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Note

Leaf gas exchange and carbohydrate concentrations in *Pinus pinaster* plants subjected to elevated CO_2 and a soil drying cycle

Catherine Picon-Cochard*, Jean-Marc Guehl

Unité de recherches en écophysiologie forestière, Équipe bioclimatologie-écophysiologie, Inra Nancy, 54280 Champenoux, France

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Abstract – Plants of maritime pine (*Pinus pinaster* Ait.) were acclimated for 2 years under ambient (350 µmol mol^{-1}) and elevated (700 µmol mol^{-1}) CO_2 concentrations ([CO_2]). In the summer of the second growing season, the plants were subjected to a soil drying cycle for 6 days. Drought reduced plant transpiration rate and net CO_2 assimilation rate (A) by about 80 %. Elevated [CO_2] induced a substantial increase of A (+105 % and +229 % in well-watered and in droughted plants, respectively) and of the needle starch (+145 %) and sucrose (+20 %) concentrations, whatever the watering regime. Drought did not significantly affect starch and sucrose concentrations, while hexose concentrations were slightly increased in the most severe drought condition (predawn water potential value equal to –1.5 MPa). The stimulating effect of elevated [CO_2] on A was maintained along the drying cycle, whereas no significant CO_2 effect was observed on the soluble carbohydrate concentration. These compounds did not contribute to an enhancement of osmotic adjustment under elevated [CO_2] in *P. pinaster*. (© Inra/Elsevier, Paris.)

elevated [CO_2] / drought / leaf gas exchange / carbohydrate / *Pinus pinaster*

Résumé – Échanges gazeux foliaires et concentrations en glucides de plants de *Pinus pinaster* soumis à un enrichissement en CO_2 de l'air et à un dessèchement du sol. Des semis de pin maritime (*Pinus pinaster* Ait.) ont été acclimatés pendant deux ans à 350 et à 700 µmol mol^{-1} de concentrations en CO_2 atmosphérique [CO_2]. Au cours de l'été de la deuxième saison de croissance, les plants ont été soumis à un dessèchement du sol pendant 6 j. La sécheresse a réduit d'environ 80 % la transpiration de la plante entière et l'assimilation nette de CO_2 (A). L'enrichissement en CO_2 de l'air a induit une augmentation marquée de l'assimilation nette de CO_2 (+105 % et +229 % en conditions de bonne alimentation hydrique et de sécheresse, respectivement), ainsi que des concentrations en amidon (+145 %) et en saccharose (+20 %), quelle que soit l'alimentation hydrique. Le traitement sécheresse n'a pas significativement affecté les concentrations en amidon et en saccharose, tandis que les concentrations en hexoses ont légèrement augmenté en condition de sécheresse sévère (valeur du potentiel hydrique de base égale à –1.5 MPa). L'effet stimulant de la [CO_2] sur A était maintenu au cours du dessèchement du sol, alors que cela n'était pas observé pour la concentration en glucides solubles. Ces composés ne contribuent pas à une augmentation de l'ajustement osmotique par l'enrichissement en CO_2 de l'air chez *P. pinaster*. (© Inra/Elsevier, Paris.)

enrichissement en CO_2 / sécheresse / échanges gazeux foliaires / glucides / *Pinus pinaster*

1. INTRODUCTION

Maritime pine (*Pinus pinaster* Ait.) is recognised as a drought-avoiding species with a high stomatal sensitivity to soil drought, since stomatal closure occurs before any alteration of leaf water status [6, 12]. Other regulation mechanisms may postpone water deficit effects on plant physiology, for example the maintenance of an active root growth whereas the aerial growth is reduced or stopped. At the cellular level, osmotic adjustment maintains the turgor pressure by increasing the produc-

* Correspondence and reprints
picon@clermont.inra.fr

tion of solutes, particularly organic compounds such as non-structural soluble carbohydrates (mainly glucose, fructose and sucrose) [7].

Elevated atmospheric CO_2 concentration ($[CO_2]$) generally stimulates the CO_2 assimilation rate (A) and decreases – or has no effect on – stomatal conductance in tree species [2, 4, 8]. The stimulation of A often induces starch and/or soluble carbohydrate accumulation in leaves. The analysis of the interactive effects of elevated $[CO_2]$ and drought on leaf carbohydrate concentration is particularly relevant because it was suggested that elevated $[CO_2]$ may improve drought tolerance by solute accumulation that contributes to osmotic adjustment [3]. However, few experiments have been carried out to test this hypothesis. The results concerned mainly deciduous broad-leaved species such as *Acer saccharum*, *Liquidambar styraciflua*, *Platanus occidentalis* [18] and *Quercus robur* [13, 19]. We found only one paper reporting results on a coniferous species, *Pinus taeda* [17]. Only in roots of *P. occidentalis* [18] and in leaves of *Q. robur* [13, 19] was the positive effect of drought on soluble carbohydrate concentration more pronounced under elevated than under ambient $[CO_2]$.

In a previous experiment on *P. pinaster* [12], the stimulation of CO_2 assimilation rate under elevated $[CO_2]$ was maintained along a drying cycle, but leaf carbohydrate concentrations were not assessed. In the present study, *P. pinaster* plants were grown under the interactive effects of elevated $[CO_2]$ and drought and the following specific questions were addressed: 1) Will drought induce an accumulation in soluble carbohydrates even though stomatal conductance and CO_2 assimilation rate are markedly lowered? 2) Will the stimulation of CO_2 assimilation rate by elevated $[CO_2]$ induce a carbohydrate accumulation contributing to osmoregulation and will this effect hold in droughted conditions as it was observed in the drought tolerant species *Q. robur* [12, 13], which is characterized by a lesser sensitivity of stomata to drought?

2. MATERIALS AND METHODS

2.1. Plant material and growing conditions

In March 1994, seeds of *Pinus pinaster* Ait., provenance Landes (southwest France), were individually germinated in 1 L cylindrical containers filled with a peat and sand mixture (1/1; v/v). The plants were placed in two transparent (50 µm thick, 80 % light transmission) polypropylene tunnels (5 m x 3 m x 2.3 m) located in a glasshouse. In the tunnels, the CO_2 concentration ($[CO_2]$) was maintained at 350 ± 30 and 700 ± 50 µmol mol^{-1} by an injection of CO_2 from a cylinder (100 % CO_2). A more complete description of this system is given in Picon et al [13]. Air temperature (T_a), photosynthetic photon flux density (I_p) and vapour pressure deficit (VPD) inside the tunnels were measured continuously. T_a ranged from 10 °C (minimum night temperature) to 31 °C (maximum diurnal temperature) during the experimental period. VPD ranged from 7 to 31.5 hPa during the day. The plants were grown under natural photoperiod. In sunny conditions, I_p was about 1 200 µmol m^{-2} s^{-1} at plant level (upper leaves). Plants were rotated between the two tunnels every month and the $[CO_2]$ were switched accordingly between tunnels. Linear regressions between the two tunnels determined for T_a, I_p and VPD were not different ($P < 0.05$) from 1:1 lines.

In December 1994, the plants were transplanted in 3 L containers filled with the same substrate as described above. At the same time and in June 1995, a complete fertilisation (5 kg m^{-3} of slow release fertiliser, Nutricote; N, P, K; 13, 13, 13, + trace elements) was given to provide adequate nutrition conditions.

From the beginning of the experiment, ten plants grown under 350 µmol mol^{-1} and ten plants grown under 700 µmol mol^{-1} were watered with deionized water every day or every 2nd day to restore soil water content to field capacity. On 6 July 1995 (day of year [DOY] 187), six plants per CO_2 treatment were subjected to a soil drying cycle by withholding water supply for 6 days. These plants were rewatered on 12 July (DOY 193) and kept well-watered until the end of the experiment, i.e. on 9 October (DOY 252). Soil water content was controlled by weighing the pots every day or every 2nd day and soil water evaporation was limited by covering the soil surface with waxed cardboard disks. Predawn leaf water potential (Ψ_{wp}, MPa) was measured four times during the soil drying cycle with a Scholander chamber on the 1-year-old needles ($n = 4$ to 6).

2.2. Gas-exchange measurements

Carbon dioxide assimilation rate (A, µmol m^{-2} s^{-1}) was measured in situ in the two CO_2 treatments with a portable system (Li6200; LiCor, Inc., Lincoln, NE, USA). Between 1200 and 1300 hours (solar time), four 1-year-old pseudophylls were enclosed into the 1 L chamber of the Li6200. The needles were placed across the width of the chamber in order to have a fixed leaf area. Measurements were made daily on four plants that were well-watered and on six plants that were subjected to drought in each $[CO_2]$. Two distinct measurements were made per plant. The carbon dioxide assimilation rate was related to the total external needle surface by multiplying the projected area by 2.57, because the needles were assimilated to a semi-cylinder. During the

measurements, the photosynthetic active radiation (PAR) values ranged from 900 to 1 200 µmol m^{-2} s^{-1}; air temperature from 28 to 32 °C; VPD about 28.9 hPa and the atmospheric [CO_2] 380.2 ± 1.1 µmol mol^{-1} and 707.7 ± 2.5 µmol mol^{-1}.

2.3. Leaf carbohydrate analyses

Needles were collected from DOY 188 to DOY 200 at predawn (0300 hours solar time), except for DOY 190, and in the afternoon (1500 hours solar time) on the needles used for Ψ_{wp} and gas-exchange measurements, respectively. After collection, the needles were cut and rapidly frozen in liquid nitrogen and stored at −18 °C.

Two to four needles (corresponding to 2–8 cm^2 projected needle area) were boiled at 80 °C for 30 min in 5 mL of aqueous ethanol 80 % (v/v). After rapid cooling, 1 mL of the soluble fraction was purified with 5 mg activated charcoal by centrifugation for 2 min (Sigma St Louis, USA, 201 M, 12 620 *g*). Thirty µL of the supernatant were used for glucose, fructose and sucrose enzymatic assays with a sequential analysis described by Stitt et al. [15, 16].

The colourless needles were then smashed in liquid nitrogen, washed and centrifuged three times (3 min, 12 620 *g*) with 1 mL of nanopure water. After 3 h of autoclave (120 °C, 1 bar, SanoClav), 100 µL of the extracted solution were reacted 14 h with a-amylase and amyloglucosidase (Boehringer Mannheim, Basel, Switzerland) at 37 °C in order to digest starch in glucose molecules, and assayed as for glucose.

The optical density of reduced nicotinamide-adenine dinucleotide phosphate (NADPH) was measured at 340 nm using a Jobin Yvon Hitachi 100-60 spectrophotometer Spex, Paris, France. The results were expressed in µmol of hexose equivalents per cm^2 (projected area).

3. RESULTS AND DISCUSSION

Global radiation and air temperature were very variable during the experimental period which caused important fluctuations of soil water content (SWC) and plant transpiration rate (*figure 1*). Four days after the drought onset, plant transpiration rate and CO_2 assimilation rate were reduced by about 80 % (*figures 1* and *2*), as expected for a drought-avoiding species.

Drought increased hexose concentrations only during severe stress (Ψ_{wp} = −1.5 MPa on DOY 191) whereas sucrose and starch afternoon concentrations values were not significantly affected ($P > 0.05$) (*table I*). For these two carbohydrates, the predawn values matched those of the afternoon on DOY 191 in both [CO_2] (*figure 3*), suggesting a decrease of leaf carbohydrate export rate. However, there was neither an accumulation of soluble carbohydrates nor a starch depletion in needles during the drying cycle (*table I*). Thus, in *P. pinaster*, no clear shift in the partitioning between carbon pools occurred during drought as it was observed in the drought-tolerant oak species [1, 5, 11]. These results may suggest that *P. pinaster* needles do not display osmotic adjustment in response to drought. However, the duration and the intensity of the drought treatment play an important role in the intensity of cellular osmotic adjustment [7]. In our experiment, pronounced drought conditions were induced over a short period (about 6 days) and it took about 1 week for A and plant transpiration rate to recover the pre-stress values (*figures 1* and *2*).

In contrast to our results obtained on needles, Nguyen and Lamant [9, 10] found osmotic adjustment of about 0.3 MPa, by a two-fold increase of pinitol in fine roots of *P. pinaster* seedlings grown in mineral solution, as it was also mentioned by Popp and Smirnoff [14] in *Cajanus cajan*. Can results obtained in such conditions extrapolate to more realistic drought induction situations? Measuring the osmotic potential at full turgor in needles or in fine roots of *P. pinaster* subjected to soil and climatic conditions similar to ours, Wartinger, Garbaye and Guehl (personal communication) did not observe any osmotic adjustment when a long-lasting soil drought was applied, whatever the [CO_2].

Increasing [CO_2] induced a large increase of A (+105 % and +229 % in well-watered and in droughted conditions, respectively). This stimulation was maintained along the soil drying cycle even at the lower values of Ψ_{wp} (*figure 2*), as it was observed in the same species by Picon et al. [12]. This effect was not linked to higher values of leaf water potential either measured at dawn (*figure 1*) or in the afternoon (data not shown). Despite this sharp stimulation of A in droughted conditions, we did not observe a significant [CO_2]-promoted increase of hexose or sucrose concentrations as shown by the absence of CO_2 x drought interaction (*figure 3, table I*). It is also noteworthy that the higher needle starch concentrations induced by elevated [CO_2] in *P. pinaster* did not lead to significant hydrolysis (i.e. decreasing starch concentration) during drought. This result is in contrast with the results we obtained in *Q. robur* for which the positive effect of drought on soluble carbohydrate concentration was more pronounced under elevated than under ambient [CO_2] [13].

In conclusion, we showed that increasing the atmospheric [CO_2] increased the CO_2 assimilation rate and needle starch concentration all along the soil drying cycle in *P. pinaster*. However, in this drought-avoiding

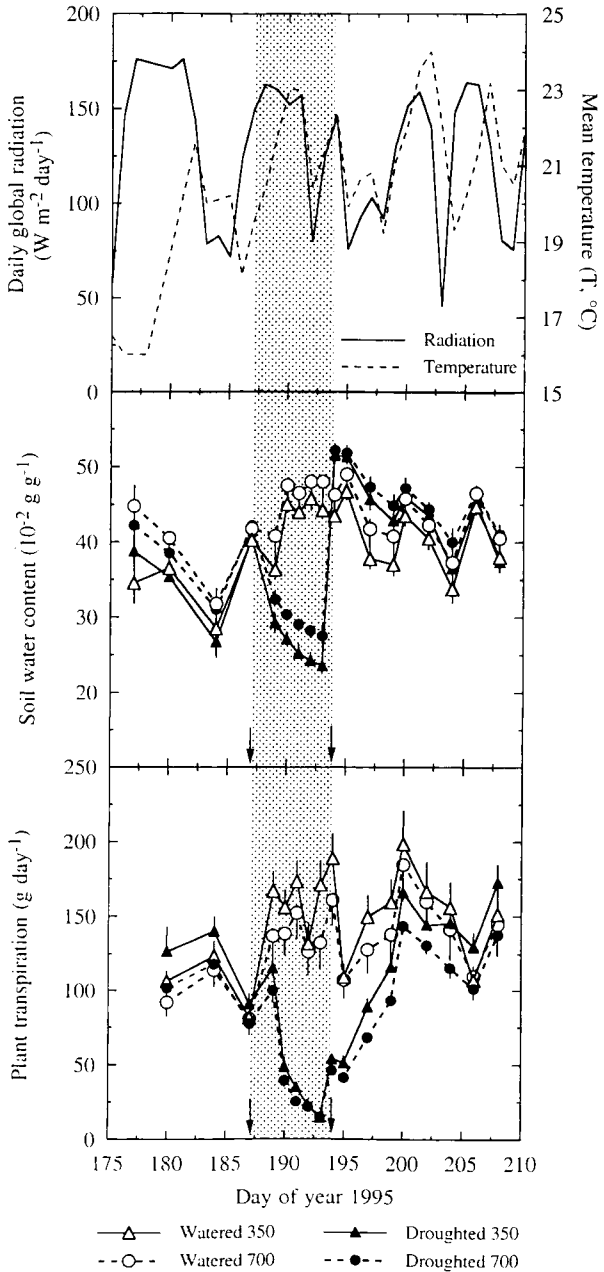

Figure 1. Time courses of daily global radiation and mean air temperature of the tunnels, soil water content and plant transpiration of *Pinus pinaster* plants in the different treatments during the experiment. Vertical bars denote 1 SEM (n = 4 to 6). The arrows and the dashed area correspond to the onset of the drought treatment and to the rewatering of the plants and to the drought period, respectively.

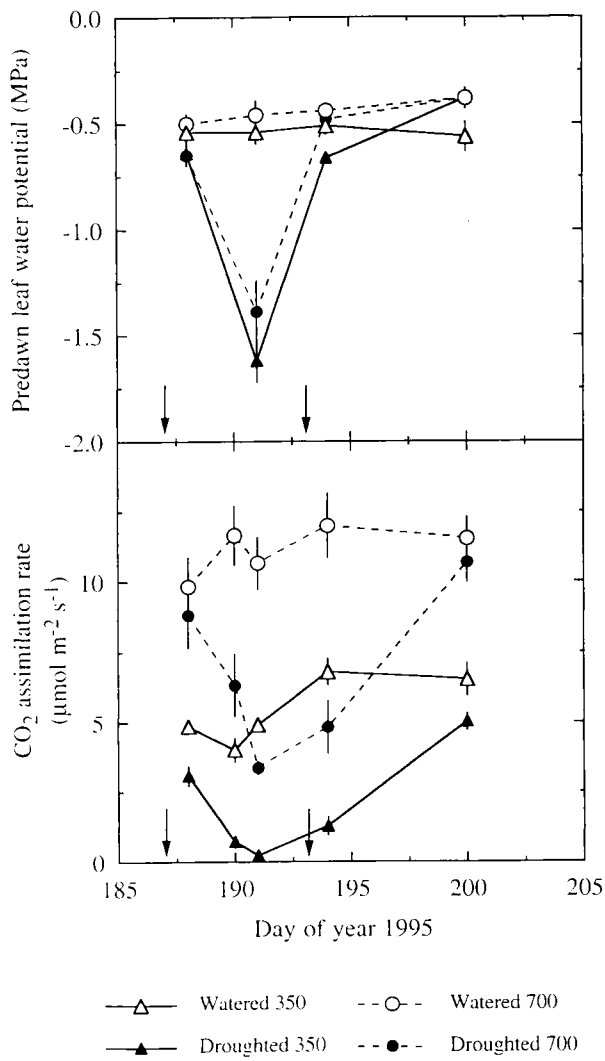

Figure 2. Predawn leaf water potential (Ψ_{wp}) and net CO_2 assimilation rate (A) of *Pinus pinaster* plants in the different treatments during the drying cycle. Ψ_{wp} was not measured on day of year (DOY) 190. Vertical bars denote ± 1 SEM (n = 4 to 6). The arrows corresponded to the onset of the drought treatment and to the rewatering of the plants.

species, no soluble carbohydrate accumulation occurred in the needles, contrary to the observations made in similar experimental conditions for leaves of *Q. robur* [13], a drought-tolerant species. These results may emphasize major differences between the two species for osmotic adjustment in response to elevated [CO_2] which could be of importance for their drought tolerance in the context of global change. Whether this difference between species can be generalised to drought-avoiding and drought-tolerant species is still an open question.

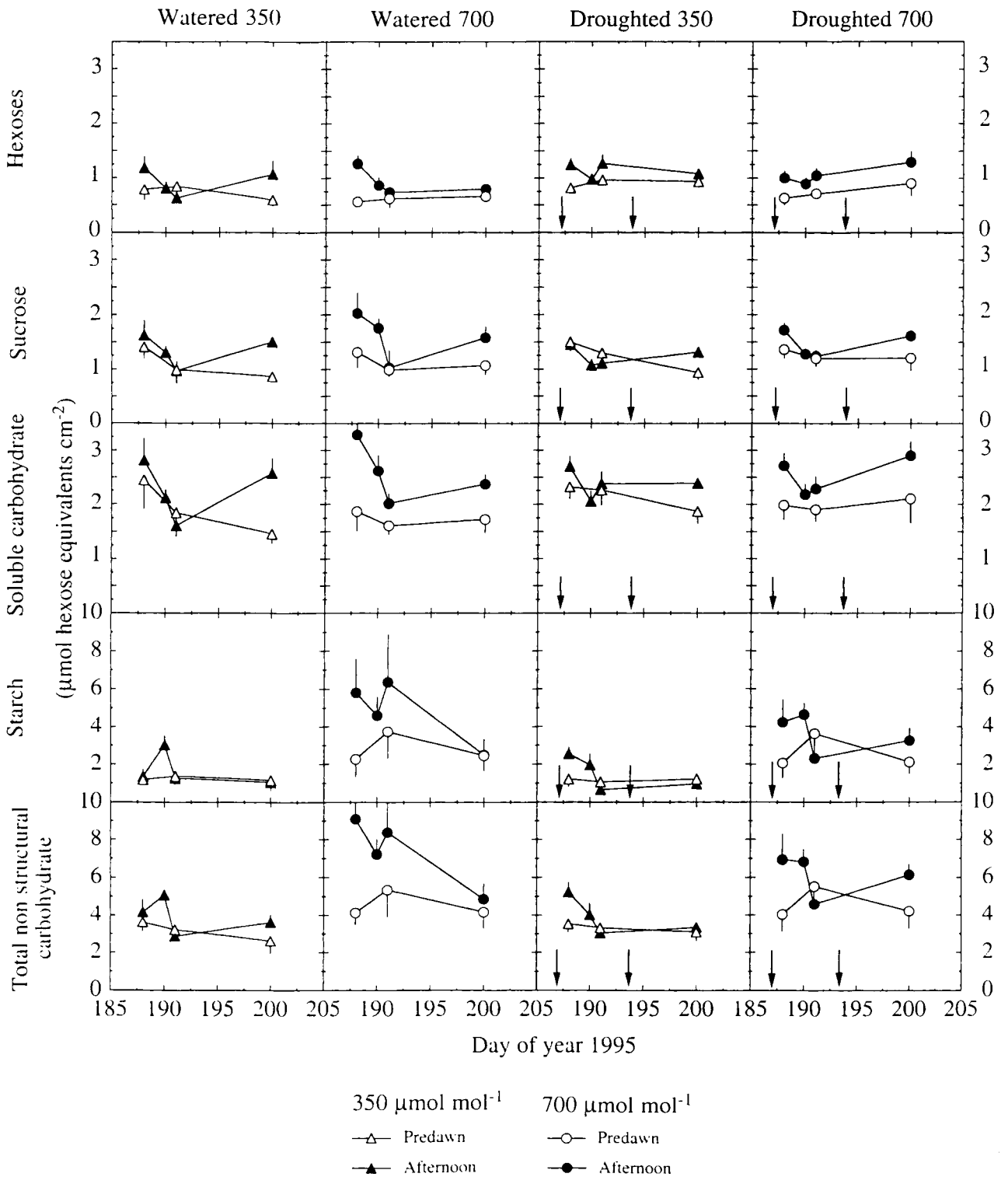

Figure 3. Hexose (glucose + fructose), sucrose, soluble carbohydrate (hexoses + sucrose) and starch concentrations (μmol hexose equivalent cm^{-2}) of 1-year-old needles harvested at predawn (open symbols) and in the afternoon (closed symbols) of *Pinus pinaster* plants in the different treatments during the drying cycle. Vertical bars denote 1 SEM (n = 4 to 6). The arrows correspond to the onset of the drought treatment and to the rewatering of the plants.

Table I. Variance analysis (ANOVA) for the effect of CO_2 concentrations ($[CO_2]$) and drought on leaf predawn water potential (Ψ_{wp}), CO_2 assimilation rate (A), hexose (glucose + fructose), sucrose, starch, soluble and total non-structural carbohydrate (TNC) afternoon concentrations of *Pinus pinaster* plants grown under ambient and elevated $[CO_2]$.

Leaf water status	Moderate drought $\Psi_{wp} = -0.5$ MPa			Severe drought $\Psi_{wp} = -1.5$ MPa		
ANOVA	$[CO_2]$	drought	CO_2 x drought	$[CO_2]$	drought	CO_2 x drought
Ψ_{wp}	ns	ns	ns	ns	**	ns
A	**	*	ns	**	**	**
Hexoses (1)	ns	ns	ns	ns	**	ns
Sucrose (2)	*	ns	ns	**	ns	ns
Starch (3)	**	ns	ns	**	ns	ns
Soluble carbohydrates (1 + 2)	ns	ns	ns	ns	ns	ns
TNC (1 + 2 + 3)	**	ns	ns	**	ns	ns

Two drought intensities were distinguished: moderate drought, $\Psi_{wp} = -0.5$ MPa and severe drought, $\Psi_{wp} \leq -1.5$ MPa. $^*P < 0.05$; $^{**}P < 0.01$; ns: not significant.

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