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RESEARCH PAPER

Growth, fructan yield, and quality of chicory (*Cichorium intybus* L.) as related to photosynthetic capacity, harvest time, and water regime

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Abstract

Fructans are polymers that are widely used in several industrial applications. In the last few years they have received increasing interest because of their positive effects on health. At present, fructans are mostly supplied by chicory, which is only grown and processed in The Netherlands, France, and Belgium. It would therefore be an attractive concept to expand its cultivation to the southern European countries, although water shortage and high temperatures may hinder its growth and yield. So far, few experiments have been carried out on the effects of water, so the present research was focused on the course of growth and fructan quality in rainfed (W_0) and well-watered (W_1) conditions. The positive effects of water restoration mostly concerned the above-ground dry weight (*ADW*), whereas the root dry weight (*RDW*) was less influenced. No significant differences on *RDW* were found in 1999, whereas it was 14% higher (P <0.01) in W_1 in 2000. The effect of water was very clear on assimilate allocation: the overall priority at the whole plant scale seemed to be root structures, then storage reserves, and finally *ADW*. Therefore, the fructan content was higher in W_0, and insignificant differences between W_0 and W_1 were found on fructan yield at the final harvests. The only significant effect of the water regime on fructans was to speed up their storage. The leaf photosynthetic capacity (*A*) was poorly affected by water availability, whereas it appeared consistently modulated by leaf temperature and leaf nitrogen content. Stomatal conductance appeared to be mostly affected by the soil water content and it was mostly related to *A* up to about 300 mmol m^{-2} s^{-1}. The fructan chain length (DP) was not affected by water regime. Besides, DP classes showed a normal statistical distribution; skewness and kurtosis significantly changed only when the harvest was very late. Equally, a very late harvest time significantly lowered DP.

Key words: Chicory, fructan, inulin, photosynthesis.

Introduction

Fructans are polydisperse polysaccharides consisting of β(2,6) fructosyl-fructose units with one glucose unit at the reducing end (Fuchs, 1990). Within the last several years, there has been a renewed interest in fructans as they are in increasing demand in several industrial applications such as detergents and pharmaceuticals (Fuchs, 1990). In addition, fructans have been found to have positive effects on digestive health. The ingestion of moderate amounts of fructans can promote a healthy digestive system as they pass intact through the stomach and reach the intestine where they promote the intestinal microflora (De Leenheer, 1994; Roberfroid and Delzenne, 1998). Fructans may also have beneficial effects on reducing post-prandial glycaemia, insulinaemia, triglyceridaemia, and total cholesterol level (Roberfroid and Delzenne, 1998).

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Abbreviations: *A*, leaf net photosynthesis; *ADW*, above-ground dry weight; DP, degree of polymerization; *Et*, evapotranspiration; *FC*, fructan content; *FSI*, free sugars index (free sugar content over total carbohydrate content); *FY*, fructan yield; g_s, stomatal conductance; GDD, growing degree days; K, kurtosis; *LAI*, leaf area index; *LDMC*, leaf dry matter content; *NAR*, net assimilation rate; N_{leaf}, leaf nitrogen content; *PI*, pure fructan index (total carbohydrate content expressed over the refractometer index); *RDW*, root dry weight; *RI*, refractometer index; *RUE*, radiation use efficiency; *RWC*, relative water content; *SLA*, specific leaf area; SK, Pearson's skewness; T_{leaf}, leaf temperature; TDR, time domain reflectometry; *VIF*, variance inflation factor; W_0, rain-fed plants; W_1, irrigated plants.

Despite the 36 000 fructan-containing species (Pignatelli, 1998), curently only three crops are practically processed: blue agave (for the tequila-distilling industry), Jerusalem artichoke, and chicory. The greater interest in chicory compared with Jerusalem artichoke is mainly due to its high fructan yield and quality (Meijer and Mathijssen, 1992). Chicory has been extensively grown in Europe since the beginning of the 19th century and today is by far the most commonly used, but nowadays it is only grown in The Netherlands, Belgium, and France on approximately 15 200 ha (De Bruyn *et al.*, 1992). It would therefore be an attractive possibility to expand its cultivation into southern European countries. However, Jerusalem artichoke was found to be quite tolerant to the water-stress conditions (Monti *et al.*, 2005), so chicory might be not the most cost-effective crop in southern Europe, especially when irrigation is not feasible. Moreover, Jerusalem artichokes accumulate fructans in their stems before their translocation into tubers, thus a summer harvest of stems, when soil conditions are generally more favourable, may be proposed for Jerusalem artichoke.

To the best of the authors' knowledge, many experiments have been performed on genotype screening, effects of harvest date, nitrogen supply, and plant density on root and fructan yields of chicory (Meijer and Mathijssen, 1992; Baert, 1997; Amaducci and Pritoni, 1998; Demeulemeester *et al.*, 1998; Desprez *et al.*, 1999; Monti *et al.*, 2002); it was, however, surprising to find that little is known about the effects of water stress. Moreover, the majority of the experiments on chicory was carried out in North-Central Europe where water availability and temperature do not generally represent serious constraints for growth. By contrast, the severe and durable water stress, that commonly occurs during the summer in southern European countries, could be strongly detrimental to chicory yield (Schittenhelm, 1999; Danuso, 2001). In very interesting research on fructan-containing crops, Schittenhelm (1999) found negative effects of water deficit, particularly on above-ground dry matter (leaves without crown). These results were confirmed by Skinner *et al.* (2002), although the authors never explored the effects of water deficit on fructan accumulation and degree of polymerization. The effect of high temperatures, which are known to inhibit photosynthesis and yield, was not examined (Salvucci and Crafts-Brandner, 2004; Wise *et al.*, 2004).

This research was therefore focused on the effects of water stress on fructan yield and their degree of polymerization (DP, i.e. the fructan chain length), that is a basic parameter for the industrial applications. Parallel to DP, the pure index (PI) and free sugar index (FSI) of fructan were also determined in order to assess the course of depolymerization over the growing season. The depolymerization process is modulated by two enzymes: fructan-exo-hydrolase (FEH) and fructosyl-fructosyl transferase (FFT) (Van den Ende and Van Laere, 2002) that remove terminal fructose residues from inulin chains resulting in shorter fructan chains. The activity of the two enzymes is mostly modulated by several factors such as temperature and sucrose availability (Van den Ende and Van Laere, 2002). In particular, FEH was mostly found to be active after leaf senescence (Khuri and John, 2000) which may be linked with sucrose availability. It is well known that water stress reduces photosynthetic rate and thus, possibly, the sucrose loading to the taproot; similarly, harvest time is related to leaf senescence.

The fructan average DP of a single chicory root (commonly 10–20) is the average of short (fewer than 10 units) and long fructan chains (about 60, Roberfroid and Delzenne, 1998). Therefore, the statistical distribution of DP classes is necessary to have a reliable DP profile into a root or sample. It is shown here that the shape of the statistical distribution of the DP classes depends on the harvest time and not the water regime.

Materials and methods

Experimental site and treatments

The experiments were carried out in a flat soil classified as Haplic Calcisol (FAO), in Bologna, Po Valley (Lat. 44° 03′, Long. 11° 02′, 33 masl) in 1999 and 2000. Rainfed (W_0) and well-watered (W_1) plants were compared according to a randomized block design with four replications. Sowing was carried out with a pneumatic seed drill placing seeds at 0.5 cm depth, with a row spacing of 45 cm. Thinning was carried out to a final plant population of 15 plants m^{-2}. A drip irrigation system was placed on every other row. Water restorations occurred whenever evapotranspiration (*Et*) reached 30 mm. *Et* was calculated daily by multiplying evaporation (class-A evaporation pan) by the specific crop coefficients (Allen *et al.*, 1998). The soil moisture contents of the W_0 and W_1 plots were measured weekly in three replications using the time-domain-reflectometry technique (TDR); moisture probes (CS-615, Campbell Sci., Leicestershire, UK) were placed vertically with sensors reaching 60 cm of soil depth. Biomass samples of each replication were hand-harvested over a square metre. Afterward, the most relevant morphological parameters, i.e. the leaf area, fresh and dry weights (after drying at 105 °C until constant weight) of taproots and leaves etc, were measured. An overview of the crop husbandry and sampling dates are summarized in Table 1.

Radiation use efficiency (*RUE*) was determined as the slope of the regression of the accumulated dry matter and accumulated absorbed photosynthetic active radiation (*PAR*a; Monteith, 1977). *PAR*a was obtained using the equation of Monsi and Saeki (1953) by the incoming *PAR*, leaf area index (*LAI*), and extinction coefficient (*k*). Leaf area was measured on all photosynthetically active leaves using the LI-3100C area meter (Li-Cor Inc.). Incoming *PAR* was determined by measuring the total solar radiation by placing a bi-metallic pyranograph (Robitzsch SO 2800, SIAP, Italy) near the crop, assuming that 50% of the solar radiation is *PAR* (Monteith and Unsworth, 1990). Since *k* of chicory was not found in the literature, that of lettuce (0.66) which is of a similar plant density and *LAI* was used (Tei *et al.*, 1996). Since chicory has a random azimuthal distribution of leaves the variation of *k* over time was ignored.

Leaf traits and gas-exchange measurements

Eight leaf gas-exchange measurements were performed from May to August of the second year using a portable infrared gas analyser

Table 1. *Crop husbandry of 1999 and 2000*

	1999	2000
Cultivar	Bergues	Bergues
Plot size (m^2)	36	36
Previous crop	Wheat	Wheat
Soil tillage	Plough (0.3 m)	Plough (0.3 m)
Seedbed preparation	Harrowing	Harrowing
Date of sowing	18 March	16 March
1000 seed weight (g)	11	11
Seed density (no. m^{-2})	40	40
Date of emergence	1 April	5 April
Weed control (hoeing)	14 April	22 April
Fungicide (kg ha^{-1})		
Forge	6+19 April	
TMTD[a]		Seed application
Thinning out	7 May	6 May
Plant density (no. m^{-2})	15	15
Irrigation (mm)	317	134
Fertilization (kg ha^{-1})		
P	43 (18 March)	43 (16 March)
N	100 (11 May)	100 (2 May)
Sampling dates	4, 11, 18, 25 May; 1, 8, 23 June; 13 July; 3, 31 August; 28 September; 12 October; 30 November	16, 30 May; 20 June; 18 July; 8 August; 5 September; 10 October; 7 November

[a] Tetramethylthiuram disulphide.

(IRGA, CIRAS-1, PP Systems, Hertfordshire, UK). Leaf photosynthetic capacity (*A*), stomatal conductance (g_s) and intercellular CO_2 (C_i) were calculated as given by von Caemmerer and Farquhar (1981). The leaf gas exchange data referred to the most recently fully expanded leaves up to 11.30 h in order to escape the midday depression on *A*. During the measurements, the average *PAR* was 1600±48 μmol (quanta) m^{-2} s^{-1}; the CO_2 concentration in the air entering the chamber was 350±11 μmol mol^{-1} (of air); the entering air flux was 250±4 cm^3 min^{-1}. Some functional leaf traits were also measured on the same leaves chosen for gas-exchange measurements in order to investigate their influence upon *A*: nitrogen content (N_{leaf}, Kjeldahl, 1883); the relative water content (*RWC*), i.e. the ratio of water contents in fresh to turgid leaves; the specific leaf area (*SLA*), i.e. the ratio of leaf area to leaf dry mass; the leaf dry matter content (*LDMC*).

Fructan analysis

Root fructan content was measured at each harvest time in 2000, but in 1999 it was only measured in the two final harvests. Root samples (about 500 g) were frozen (−18 °C) and milled in dry ice. Fructans were extracted in hot water (80 °C) for 60 min. Fructan content (*FC*) was determined as given by Baert (1997): $FC=(F+G)-(f+g+s)$; where *F* and *G*, are the total fructose and glucose after acid hydrolysis (HCl) and *f*, *g*, and *s* are the reducing free sugars fructose, glucose, and sucrose before the acid hydrolysis. The amount of reducing sugars was determined by HPLC (high-performance-liquid-chromatography). The column was Rezex 8% Ca (30×0.78 cm); column temperature was 75 °C. Water with a flow rate of 0.6 ml min^{-1} was used as the mobile phase. An analytical differential refractive index (*RI*) detector was used. The average fructan chain-length (*DP*), the pure fructan index (*PI*) and the free sugars index (*FSI*) were calculated as follow (Baert, 1997): $DP=F/G$; $PI=[(F+G)/RI]\times100$; $FSI=[(f+g+s)/(F+G)]\times100$, where *RI* (Brix %) is the refractive index of solids measured on taproot sap (Palm Abbe 200, Misco, Cleveland, OH). To determine the DP classes, the samples were extracted in a hot (80 °C) water–ethanol mix (33% v/v); afterwards a 40 mg subsample of the extract was diluted in 1 ml of distilled water and than analysed using a size-exclusion-chromatography. The DP classes were based on exclusion 30 s time-intervals.

Statistical analysis

All the measured and derived data were subjected to the analysis of variance (ANOVA) carried out with the Systat package (Systat Software Inc.). Bartlett's and Kolmogorov–Smirnov's tests were used, respectively, to verify the homogeneity of variance and the normal distribution of data. The relevance of leaf traits upon photosynthesis was tested by a sensitivity analysis using the adjusted multiple regression coefficient as the discriminating parameter. The variance inflation factor (*VIF*) was used to measure the multicollinearity, that is the 'inflation' of a regression parameter for an independent variable due to the redundant information in other independent variables. Briefly, the higher the *VIF*, the more redundant are the variables in the regression model.

The statistical distribution of fructan DP was assessed using the Kolmogorov–Smirnov's test. Pearson's skewness (SK), i.e. the ratio of the difference between mean and mode to standard deviation, was considered as an indicator of the asymmetric distribution: if the ratio of SK to its standard error was greater than 2 the distribution was considered asymmetric. A positive SK indicates a left long tail. Kurtosis (K) was taken as a measure of the flat distribution: negative K indicates a flatter shape. Again, K was considered significant when the ratio of K to its standard error was higher than 2.

Results

Weather data

The seasonal rainfall distribution varied between the two years. It was higher from April to May in 1999 (+93 l m^{-2}) and from June to August in 2000 (+88 l m^{-2}). Air temperatures was generally higher (on average by 2 °C) in 2000, especially in the early part of the cycle. Water deficit, i.e. the difference between *Et* and rainfall, during the treatment time (1068–2858 GDD and 882–2384 GDD, in 1999 and 2000, respectively) were similar in the two years: 374 l m^{-2} (1999) and 358 l m^{-2} (2000). Water table depth was also similar in the two years and was not affected by irrigation (from 1.4 to 2.3 m depth from the start to the end of treatment). Conversely, during the treatment the average soil water content of W_0 was significantly lower than that of W_1 (−4.7% and −4.0% (v/v), respectively, in 1999 and 2000).

Dry matter accumulation and photosynthesis

The consistently higher biomass yield of 1999 (Fig. 1) was probably explained by the early rainfall which speeded up the growth of the young roots and leaves with a consequently higher intercepted solar radiation and water supply; water table being more superficial in the early growing cycle of 1999. The effects of water regime was clearly more visible on above-ground dry weight (*ADW*) and leaf area index (*LAI*) than on root dry weight (*RDW*). *ADW* was, on average, 50% higher in W_1, whereas *RDW* was only 5% higher (Fig. 1). The early leaf area development allowed W_1 to absorb only 5% more incident photosynthetic active radiation (*PAR*) than W_0 (75% versus 80%). However, the

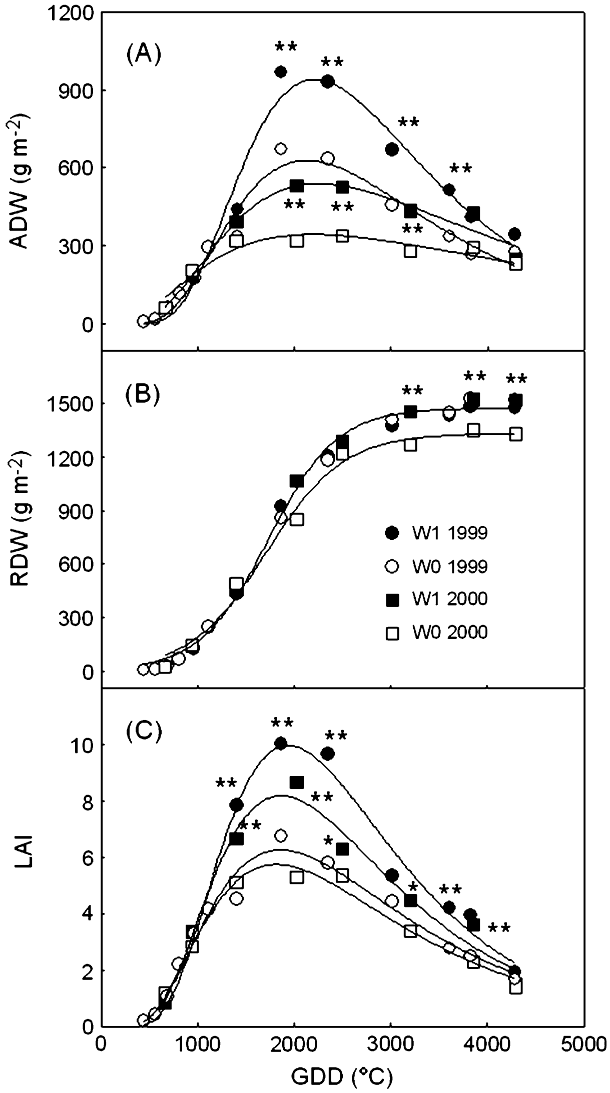

Fig. 1. Above-ground dry weight (*ADW*), root dry weight (*RDW*), and leaf area index (*LAI*) in 1999 and 2000. W_1 and W_0 represent well-watered and rainfed plants. GDD are the growing degree days (base temperature 0 °C). Log-normal equations with three parameters were used to fit *ADW* and *LAI* data; a sigmoid equation with three parameters was used to fit *RDW* data. Since the *RDW* data of W_1 and W_0 of 1999 and W_1 of 2000 were not statistically different, only one fitting on grouped data was done. All regressions were statistically significant with R^2 ranging from 0.96 to 0.99. Regression parameters were always statistically significant for $P \leqslant 0.05$; * and ** mean significant differences between treatments for $P \leqslant 0.05$ and 0.01, respectively.

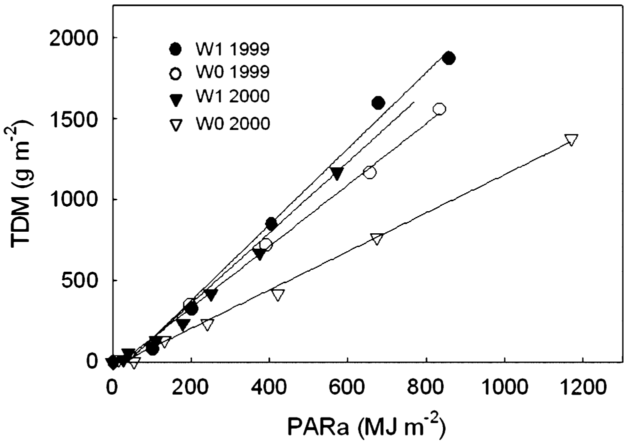

Fig. 2. Relationships between cumulative dry matter and absorbed *PAR* by the crop canopies in the years 1999 and 2000. The slopes of these linear regressions represent the radiation use efficiency (*RUE*, g MJ^{-1}) of W_0 and W_1. Fitted equations are: $y=2.34x-92.5$, $r^2=0.98$; $y=2.18x-77.9$, $r^2=0.99$; $y=2.04x-59.2$, $r^2=0.97$; $y=1.89x-44.2$, $r^2=0.98$.

Table 2. *Correlation matrix among leaf traits (upper part of the table) and sensitivity analysis (lower part)*

* and ** mean statistical significance for P <0.05 and 0.01. Adjusted R^2 measures the ability of variables to predict the leaf photosynthesis. Thus from up to down, a forward stepwise for the best subset is shown. That is the number of + symbols in the same row mean the number of variables included in the multiple regression model, and the increasing of adj. R^2 represents the variable contribution in predicting *A*. Variance Inflation Factor (*VIF*) is the estimate of multicollinearity: variables with values larger than four are considered too redundant.

	g_s	T_{leaf}	*SLA*	*LDMC*	N_{leaf}	*RWC*	Adj. R^2
A	0.38*	−0.84**	0.66**	−0.67**	0.70**	−0.61**	
g_s		−0.37*	0.03	0.02	0.11	0.19	
T_{leaf}			−0.65**	0.74**	−0.26	0.59**	
SLA				−0.85**	0.60**	−0.76**	
LDMC					−0.66**	0.79**	
N_{leaf}						−0.67**	
RWC							
Sensitivity analysis		+					0.70
		+			+		0.78
	+	+			+		0.80
		+		+	+		0.82
	+	+		+	+		0.83
	+	+	+	+	+		0.84
	+	+	+	+	+	+	0.84
VIF	1.9	1.6	29.2	6.8	1.4	4.4	

radiation use efficiency (*RUE*), which ranged from 2.6 g MJ^{-1} to 1.9 g MJ^{-1}, was always clearly higher in W_1 (Fig. 2).

The leaf net photosynthesis (*A*) was poorly influenced by the water regime. Leaf photosynthesis appeared significantly correlated to nearly all the leaf traits (Table 2), thus a sensitivity analysis was performed to find the traits that had the most influence upon *A*. T_{leaf} appeared the most relevant factor, explaining 70% of the total *A* variation. N_{leaf} added 8% to the predicting capacity, whereas all the other variables considered had little influence upon *A* (Table 2). T_{leaf} showed a close linear relation with *A* within the explored temperatures (from 28 to 38 °C) (Fig. 3). Conversely, it was unexpected to find g_s weakly correlated to *A*. However, the Pearson's correlation coefficient measures the linear predictability, while a clear non-linear association between *A* and g_s was observed. Specifically, a strong correlation was found between *A* and g_s up to 300 mmol m^{-2} s^{-1}, whereas the correlation was much weaker above this threshold (Fig. 3). Finally, g_s appeared not to be significantly related to the other leaf traits, whereas it was

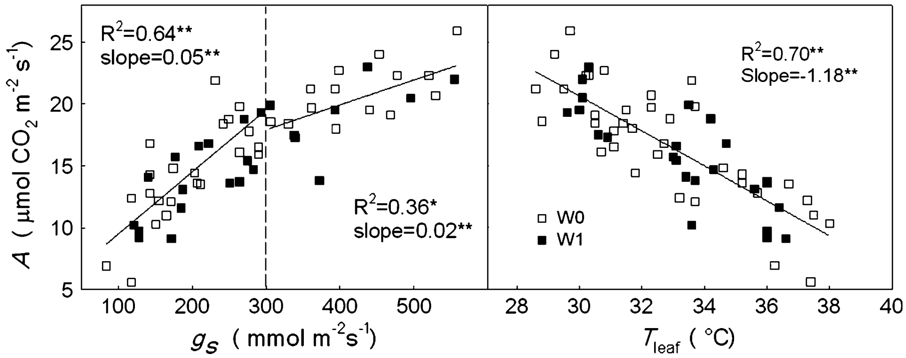

Fig. 3. Relationships between leaf net photosynthesis (A) and stomatal conductance (g_s, left) and leaf temperature (T_{leaf}, right). Two separate regressions A versus g_s were fitted below and over 300 mmol m^{-2} s^{-1}. W_0 and W_1 represent rainfed and well-watered plants, respectively. R^2 is the regression coefficient; * and ** mean significance level for $P \leqslant 0.05$ and 0.01.

affected by soil drying (r=0.69**). Thus, soil drying somewhat modulated A as well (r=0.47*).

Fructan accumulation

The significant differences in fructan contents (FC) and fructan yields (FY) between W_0 and W_1 were limited to the three first irrigations, W_1 showing a faster fructan accumulation than W_0 (Fig. 4). At about 2000 thermal units, FC was 42% higher in W_1, while FY was still higher (78%) due to the higher RDW of W_1. However, a few days later (2500 GDD) W_0 and W_1 showed similar FY values, and at the last harvest no significant differences were found between treatments or years.

Water regime did not significantly modify the maximum DP that was 13.7 in W_0 and 14.2 in W_1. Since DP was strictly correlated with FC (r=0.92**), the maximum DP occurred 60 d earlier in W_1.

Since DP merely represents the average value of fructan chain length, the statistical distribution of DP classes around the mean was also investigated. DP strongly varied from 2 to more than 100, showing a natural trend platykurtic distribution of the DP classes (negative kurtosis). Generally, the statistical distribution of DP classes did not deviate from the Gaussian curve (Fig. 5). Water regime did not affect the Pearson's skewness (SK) and K. Conversely, harvest time appeared to have a strong influence upon the fructan chain length and the statistical distribution of DP (Fig. 5). Specifically, during the final harvest of 1999, the average DP decreased from 12 to less than 4, while the DP distribution showed a significant low K (−1.94), i.e. a long right tail. This only included 36% of the total DP values in the two central classes, compared with 47–49% at the other harvest times in the same or in the following year.

The reducing free sugars were measured at each sampling date and represented as pure (PI) and free sugar indices (FSI). FSI and PI indices appeared neither correlated to each other nor changing over time and treatments. FSI ranged from 4% to 7%; PI from 85% to 89%. Similar values were found also by Baert (1997). Sucrose, glucose, and fructose hardly changed over the growing season, being less than 2%. Free sugars were also significantly related to the refractometer index (RI) (r=0.83**, 0.91**, and 0.63*, respectively, for sucrose, glucose, and fructose), that increased over time from 8.5 (900 GDD) to 13.6 °Brix (4300 GDD). Unfortunately, RI appeared to be ineffective in predicting DP or FC.

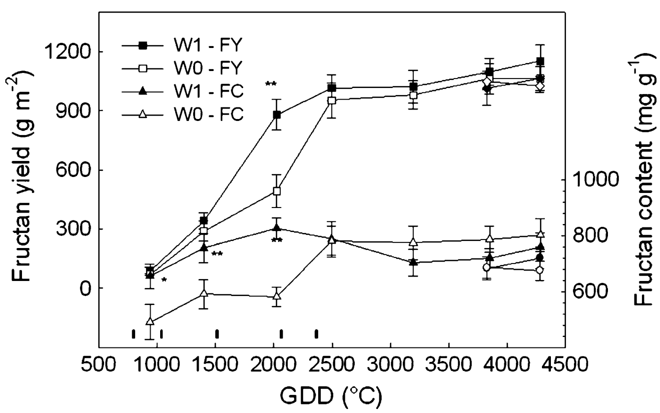

Fig. 4. Course of fructan yield (FY) and fructan content (FC). Circle and diamonds (filled symbols = W_1; unfilled symbols = W_0) represent FY and FC measured in the two last harvests of 1999. Vertical bars are the standard error of means; * and ** indicate the statistically significant difference between means for $P \leqslant 0.05$ and 0.01, respectively. Vertical marks correspond to each irrigation day.

Discussion

According to Hsiao (1973), the effects of the higher water availability were mostly on the above-ground biomass. Conversely, root growth was hardly affected by water regimes. W_0 allocated higher proportional amounts of assimilates to the taproot than W_1 which, in turn, showed 50% higher source. By contrast, fructan accumulation was much faster in W_1 than W_0. Therefore, even if the mechanism of assimilate distribution among the different biomass

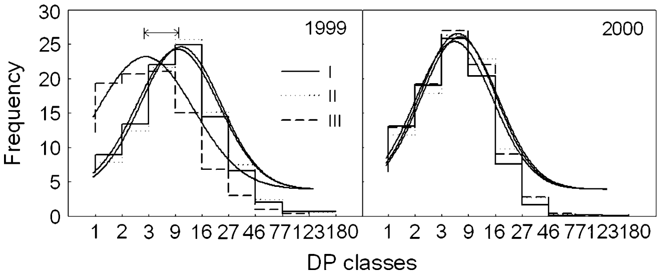

Fig. 5. Statistical distribution of the nine DP categories (classes) obtained with an exclusion time-interval of 30 s. Each histogram represents a class of DP during the three last harvests of 1999 an 2000. Gaussian curves fitted on real data were highly representative, except for the last harvest of 1999 which also determined a DP reduction. I, II, and III represent the three harvest times of 1999 and 2000: 1 September, 12 October, and 1 December 1999; 6 September, 10 October, and 6 November 2000.

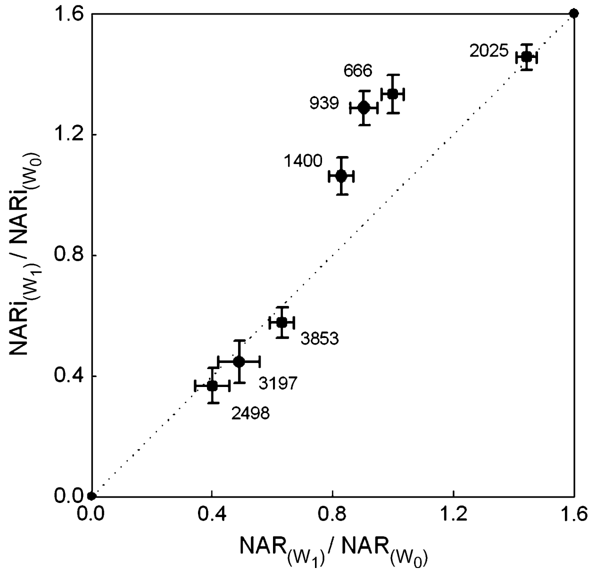

Fig. 6. Effect of water restoration weighted on total dry matter or fructans. *NAR*i and *NAR* are the net assimilation rate weighted on fructans and dry matter, respectively. W_1 and W_0 represent well-watered and rainfed plants. The distance of points from the dashed line (1:1 ratio) is a measure of the different effect of water restoration on total dry matter and fructan accumulation. Thus there is a strong effect of water regime on fructans in the early period (666, 939, and 1400). Numbers beside each point indicate the corresponding growing degree days (GDD). Vertical and horizontal bars are the mean standard errors of *NAR*i and *NAR* ratios.

parts is still not well known, the order of priority at the whole plant level of chicory seems to be root structural parts, then reserves (fructan), and finally the above-ground biomass. Therefore, it may be supposed that the larger source of W_1 triggered off a fast fructan loading, afterward the assimilates were used to form new leaves when the sink became limiting. The approach of this research was at too large a scale to allow a more detailed mechanistic explanation of the source–sink relationships.

Radiation use efficiency was higher in W_1. However, despite the higher biomass yield of W_1 the intercepted *PAR* of W_1 was only 5% higher than W_0 (+32 and +57 MJ m^{-2} in 1999 and 2000, respectively). Therefore, as given by *RUE*, W_1 was expected to accumulate 83 g m^{-2} (1999) and 148 g m^{-2} (2000) more dry biomass than W_0, i.e. 20% and 50% less than that measured on total accumulated biomass. A higher photosynthetic capacity of W_1 was therefore suspected, but this was only weakly demonstrated by the leaf gas exchange measurements.

The effect of water regime was more evident on marketable products (fructans) than on total dry matter. In fact, when *RUE* was weighted on fructans instead of the total dry matter, it appears very similar in W_0 and W_1 (1.0 and 1.1 g (fructan) MJ^{-1}). Similarly, the net assimilation rate (*NAR*) weighted on *FY* (i.e. by multiplying *NAR* per *FC*) appeared frequently higher in W_0 (Fig. 6). Therefore, all things being equal, the crop effectiveness on fructan accumulation was not reduced by the water deficit.

Among the leaf traits, T_{leaf} showed the highest influence upon *A*. For every additional degree of the explored T_{leaf} the photosynthetic capacity was expected to be reduced by 1.4 μmol m^{-2} s^{-1}, thus 56% less photosynthetic capacity was observed from 28–38 °C. The negative effects of high T_{leaf} were found both in the early and late parts of the growing cycle, therefore the suspected combined effects of T_{leaf} and age were not confirmed. Conversely, a lower N_{leaf} was generally found in old leaves that showed a low photosynthetic capacity as well. Thus a linear regression model could be considered between *A* (dependent variable) and T_{leaf} (driving variable), whereas N_{leaf} seemed simply to run in parallel to *A*. However, although the effects of N_{leaf} on the carboxylation activity of Rubisco is largely known (Sage and Pearcy, 1987), poor mechanistic explanations may be given in this research as the N_{leaf} and leaf ageing effects cannot be separated. Still, as g_s is poorly correlated with T_{leaf} over 33 °C while *A* still decreased up to 38 °C, non-stomatal limitations of high T_{leaf} upon *A* seemed highly probable (Farquhar and Sharkey, 1982). Since 54% (1999) and 47% (2000) of days showed temperatures over 28 °C degrees during the growing season, summer temperatures might be a basic constraint for chicory grown in this area, independently from the water regime.

Generally, the DP did not appear affected statistically by the water regime and the harvest time. The only exception concerned the final harvest of 1999 that significantly lowered the DP. This was not found in the final harvest of 2000 which, however, was performed one month earlier than in 1999. Since the depolymerization process is modulated by the enzymes FEH and FFT, that are mostly active when low temperatures occur and no more sucrose is provided (Van den Ende and Van Laere, 2002; Denoroy, 1996), it was not surprising to find a strong reduction of DP in 1999 since this harvest occurred when night temperatures were low and many days after total leaf senescence. This study's results were corroborated by those of Wilson *et al.* (2004) that found the DP>20 category to decline from 23% to 13% a few days after the first frost.

To sum up, despite the fact that water availability consistently increased biomass accumulation, water restoration seems not to be worthwhile in this area. *RUE* and *NAR* weighed on fructan clearly showed the reserves to override the above-ground structural parts. Thus the dry biomass accumulation did not run in parallel to the fructan storage, that was not significantly lower in rainfed conditions. The only significant effect of water regime was to speed up the fructan accumulation, probably related to the sink-to-source ratio. The harvest time was found to be strongly detrimental of the quality, both on fructan chain length and normal distribution of chain classes. Among the leaf traits, temperature appeared to be the most influencing parameter upon photosynthetic capacity. Therefore, since durable high temperatures generally occur in this region during the growing cycle, they may represent a basic constraint for chicory yield, independently from the water regime.

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