, fruits were by far the largest carbohydrates sinks, importing up to 80% of the total ^{14}C exported from the fed leaf (Fig. 4B). The strength of the fruit as a carbohydrate sink changed the pattern of distribution, reducing drastically the relative importance of others sinks such as shoots, trunks and roots. In these plants, water stress induced some

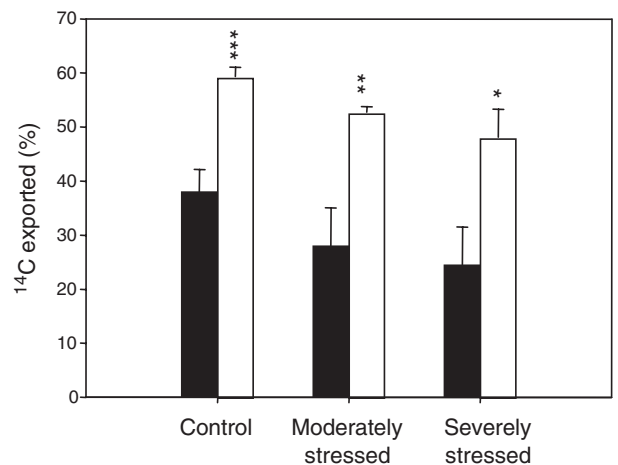


Fig. 3. Percentage of ^{14}C export out of $^{14}CO_2$ -fed leaf in three treatments: control, well watered, moderately water-stressed, and severely water-stressed Tempranillo plants with (white bars) and without fruit (black bars). Data are means \pm SE of three plants. Statistical significance of differences between fruiting and non-fruiting plants are given as: *, ($P < 0.05$); **, ($P < 0.01$); ***, ($P < 0.001$).

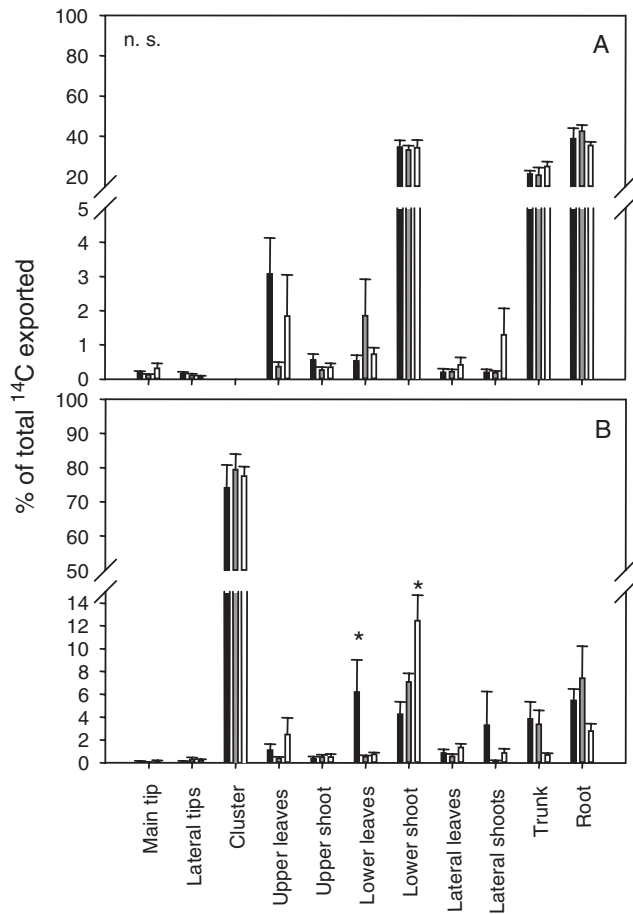


Fig. 4. Distribution of ¹⁴C-assimilates exported out of the fed leaf to the different grapevine parts, in three treatments: control, well-watered (black bars), moderately water-stressed (grey bars) and severely water-stressed (white bars) Tempranillo plants without fruit (A) and with fruit (B). Data are expressed as a percentage of total activity excluding fed leaf. Data are means \pm SE of three plants. Statistical significances for differences between treatments are given: *, ($P < 0.05$).

significant differences in the carbon allocated to the lower part of the main shoot (Fig. 4B). Lower leaves contained relatively larger amount of ¹⁴C in control plants than in water stressed plants. In contrast, ¹⁴C recovery in the lower part of the shoot of the severely water stressed plants was significantly higher than in control or moderately water stressed plants.

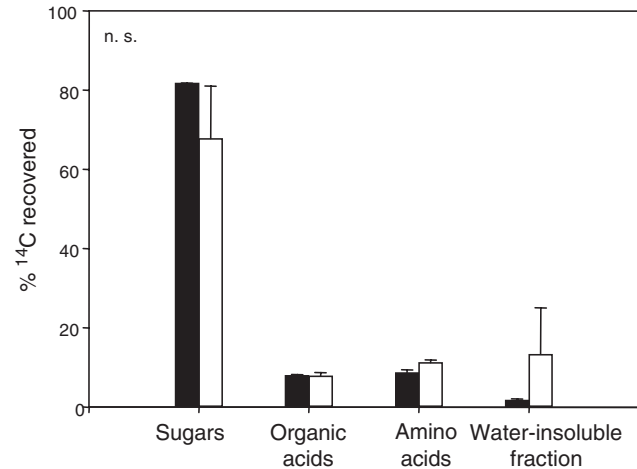


Fig. 5. Carbon partitioning into different chemical fractions in berries from Tempranillo plants in two treatments: control, well-watered (black bars) and severely water-stressed (white bars) plants.

Carbon partitioning to different metabolite fractions of the fruit in Tempranillo plants

Partitioning of ¹⁴C into soluble carbohydrates, organic acids, amino acids and the water-insoluble fraction (mainly lipids) in the control and severely stressed Tempranillo plants is shown in Fig. 5. Non-significant differences were found between the two treatments; the percentage of ¹⁴C recovered in the sugar fraction was approximately 17% lower for water stressed plants compared with the control.

Experiment with the Alfonso Lavallée cultivar

An additional experiment with another cultivar, Alfonso Lavallée, yielded somewhat different results. Only the severe water stress treatment was applied (33% of the ET).

Leaf water potential was strongly reduced by the effect of water stress, reaching values substantially lower than observed in severely stressed Tempranillo. However, reductions in RWC were non-significant. Water stress also induced important reductions in gas-exchange parameters. Net assimilation rate and g_s were reduced by approximately 66% and 83%, respectively (Table 4). Control plants of Alfonso Lavallée showed lower assimilation rate but higher stomatal conductance when compared to control plants of Tempranillo with fruit (Tables 2 and 3). In both cultivars,

Table 4. Pre-dawn leaf water potential (Ψ), leaf relative water content (RWC), net photosynthesis (A_N) and stomatal conductance (g_s) in mature leaves of Alfonso Lavallée plants with fruit. Data are the mean \pm SE of 9–13 replicates. Within each column, values with different superscripted letters are significantly different according to Duncan's test ($P = 0.05$)

Treatment	RWC (%)	Ψ (MPa)	A_N ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$)	g_s ($\text{mmol H}_2\text{O m}^{-2} \text{ s}^{-1}$)
Control	87.06 \pm 1.48 ^a	-0.25 \pm 0.01 ^a	11.30 \pm 0.33 ^a	280.91 \pm 0.03 ^a
Drought	83.65 \pm 2.50 ^a	-1.02 \pm 0.15 ^b	3.81 \pm 0.51 ^b	48.18 \pm 0.01 ^b

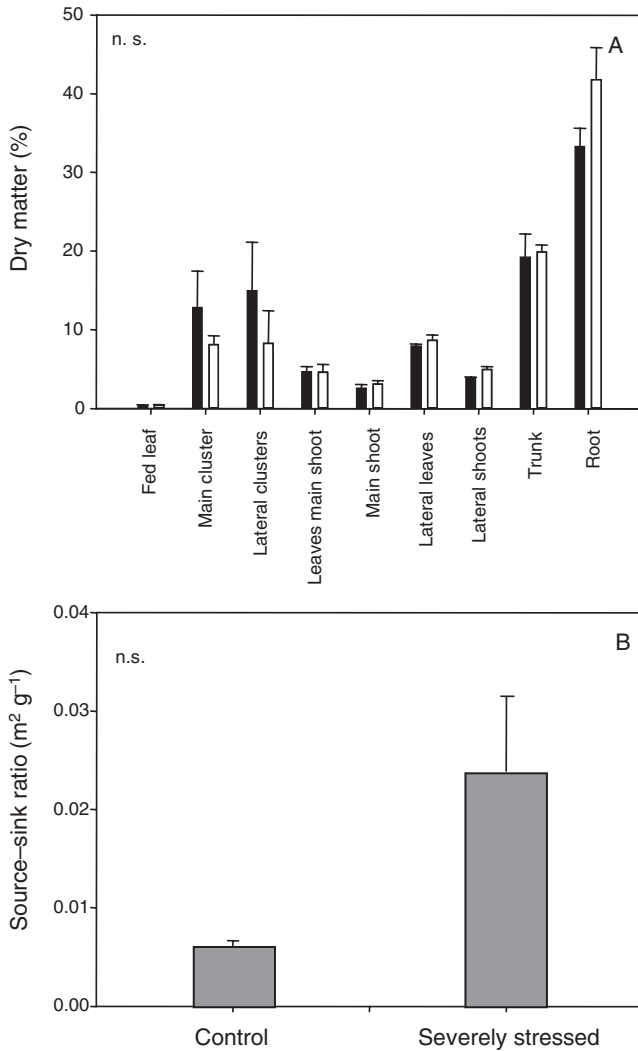


Fig. 6. Percentage of dry matter partitioning (A) and source-sink ratio, expressed as m² of leaf area per g of fruit dry weight (B), in cv. Alfonso Lavallée in two treatments: control, well-watered (black bars in A) and severely water-stressed (white bars in A) plants. Data are means ± SE of three plants.

the consistency of midday RWC suggests an isohydric behaviour. Previous results of Flexas *et al.* (2002) have shown that Tempranillo always demonstrates isohydric behaviour, except under stress situations more severe than those in the present study.

The DM partitioning pattern in Alfonso Lavallée plants was similar to that observed in Tempranillo fruiting plants, except that the percentage of DM in roots was higher (Figs 1B, 6A). As in Tempranillo, no effects of water stress were observed in the DM distribution pattern. Regarding source-sink, an increasing tendency under water stress seemed to be clearer in Alfonso Lavallée than in Tempranillo, although non-significant in any case (Figs 2, 6B).

The percentage of ¹⁴C exported from the fed leaf to other parts of the plant was significantly lower ($P=0.001$) in water

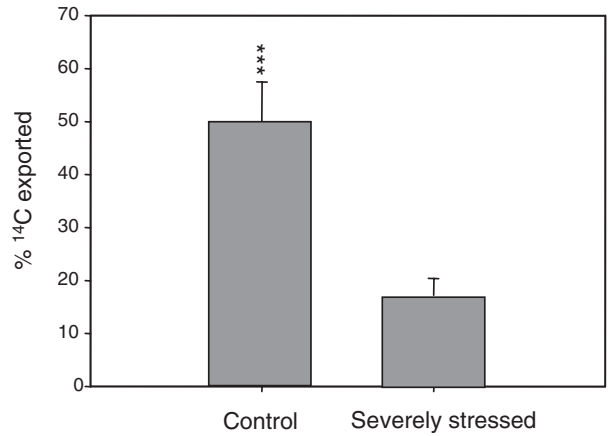


Fig. 7. Percentage of ¹⁴C export out of ¹⁴CO₂-fed leaf in two treatments: control, well watered (black bar) and severely water-stressed (white bar) Alfonso Lavallée plants with fruit. Data are means ± SE of three plants. Statistical significance of differences between treatments are indicated: ***, $P=0.001$.

stressed plants than in the controls. Almost 50% of ¹⁴C was found out of the fed leaf in control plants, compared with less than 20% in water stressed plants (Fig. 7). Water stress also affected the distribution of ¹⁴C-labelled assimilates (Fig. 8). In control plants, the main cluster contained a similar percentage (80%) of labelled carbon to Tempranillo, despite the presence of additional clusters in Alfonso Lavallée plants. However, unlike Tempranillo, this percentage was

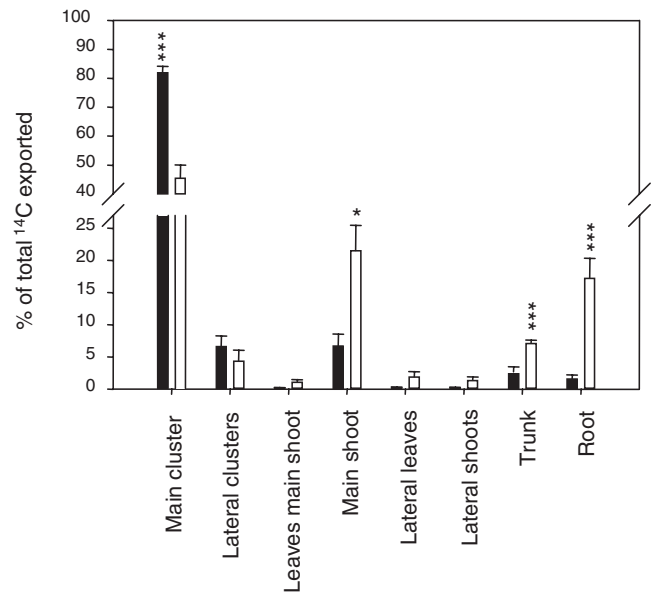


Fig. 8. Distribution of ¹⁴C-assimilates exported from the fed leaf to the different grapevine parts, in two treatments: control, well watered (black bars) and severely water-stressed (white bars) Alfonso Lavallée plants with fruit. Data are expressed as a percentage of total activity excluding fed leaf. Data are means ± SE of three plants. Statistical significance of differences between treatments are given: *, $P<0.05$; ***, $P<0.001$.

strongly and significantly reduced ($P < 0.001$) to 45.5% under water stress. Besides the cluster, the remaining ^{14}C -labelled assimilates were exported primarily to the main shoot, trunk and roots. The accumulation of ^{14}C -labelled compounds in these woody fractions was higher in water stressed plants. In the main shoot fraction, water stress increased total ^{14}C recovery by 3-fold in comparison to control plants (21.5% and 6.7%, respectively). Highly significant differences ($P < 0.001$) were also found in trunk and roots. In water stressed plants, approximately 7% of total ^{14}C exported was recovered in the trunk, compared with only 2.4% in the controls. In roots, the differences were even stronger, with a 17.2% of ^{14}C recovered in water stressed plants and only 1.6% in controls (Fig. 8).

Discussion

Effects of fruiting and water stress on leaf ^{14}C export

The presence of fruit in Tempranillo plants strongly stimulated carbohydrate export in all treatments (Fig. 3), despite similar assimilation rates between fruiting and non-fruiting plants (Table 2). Therefore, these results suggest that the presence of a strong sink stimulates the source activity; not increasing photosynthesis, but increasing phloem-loading rate. Untreated fruiting Alfonso Lavallée plants showed similar percentages of export than untreated fruiting Tempranillo plants.

The imposition of moderate or severe water stress in Tempranillo resulted in progressive, although non-significant, reductions of carbon export out of the fed leaves (Fig. 3). By contrast, in Alfonso Lavallée plants, water stress resulted in a strong and highly significant reduction of export (Fig. 7).

Although it is generally assumed that carbon transport is reduced by water stress, these results suggest that water stress affects the source activity (photosynthesis) more strongly than it does than phloem translocation. It is commonly observed that the translocation process is more tolerant to water stress than photosynthesis, so that changes in the carbohydrate transport and partitioning usually occur at lower water potentials than reductions of photosynthesis (Wardlaw 1976; McPherson and Boyer 1977; Sung and Krieg 1979; Krieg 1983; Zrenner and Stitt 1991). However, a lower export rate could increase the accumulation of carbohydrates and lead to a decrease in the capacity for photosynthesis (Sharkey 1985). Quick *et al.* (1992) observed in four different species, including grapevine, a decreased rate of carbon export, maintaining high levels of sucrose in leaves, despite having lower rates of photosynthesis. In fact, sucrose accumulation could actually lead to a feedback inhibition of photosynthesis, which has been reported in some species (Azcón-Bieto 1983; Plaut *et al.* 1987; Krapp *et al.* 1991, 1993), but not in others (Woodrow and Berry 1987). In contrast to the observations by Quick *et al.* (1992), in fruiting plants of Tempranillo, the absolute amount of ^{14}C remaining in the

fed leaf after 24 h decreased relative to control plants by approximately 300% and 60% in moderately and severely stressed plants, respectively. Therefore, these results suggest that reductions of A_N are not caused by sugar accumulation in source leaf, nor even in the severely stressed plants. In these plants, reduced carbon assimilation under relatively high CO_2 (500–1000 $\mu\text{mol CO}_2 \text{ mol}^{-1}$) could suggest the presence of some non-stomatal limitations to photosynthesis. The present results are in agreement with previous suggestions that sugar accumulation in leaves is not a generalised limitation to photosynthesis in water stressed grapevines, especially in vines with fruits, which act as strong carbohydrate sinks (Downton and Grant 1992; Chaumont *et al.* 1994).

The reduction of ^{14}C export from the leaves in water stressed plants could be primarily due to reductions in carbon uptake in the labelling phase, but effects on phloem loading cannot be disregarded. The inhibition of sugar unloading by water stress has been reported in grape berries (Wang *et al.* 2003) and this could also explain the reduction in carbon export, especially in Alfonso Lavallée plants, where the results strongly suggest a significant water stress effect on sink activity (Fig. 8).

The differences between the results obtained with Tempranillo and Alfonso Lavallée could be cultivar-dependent, but may also be due to the degree of stress achieved. Dry mass partitioning among organs and the source–sink ratio were similar among cultivars and treatments. Also, the treatment application and environmental conditions were similar in both experiments. Nevertheless, in Alfonso Lavallée plants, total plant leaf area was 2.5-times that of Tempranillo, which resulted in a higher plant daily transpiration and lower Ψ values in water stressed plants than in severely water stressed Tempranillo (Tables 1, 3).

Effects of fruiting and water stress on the distribution of ^{14}C -assimilates among different plant organs in Tempranillo

In the absence of fruit, predominant sinks were the storage organs (roots, trunk and lower shoot, Fig. 4A). These three organs represented approximately 80% of total plant DM (Fig. 1A). Hence, the fact that they import up to 90% of total ^{14}C exported could be simply related to sink size rather than to a high sink activity of these organs. This pattern, which usually occurs during other stages of development, could play an essential role for future assimilate requirements in the following growth season, when the stored carbohydrates will be translocated to support the demand of the early stages of development (Mullins *et al.* 1992; Martínez de Toda 1991).

The presence of fruit in Tempranillo plants showed how strongly the translocation of assimilates is affected by sink–source relationships (Fig. 3). Even when total cluster DM accounts for only around 25% of plant dry mass (a percentage similar to trunk and roots, Fig. 1B), they

import up to 75% of total ^{14}C exported, relegating storage organs to a minor role as carbon sinks (Fig. 4B). During fruit set and later after veraison, translocation becomes markedly directed towards the clusters (Hale and Weaver 1962; Hunter and Visser 1988a), and during this period berries accumulate a large amount of sugars (Coombe 1992). This is supported by the present results, and expected from the water-potential-gradient mechanism, based on the low turgor of fruits after veraison (Lang and Thorpe 1989; Lang and Düring 1991). This characteristic makes the fruit a strong carbohydrate sink.

Some studies in woody plants have shown that water stress increases carbohydrate reserves in typical reserve organs: roots, trunk and shoots (Bradford and Hsiao 1982; Loescher *et al.* 1990). However, in the present study, no significant differences between treatments were found in any of the fractions of Tempranillo plants without fruit (Fig. 4A). In addition, although the presence of fruit notably changed the distribution pattern, the effects of water stress were only significant for a few fractions in Tempranillo plants with fruits. Significant differences between irrigated and water stressed plants were found in lower leaves and lower shoot fractions (Fig. 4B). This could indicate that the decline in photosynthesis and assimilate export, as well as a highly maintained fruit cluster sink demand, resulted in an increase of competition between low-priority sinks (e.g. lower leaves, lower shoot). An alternative explanation is that water stress reduced the kinetic constant of carbohydrate transport, thus resulting in an accumulation of labelled carbon in organs that are situated in intermediate steps of the phloem pathway. The maintenance of strong sink activity of fruits under water stress may be explained by the presence of a large water potential gradient between source and sink. This explanation is supported by the reduction in conductivity of the berry xylem vasculature from the time of onset of ripening (Düring *et al.* 1987; Findlay *et al.* 1987), which makes the fruit relatively independent of the plant water status.

Therefore, the present results suggest that, even when water stress affects directly the source activity (photosynthesis), the strength of the predominant sink was not significantly affected, resulting in similar patterns of distribution in all treatments (Fig. 4).

The metabolite fraction analysis in Tempranillo fruits suggested that water stress did not strongly alter the grape composition (Fig. 5). These results are in agreement with the high stability of fruit quality parameters under different water stress intensities that has been shown for this cultivar during a 10-year experiment under field conditions (Medrano *et al.* 2003). Potential wine quality is largely determined by the composition of the fruit. In field studies, it has been widely reported that moderate water deficits can increase the rate of sugar accumulation, but it is likely that the increases in sugar at the end of the season are mostly driven by lower berry expansion and by some berry dehydration,

rather than sugar production. In the present experiment, water stress led to some reduction of sugar accumulation and some increase of the water-insoluble fraction in the berry, even though differences between treatments were not statistically significant. In berries of control plants, absolute ^{14}C accumulation in the sugar fraction was more than three times that of water-stressed plants. It has been recently demonstrated that water stress applied to the vine inhibits sugar unloading in grape berries of cv. Shiraz (Wang *et al.* 2003). Nevertheless, small differences between percentages of ^{14}C exported to the cluster and those recovered in the sugar fraction of the fruit suggested that water stress reduced carbon assimilation rather than phloem transport rate or phloem unloading.

Effects of water stress on the distribution of ^{14}C -assimilates among different plant organs in Alfonso Lavallée

Fruits of control Alfonso Lavallée plants were the major carbohydrate sink, importing up to 80% of total ^{14}C exported (Fig. 8). As in Tempranillo, the strong sink activity of fruits cannot be attributed to sink size, since they account for only approximately 10% of total plant DM (Fig. 6A). However, in contrast to Tempranillo, the photosynthate distribution pattern in Alfonso Lavallée plants was strongly affected by water stress. Although fruits of stressed vines were still actively attracting photosynthates from leaves, they only imported 45% of total ^{14}C exported. However, under drought conditions, shoots, trunk and roots significantly increased the percentage of exported ^{14}C -labelled photosynthates and became important carbon sinks (Fig. 8). These changes in partitioning cannot be attributed to differences in sink size between treatments, since these were not significant (Fig. 6A); as expected from the short duration of the water stress treatment. Therefore, the observed differences should be attributed to other factors. Water-stressed Alfonso Lavallée plants reached water potential values substantially lower than those observed in severely stressed Tempranillo plants. Assuming that pre-dawn leaf water potential is close to the root water potential, a likely explanation for the observed differences is that the greater water potential gradient could increase translocation towards the trunk and root (Lang and Thorpe 1986; Lang and Düring 1991), as increases in carbohydrate reserves under drought conditions have been suggested in other woody plants (Bradford and Hsiao 1982; Loescher *et al.* 1990).

Concluding remarks

The present results show that water stress can affect not only photosynthesis, but also photoassimilate export out of the leaves, and photoassimilate transport and partitioning within the plant in *V. vinifera*. However, reduced carbon export seems to not account for feedback inhibition of photosynthesis in the two cultivars studied.

In Tempranillo, water stress caused important reductions on source activity (photosynthesis), while the assimilate export and sink activity was only slightly altered. By contrast, in cv. Alfonso Lavallée, water stress altered the partitioning pattern primarily through reductions in the strength of the main cluster concurrently with increases in strength of trunk and root sinks. A similar pattern of partitioning has already been suggested for other plant species (Wardlaw 1976), and reflects differential sensitivities to water stress between organs, with stronger accumulation of carbohydrates in sinks other than the cluster. These differences observed among cultivars may be caused by the large water potential gradient between source and sink attained in Alfonso Lavallée (Lang and Düring 1991).

Alternatively, these differences could be cultivar-dependent, and future studies should be developed to extend the knowledge of how water stress affects translocation and partitioning to other cultivars. It should also aim to search for closer links between these cultivar-dependent responses and the cultivar-dependent stability of fruit yield and quality under water stress conditions.

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