

Short note

Starch and soluble carbohydrates in leaves of water-stressed oak saplings

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Summary — Four-year-old potted saplings of *Quercus petraea* (Matt) Liebl were exposed to water shortage by withholding irrigation. After 10 days, predawn leaf water potential was decreased to -2.0 MPa and leaf photosynthesis was reduced by 55%. At this stage, starch and sucrose concentrations were decreased by 47 and 48%, respectively. A five-fold increase was observed in glucose and fructose concentrations of water-stressed saplings compared with well-watered plants. These results suggested that drought-induced changes in sugar composition contribute to osmotic adjustment in this species.

oak / *Quercus* / soluble carbohydrate / starch / sucrose / water stress

Résumé — Amidon et glucides solubles dans les feuilles de jeunes plants de chêne soumis à un déficit hydrique. Des jeunes plants en pot de *Quercus petraea* (Matt) Liebl âgés de 4 ans ont été soumis à un déficit hydrique en supprimant l'irrigation. Après 10 jours, le potentiel hydrique en fin de nuit était de $-2,0$ MPa et la photosynthèse foliaire réduite de 55 %. À ce stade, les concentrations en amidon et en saccharose étaient diminuées de 47 et 48 % respectivement. Les concentrations foliaires en glucose et en fructose des plants soumis à la sécheresse étaient augmentées d'un facteur 5 par rapport aux plants bien irrigués. Ces résultats suggèrent que les changements de la composition glucidique des feuilles lors d'un déficit hydrique contribuent à un ajustement osmotique chez cette espèce.

chêne / *Quercus* / déficit hydrique / amidon / saccharose / glucide soluble

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INTRODUCTION

Sessile oak (*Quercus petraea*) is widely distributed in plain forests all over France and represents one of the major species used for timber production. Even if lowland forests of western Europe are submitted to temperate climate with rather important precipitations, they encounter periods of severe drought which are known to be involved in decline processes and to limit forest primary productivity and tree growth (Aussenac, 1978; Becker and Levy, 1982).

Since leaf photosynthesis is a major component of primary production, its decrease during periods of water shortage has often been investigated in oaks (Epron and Dreyer, 1990, 1993a, b). From the last decade, it has been recognised that stomatal control of CO₂ diffusion is the main factor involved in the depression of net CO₂ assimilation in water-stressed plants and that the photosynthetic apparatus is rather resistant to leaf dehydration per se (Kaiser, 1987; Cornic et al, 1989; Epron and Dreyer, 1992). Sessile oaks clearly display maintenance of substantial stomatal conductance and CO₂ assimilation during drought progression (Epron and Dreyer, 1993a) which, together with their deep rooting capacity (Bréda et al, 1993) and low susceptibility to cavitation (Cochard et al, 1992), reflects their ability to tolerate long periods of drought.

The ability of plants to tolerate water deficits has been frequently attributed to their capacity for osmotic adjustment through accumulation of organic compounds such as amino acids or soluble carbohydrates (Turner and Jones, 1980; Morgan, 1984). We studied the effect of a moderate soil drought on the rate of CO₂ assimilation and the amount of soluble and insoluble carbohydrates in leaves of 4-year-old saplings of *Q. petraea*. The aim of these experiments was to assess whether the decline in leaf photosynthesis was accompanied by a

change in the partitioning of photosynthates and whether this change reflected an increased requirement of soluble carbohydrates for osmotic adjustment.

MATERIALS AND METHODS

Plant material

Four-year-old *Quercus petraea* (Matt) Liebl saplings (seed origin: forêt domaniale d'Amance, northeast of France) were grown in 7 L pots on a 1:1 v/v mixture of brown sphagnum peat and sandy soil in a naturally illuminated greenhouse. They were fertilised four times each year with a complete nutrient solution and irrigated twice per week (see Epron and Dreyer, 1990, for details). One week before the onset of the experiments, the saplings were transferred into a growth cabinet with 22/16 °C day/night temperature, 70/95% day/night relative humidity and a 16 h photoperiod with a photon flux density of 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$ in the photosynthetically active radiation (PAR) region. Drought was imposed by withholding water supply.

Water status and photosynthesis

Predawn leaf water potential (Ψ_{wp}) was measured at the end of the 8 h dark period using a Scholander pressure chamber. Net CO₂ assimilation rate (A) was recorded with a gas exchange system described in Epron and Dreyer (1990). Two or three leaves were inserted in a 2 L ventilated cuvette and CO₂ exchange was monitored by a differential infrared gas analyser (Binos, Leybold Heraeus, Germany). Air temperature, leaf-to-air vapour mole fraction difference, ambient CO₂ mole fraction and photon flux density were, respectively, 22 °C, 8 mmol mol⁻¹, 350 $\mu\text{mol mol}^{-1}$ and 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$.

Leaf carbohydrates

Starch and soluble carbohydrates were determined in leaf samples frozen in liquid nitrogen, freeze-dried under vacuum, ground and stored

at -30°C . Sucrose, glucose and fructose were analysed using the spectrophotometric method as described by Jones et al (1977). Soluble sugars were extracted from 0.02 g of powdered leaf samples in frozen 1 M HClO_4 . Sucrose was hydrolysed into glucose and fructose by an invertase (E.C. 3.2.1.26). Glucose and fructose were phosphorylated to glucose-6-P and fructose-6-P using a hexokinase (E.C. 2.7.1.1). Fructose-6-P was transformed into glucose-6-P using phosphoglucosomerase (E.C. 5.3.1.9) and then into 6-phosphogluconate by glucose-6-P dehydrogenase (E.C. 1.1.1.49). The simultaneous reduction of NADP was spectrometrically followed at 340 nm. The assay was employed for sequential determination of glucose, fructose and sucrose. Starch analysis was performed as described in Guehl et al (1993). Extractions were carried out on 0.2 g of leaf powder by incubating in $\text{HCl/DiMethylSulfoxide}$ for 30 min at 60°C . Starch was hydrolysed by amyloglucosidase (E.C. 3.2.1.3) to glucose which was determined as described earlier. Enzymes and buffer media were provided by Boehringer Mannheim.

RESULTS AND DISCUSSION

Two weeks after withholding water supply, predawn leaf water potential decreased to -2.0 MPa. Net CO_2 assimilation was reduced by 55% (table I). Maintenance of still substantial rates of A despite the strong water stress was consistent with results obtained previously on many European oaks

Table I. Predawn leaf water potential (Ψ_{wp}) and net CO_2 assimilation rates (A) in control and water-stressed leaves of *Quercus petraea* saplings (mean of four replicates \pm standard error).

Treatment	Ψ_{wp} (MPa)	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)
Control	-0.2 ± 0.0	4.9 ± 0.4
Stressed	$-2.3 \pm 0.1^*$	$2.2 \pm 0.4^*$

* Significant differences ($P < 0.05$, Student's t -test) between control and stressed saplings.

either under controlled (Epron and Dreyer, 1990; Epron et al, 1993) or under natural conditions (Epron and Dreyer, 1993a; Valentini et al, 1994). This is in agreement with the hypothesis that oaks are rather drought tolerant (Abrams, 1990; Dreyer et al, 1993).

Starch and sucrose concentrations in leaves (fig 1) were strongly reduced by water stress (-47 and -48% , respectively). Decreased starch concentrations in response to soil drying have frequently been reported in soybean, sunflower, lupin, eucalypt, apple or grapevine (Bensari et al, 1990; Fredeen et al, 1991; Quick et al, 1992; Wang and Stutte, 1992; Rodrigues et al, 1993). Decreased sucrose concentrations were also observed in apple trees or grapevine (Wang and Stutte, 1992; Rodrigues et al, 1993). In contrast, foliar sucrose concentrations increased in water-stressed soybeans, eucalypts or sunflowers (Bensari et al, 1990; Fredeen et al, 1991; Quick et al, 1992) or remained at levels close to those of control plants in lupin or grapevine (Quick et al, 1992).

In contrast to starch and sucrose, a five-fold increase was observed in the hexose

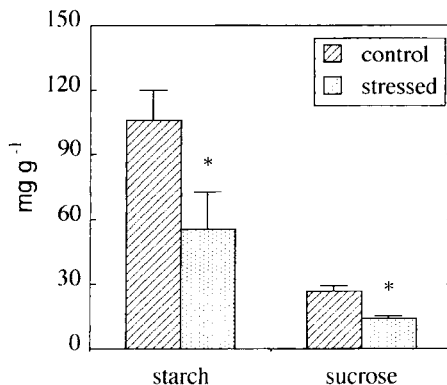


Fig 1. Starch and sucrose concentrations in control and water-stressed leaves of *Quercus petraea* saplings. Vertical bars indicate the standard error of the mean of four replicates and asterisks indicate significant differences ($P < 0.05$, Student's t -test) between control and stressed saplings.

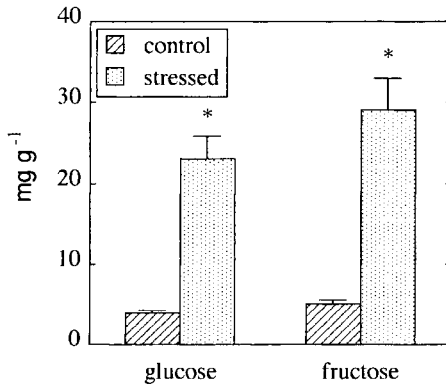


Fig 2. Glucose and fructose concentrations in control and water-stressed leaves of *Quercus petraea* saplings. Vertical bars indicate the standard error of the mean of four replicates and asterisks indicate significant differences ($P < 0.05$, Student's *t*-test) between control and stressed saplings.

(ie, glucose and fructose) concentrations in leaves of water-stressed saplings (fig 2). This result contrasted with the lack of drought effects on hexose in grapevine (Rodrigues et al, 1993), but was similar to the seven- to 14-fold increase in glucose concentration in sunflower leaves observed by Fredeen et al (1991). Despite a significant decrease in sucrose concentration, total soluble carbohydrates (sucrose + glucose + fructose) greatly increased in water-stressed plants (+76%).

The large decrease in the starch/soluble carbohydrate ratio (table II) may reflect an increased starch hydrolysis in water-stressed leaves (Jones et al, 1980) and/or a change in the partitioning between starch and sucrose synthesis (Vassey and Sharkey, 1989). In spinach, this change in the partitioning between starch and sucrose synthesis was related to an increase in the activation of sucrose phosphate synthase (Quick et al, 1989; Zrenner and Stitt, 1991). In the present experiment, sucrose concentrations decreased in water-stressed leaves. It may be suggested that sucrose was diverted to the vacuole and further hydrolysed into glucose and fructose. The large increase of the hexose/sucrose ratio while the fructose/glucose ratio remained unchanged (table II) supports this hypothesis.

It is unlikely that this increase in soluble carbohydrate reflected a direct inhibition of phloem loading in response to water deficits (Hoddinot et al, 1979; Smith and Milburn, 1980). A decreased carbohydrate export from leaves may also result from a restriction of the growth in 'sink' organ (Herold, 1980). However, such kind of inhibition often occurs together with an increase in sucrose and starch (Foyer, 1988), which was not observed in this study.

It is well known that soluble carbohydrates may act as osmotic solutes and contribute to osmoregulation in water-stressed

Table II. Ratio of the concentrations of starch to soluble carbohydrates, of sucrose to hexoses and of glucose to fructose in control and water-stressed leaves of *Quercus petraea* saplings (mean of four replicates \pm standard error).

Treatment	Starch/soluble carbohydrates	Sucrose/hexose	Glucose/fructose
Control	2.9 \pm 0.4	2.8 \pm 0.3	0.8 \pm 0.0
Stressed	1.0 \pm 0.3*	0.3 \pm 0.0*	0.8 \pm 0.0

* Significant differences ($P < 0.05$, Student's *t*-test) between control and stressed saplings.

plants (Jones et al, 1980). Large increases in hexose concentrations in water-stressed oak leaves may indicate that these soluble carbohydrates largely contributed to osmotic adjustment in this species, even though other compounds such as sorbitol, amino acids or inorganic anions and cations may also account for an increase in leaf osmolality (Morgan, 1984). Osmotic adjustment in response to soil drought has been reported for many North American oak species (Abrams, 1990) including *Q. alba*, *Q. macrocarpa* and *Q. stellata* (Parker and Pallardy, 1988). A similar drift in osmotic potential has been postulated in *Q. petraea* (Epron et al, 1993) and demonstrated in *Q. robur* (Osonubi and Davies, 1981). The observed change in soluble carbohydrate content accounts for a decrease in leaf osmotic of about -0.3 MPa. This is well in the range of drought-induced osmotic adjustments that have been reported for various tree species including oaks (Dreyer et al, 1990).

In conclusion, soil drought had pronounced effects on the carbohydrate content of leaves of *Q. petraea* saplings. Decreased starch and sucrose concentrations were almost fully compensated by increased glucose and fructose. Our results suggested that a shift in sugar partitioning may contribute to drought-induced osmotic adjustment in oak leaves.

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