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ORIGINAL ARTICLE

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Effect of a strong cold storage induced desiccation on metabolic solutes, stock quality and regrowth, in seedlings of two oak species

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Abstract Bare-root seedlings of pedunculate oak (*Quercus robur* L.) and northern red oak (*Quercus rubra* L.) were lifted in January and stored at 1.8°C, at 82% relative humidity, until their fresh weight declined by 33%. Root growth potential (RGP), fine root electrolyte leakage (REL), fine root water content (RWC), shoot tip water content (SWC), starch and metabolic solute contents in root and shoot, were measured just after lifting and after treatment. Survival of treated seedlings was also assessed in a field trial. RWC, SWC, REL, RGP were dramatically affected by desiccation during cold storage. In both species, root soluble carbohydrate level, inositol level and isocitrate level increased, whereas root starch level and shoot soluble carbohydrate level decreased. In northern red oak, treated seedlings had higher root contents of soluble carbohydrates, inositol and proline than in pedunculate oak. Moreover, treatment induced proline accumulation only in northern red oak roots. These differences could explain why field survival of treated seedlings was significantly better in northern red oak than in pedunculate oak.

Keywords Carbohydrate · Nuclear magnetic resonance · Plant quality · Proline · Starch

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Introduction

Bare-root seedlings are commonly used for reforestation under temperate climates. In nursery management, cold storage allows flexibility in nursery and lifting operations, since the plants can be lifted when weather and soil conditions are favourable, and then stored until required. Unfortunately, cold storage can cause desiccation in bare rooted seedlings if not protected in sealed bags, especially in a poorly humidified cold store (Sharpe and Mason 1992). Air-drying conditions may affect plant water status and subsequent survival (Tabbush 1987; Girard et al. 1997). Although conifer seedlings are commonly stored or transported in sealed plastic bags, such a practice is rare for broadleaves, because their larger size makes this impractical. After planting, seedlings have to face a transplanting shock, mainly due to an insufficient water supply from soil to roots (Sands 1984). To overcome this shock and regain a favourable physiological status, they must be able to produce new roots (Grossnickle 1988; Haase and Rose 1993). To predict survival after desiccation, different quality parameters are commonly used, especially water status variables such as fine root water content (RWC), and fine root electrolyte leakage (REL) (McKay and White 1997; Généré and Garriou 1999; Garriou et al. 2000; Radoglou and Raftoyannis 2001).

Metabolic solutes could also play a major role. Variation in their nature, allocation and concentration can occur between seedling lots or, for a given lot, from lifting to planting time. Some types of source-sink regulation are of particular importance: metabolic regulation by sugars (soluble sugars and starch) and the effect of stress-related exogenous stimuli (such as desiccation and cold storage). Sugars not only function as a substrate to sustain the heterotrophic growth of sink tissues (root), but are also important signalling molecules that regulate both source and sink metabolism (Roitsch 1999). The use of nuclear

magnetic resonance of ^{13}C (NMR) can provide information not only on soluble sugars, but also on other molecules such as amino acids or glycols.

In the literature, we could not find any study on the effect of an abiotic stress simultaneously on seedling quality, regrowth indicators and metabolic solutes detected by NMR techniques. There is also a gap in knowledge on the effect of a strong desiccation stress (such as abiotic stress) on the previous parameters for a given seedling lot, when survival is markedly low but still possible. Lastly, studies on oak seedlings are recent and not very common, whereas nowadays, millions of them are produced in bare-root nurseries throughout the temperate zone; thus, a comparison between seedlings of two species used in reforestation could be interesting for foresters.

The aim of the present work is to compare the physiological responses of seedlings from two oak species to a strong desiccation stress during cold storage, by using stock quality variables, metabolic contents and regrowth capacity. Northern red oak (*Quercus rubra* L.) and pedunculate oak (*Quercus robur* L.), commonly used for reforestation in France, were chosen. In both species, the seed source was locally recommended, seedlings had the same age, plant type and similar dimensions, and a 33% weight loss treatment was applied. Thus, after transplanting, root growth was in close relationship with the physiological status of the plant. The treatment effect was analysed on

- Stock quality variables: plant water status [RWC, shoot tip water content (SWC)], REL;
- Regrowth indicators: root growth potential (RGP) and field survival;
- Metabolic solutes (carbohydrates, free amino acids and glycols by NMR) and starch, in shoot and root.

Materials and methods

Seedling origin, nursery and planting operations

Seedlings were grown in a private nursery at Lordonnois in France (lat. 47°54′N, long. 3°43′E, elev. 160 m). Two-year-old planting stocks (undercut at age 1) of "Loire Moyenne" and "Nord-Est" provenance were used for pedunculate oak and northern red oak, respectively. They were lifted on 7 January 1998, sealed in polyethylene bags and delivered by van to the laboratory at Nogent-sur-Vernisson (100 km). Seedlings were washed to remove excess soil, bundled by 80 seedlings, and cold stored (total darkness, temperature 1.8±0.3°C, relative humidity, RH 82±5%). Seedlings were stored bare-rooted (exposed to desiccation: treated). When the mean fresh weight declined by 33% (after 12 weeks for red oak and 9 weeks for pedunculate oak), the seedlings were sealed in polyethylene bags, in order to stop desiccation. All treated seedlings had spent 12 weeks in the cold store, when laboratory analyses and field planting were performed. At the end of the storage, ethylene levels were <0.1 ppm in the cold store and 0.1 ppm in the bags, and thus not considered to have any effect on the following results.

Seedlings were chosen for planting stock, so that both species had similar morphological characteristics. The mean (± SE) heights were 593±8 mm and 629±6 mm; root collar diameter, 8.1±0.1 mm and 6.8±0.1 mm; and number of first order roots, 12.8±0.8 and 9.4±0.4, in red and pedunculate oak, respectively. Plant water status parameters (RWC and SWC), REL, RGP, starch content and metabolic solute contents in shoot and root samples were measured on freshly lifted and treated seedlings. Treated seedlings were planted on 8 April in a local nursery at Nogent-sur-Vernisson (lat. 47°50′N, long. 2°45′E, elevation 147 m), to assess survival.

Plant water status and REL

Thirteen seedlings per treatment were randomly chosen to measure RWC, SWC and REL. RWC (fine roots, 0.5–1 mm diameter) and SWC (using the distal 30 mm of the leading shoot after discarding the terminal whorl of buds) were calculated, using fresh weight (FW) and dry weight (DW, oven dried at 105°C for 24 h). Water content (WC, %) was expressed as:

$$\mathrm{WC} = \frac{\mathrm{FW} - \mathrm{DW}}{\mathrm{DW}} \times 100$$

REL was assessed following the method of McKay (1992). For each seedling, four samples of fine roots (1.2–1.8 mm diameter, 20 mm long) were taken and washed twice in deionised water. The fine root samples were then immersed in 25 ml deionised water and agitated at room temperature for 24 h. Then, conductivity of the solution (C_i) was measured using a conductivity probe with temperature compensation (Hanna, HI 8820N). Samples were autoclaved to break cell membranes (110°C, 10 min). Total conductivity (C_t) was measured after sample cooling. REL (%) was expressed as:

$$\mathrm{REL} = \frac{C_\mathrm{i} - C_\mathrm{w}}{C_\mathrm{t} - C_\mathrm{w}} \times 100$$

where C_w is the deionised water conductivity.

Starch and metabolic solutes

Treated and control seedlings of each species were frozen (−20°C) until processed. For each treatment, 3 g of root and shoot tissue were collected from each of ten seedlings and then ground separately by tissue type.

Three powder replicates of 100 mg were used for starch analysis and mixed in 0.8 ml ethanol (80%, 30 min). The suspension was centrifuged (3,000*g*, 10 min). The supernatant was discarded and the pellet was washed again, as described previously. The remaining pellet was treated with 0.9 ml NaOH 0.02*N* (30 min at 100°C) and by 0.1 ml amyloglucosidase (EC 3.2.1.3, 30 min at 50°C) to hydrolyse the starch. Using the enzymatic method (Boehringer-Mannhein, in Bergmeyer and Bernt 1974), the free glucose obtained was phosphorylated to glucose-6-phosphate in the presence of hexokinase (EC 2.7.1.1). It was then oxidised by nicotinamide–adenine dinucleotide phosphate (NADPH), in the presence of glucose-6-phosphate dehydrogenase (EC 1.1.1.49), with the formation of reduced NADPH. The amount of NADPH formed is stoichiometrically related to the amount of free glucose formed by the hydrolysis of starch. NADPH was determined by means of light absorbance at 340 nm (630 l mmol^{-1} m^{-1}). Starch content (C; µmol g^{-1} of dry weight) was calculated by the following formula:

$$C = \frac{V}{\varepsilon \times d \times v \times \mathrm{DW}} \times \Delta A$$

V is the final volume (ml); ε is the extinction coefficient of NADPH at 340 nm; d is the cuvette light path (10 mm); v is the sample volume (ml); DW is the dry weight (g); ΔA is the variation of absorption before and after adding enzymatic solution.

To determine the metabolic solute content for each treatment and tissue type, three powder replicates of 3 g were used and mixed in 20 ml ethanol (80%, 20 min). The suspension was filtered and two

other extractions with 13 ml ethanol (80%) were carried out. The three solutions were mixed and ethanol partly evaporated at 50°C. The pellet was dissolved in 10 ml water and centrifuged (3,000*g*, 10 min). The supernatant was neutralised with 2 M KOH at pH 6.5, then centrifuged (3,000*g*, 10 min). The resulting supernatant was lyophilised and stored at −20°C. For NMR measurements, the freeze-dried material was dissolved in 500 µl D_2O containing dioxane at 5 mM. Spectra were recorded on a 7.04 T Bruker AM 300 spectrometer equipped with a 10 mm multinuclear probehead at 75.47 MHz. Experiments were performed overnight (about 10,000 scans) at 3°C, using 30° pulses with a 2 s repetition time and a spectral width of 17,857 Hz. Peak identification was carried out by analysis of pure metabolic solutes in ^{13}C NMR, to measure peak chemical shifts (Omarzad 1998; Omarzad et al. 1998). To verify these shifts, and unambiguously identify these compounds, the extract solution was spiked with traces of pure metabolic solutes. To determine the metabolic content (in µmol g^{-1} of dry weight), the area below each peak was compared to that of dioxane (fixed content).

Root growth potential and field survival

Just after lifting and after desiccation treatment, nine seedlings representing each treatment were planted in minirhizotrons (boxes of 30×300×700 mm^3 with one transparent side to follow root growth), filled with sphagnum peat. Irrigation was provided just after potting and every second day (with 40 ml of water per seedling). The minirhizotrons were placed in a growth chamber that had the following environmental conditions: temperature =20°C day/15°C night; relative humidity =71% day/92% night; photosynthetic flux density =290 µmol m^{-2} s^{-1}; CO_2 air concentration =410 µmol mol^{-1}. The length of new roots was measured along the transparent screen, 28 days after potting.

At the end of cold storage, 60 seedlings per species were planted in nursery cold frame raised beds, at a density of 20 seedlings per m^2. Soil consisted of 2.4% organic matter, 4.2% clay, 10.4% silt and 82.6% sand with a pH of 5.8. At planting, the soil was at field capacity, and thereafter, plant water needs were supplied by drip irrigation. Treatments were randomised in a four-block design, and survival was determined 100 days after planting, well after bud break was completed.

Statistical analysis

Analysis of variance (ANOVA) followed by Tukey's HSD test ($P<0.05$) was used for the effects of desiccation during cold storage on quality parameters. On survival rates, the use of ANOVA was not appropriate because of non-normalcy of the distribution, and a Chi-square test was performed to compare species at $P=0.05$, after validation on each block. On starch and identified metabolic solutes, mean value and standard error were calculated for root and shoot of each species, separately.

Results

Stock quality

The treatment dramatically lowered SWC and RWC (Table 1). Fine root water content was reduced to 26.4% in red oak versus 36.6% in pedunculate oak, but SWC showed the reverse trend (35.9% vs 22.8%). Relative water loss was very similar for fine roots (RWC) in both species and, for shoot tips, in pedunculate oak (−74% and −79%), but was less affected in red oak shoot tips (−59%). After desiccation, REL increased by more than 300% and final values were high (>75%) in both species.

Nevertheless, some significant differences occurred between species, either on freshly lifted seedlings or after treatment. Initially, SWC and RWC were higher in pedunculate oak than in red oak, but REL was independent of species. After treatment, SWC and REL were lower in pedunculate oak than in red oak, and differences between species on RWC were not significant at $P=0.05$.

Root regeneration and field survival

The length of new roots (RGP) formed by freshly lifted seedlings was over 380 mm, whereas after treatment, root elongation did not occur in pedunculate oak, or was very low (<10 mm) in red oak (Table 1). RGP results were independent of species.

In the field trial, in contrast, the survival of treated seedlings was 53% in red oak but only 10% in pedunculate oak. This difference between species was highly significant ($P<10^{-4}$) and independent of blocks.

Metabolic solutes and starch

NMR spectra profiles showed that the amount, quality and allocation of metabolic solutes differed between freshly lifted and treated seedlings (Fig. 1). Thus, some metabolic solutes were detected by NMR measurement: glucose, fructose, sucrose, inositol, arginine, glutamate, glutamine, proline, citrate, and isocitrate. Three peaks (x_1, x_2 and x_3) were not identified.

The total soluble carbohydrate content (glucose, fructose, and sucrose) decreased in shoots and increased in

Table 1 Shoot tip water content (SWC), fine root water content (RWC), fine root electrolyte leakage (REL) and root growth potential (RGP) at the time of lifting (fresh) and after desiccation in cold storage (treated, 33% weight loss), for seedlings of red and pedunculate oak. For a given variable, mean values not sharing common letters are significantly different at $P=0.05$ (Tukey's HSD test following ANOVA)

	Red oak			Pedunculate oak		
	Fresh	Treated	Variation (%)	Fresh	Treated	Variation (%)
SWC (%)	87.8 (b)	35.9 (c)	−59	109.7 (a)	22.8 (d)	−79
RWC (%)	116.8 (b)	26.4 (c)	−77	141.6 (a)	36.6 (c)	−74
REL (%)	17.2 (a)	81.3 (c)	+373	18.3 (a)	75.1 (b)	+310
RGP: root elongation in 28 days (mm / seedling)	382 (a)	4 (b)		383 (a)	0 (b)	

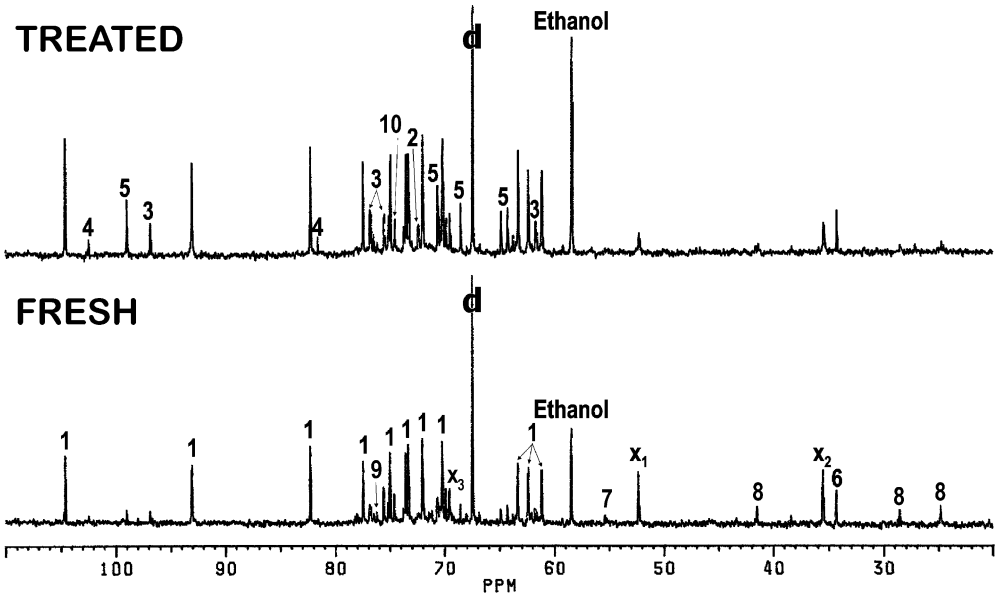

Fig. 1 Partial natural abundance proton-decoupled ^{13}C NMR (75.48 MHz) spectra (20–110 ppm) obtained from roots of freshly lifted and treated seedlings (33% weight loss in cold storage) in pedunculate oak. Each spectrum represents overnight data accumulation (ca. 10,000 scans). Peak assignments: *1* sucrose; *2* α-glucose; *3* β-glucose; *4* α-fructose; *5* β-fructose; *6* glutamate; *7* glutamine; *8* arginine; *9* citrate; *10* isocitrate; x_1, x_2 and x_3 not identified; *d* dioxane (5 mM)

roots, in both species after desiccation during cold storage (Fig. 2). Sucrose accounted for the largest proportion of total soluble carbohydrate (74–87% of soluble carbohydrate content in root). Soluble carbohydrate levels decreased more in shoots of pedunculate oak (−54% vs −29% in red oak), but increased less in the roots (+70% vs +154% in red oak). Root soluble carbohydrate content reached 290 μmol equivalent (equiv.) glucose g^{-1} (1 sucrose =2 equiv. glucose) in red oak, and only 114 μmol equiv. glucose g^{-1} in pedunculate oak. Soluble carbohydrate levels were always higher in red oak than in pedunculate oak.

The fluctuations in content of the other metabolic solutes detected (inositol, arginine, glutamate, glutamine, proline, citrate, and isocitrate) were less consistent (Fig. 3). In the shoots of treated compared with non-treated seedlings, inositol content remained unchanged in red oak, but decreased in pedunculate oak. In roots, treatment increased the content of isocitrate (10–28 μmol g^{-1} in red oak; 9–19 μmol g^{-1} in pedunculate oak) and inositol (16–36 μmol g^{-1} in red oak; 2–13 μmol g^{-1} in pedunculate oak). Moreover, in red oak, a large peak of proline was detected in the roots of treated seedlings (52 μmol g^{-1}, three times the value of freshly lifted seedlings, integrated at chemical shift of 175.3 ppm). The inositol content may be underestimated because of the proximity of a large sucrose peak in the RMN spectra (integrated at chemical shift of 72.0 ppm).

In treated or non-treated seedlings of both species, starch content was much higher in root (>80% of total value) than in shoot tissues (Fig. 4). Desiccation during cold storage reduced shoot starch content in pedunculate oak (−42%) but increased it in red oak (+53%). Treatment lowered starch content in the roots of both species (−31% and −26% in red oak and pedunculate oak, respectively).

Discussion

In both oak species, cold storage of unprotected seedlings impaired the water status in root and shoot, and increased REL. Fine roots were dramatically damaged, due to a low water content (RWC) which impaired the cell membranes (very high REL); this can account for difficult root regeneration, as shown by the very low or nil RGP of treated seedlings, and for increased mortality after planting. Similar detrimental effects have been recorded for various conifers or broadleaves (including northern red oak) exposed to drying conditions (Insley 1979; Tabbush 1987; Deans et al. 1990; Girard et al. 1997). Insley (1979)

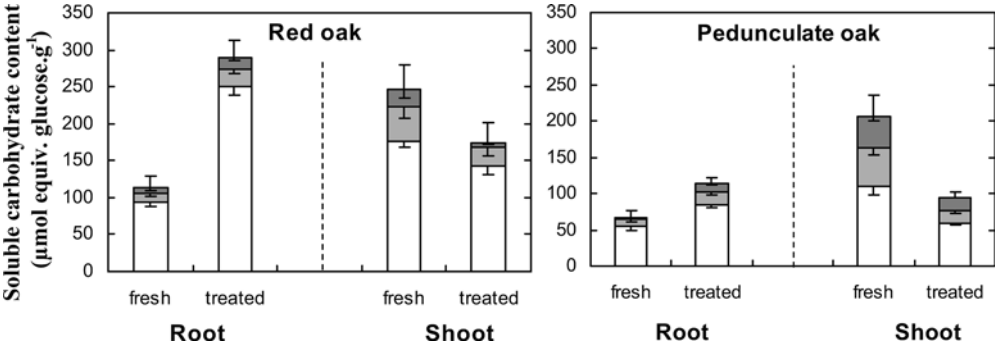

Fig. 2 Effect of cold storage desiccation (33% weight loss) on soluble carbohydrate content (in μmol equiv glucose g^{-1} of dry weight) of roots and shoots in red and pedunculate oak seedlings. *Open bars* sucrose, *pale grey filled bars* fructose, and *dark grey filled bars* glucose. *Error bars* represent ±1 SE for the mean of three replicates (*lower bars* for each carbohydrate, *upper bars* for total)

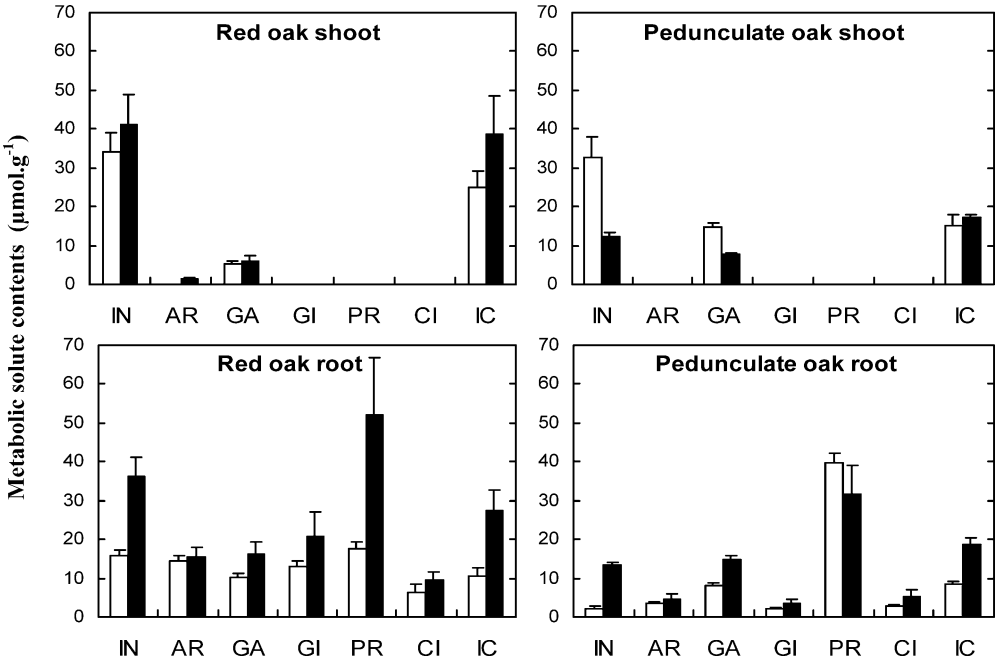

Fig. 3 Effect of cold storage desiccation (33% weight loss) on metabolic solutes (in µmol g^{-1} of dry weight) identified in root and shoot of red and pedunculate oak seedlings. *Open bars* for freshly lifted seedlings and *filled bars* for treated seedlings. Identified metabolic solutes: *IN* inositol, *AR* arginine, *GA* glutamate, *GI* glutamine, *PR* proline, *CI* citrate and *IC* isocitrate. *Upper bars* represent ±1 SE for the mean of three replicates

showed a strong negative relationship between moisture content and survival in three species. For seedlings that had an 80% moisture content in the study, only 10% survival was reported in *Nothofagus oblica*, instead of more than 60% in *Acer platanoides* and *Quercus petraea*. In *Q. rubra*, Girard et al. (1997) observed that moisture content steadily decreased with the duration of exposure to 60% RH for 12 days at 8°C, taproot moisture content was near 55% and mortality occurred. The increase in REL, due to the impairment of cell membranes (McKay 1992), was also reported in seedlings exposed to desiccation (McKay and White 1997). Within 3 h of desiccation at 20°C, 55% RH, under light, REL tripled in *Picea sitchensis* (REL=38%) and *Pseudotsuga menziesii* (REL=59%). In broadleaves exposed to desiccation for 36 h (15°C, 50% RH, in light), McKay et al. (1999) showed an increase in REL, up to a level which depended on the species: REL=30% on *Q. robur*, 40% on *Fagus sylvatica* and 50% on *Betula pendula* and *Fraxinus excelsior*. In general terms, exposure to desiccation (Feret et al. 1985; Sharpe and Mason 1992; Bates and Niemiera 1997; Girard et al. 1997) was shown to reduce root growth and survival. In contrast, Garriou (2000) and Lindqvist (2001) showed that cold storage of oak seedlings in sealed bags, not only maintains plant water status but also does not reduce growth and survival, when seedlings are lifted in December or January; these results obtained in various experiments and cold storage durations indicate that, in the present study, the decrease in RGP and survival was due to desiccation and not to cold storage. Because it impaired root cell membrane function, desiccation played the major role, as already noticed in studies combining desiccation and cold storage effects, on Douglas-fir (Généré and Garriou 1999), pedunculate and northern red oaks (Garriou et al. 2000).

Other experiments, inducing different desiccation conditions (27% weight loss instead of 33%), showed alterations in pedunculate oak fine root structures (1.5 mm diameter). Cells from cork cambium, pericycle and phloem had a flat structure, instead of a well-rounded one in fresh control. The cell structure from cambium layers was also altered (Garriou 2000).

In our experiment, the field survival of treated seedlings revealed differences between species which could not be explained by root damage: both RWC and RGP were independent of species, and red oak had a significantly higher REL than pedunculate oak. The higher vulnerability of pedunculate oak could be partly linked to a severe

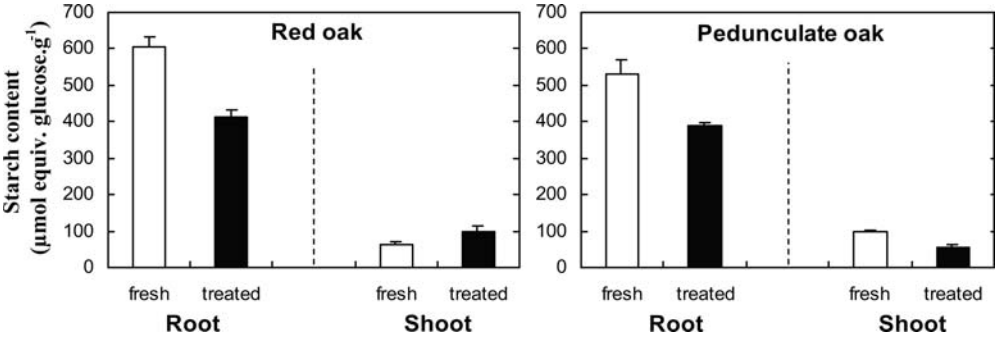

Fig. 4 Effect of cold storage desiccation (33% weight loss) on root and shoot starch content (in µmol equiv. glucose g^{-1} of dry weight), in seedlings of red and pedunculate oak. *Upper bars* represent ±1 SE for the mean of three replicates

drop in SWC between freshly lifted and desiccated seedlings (−79% vs −59% in red oak): before treatment, SWC was significantly higher in pedunculate oak than in red oak, and this result was reversed after treatment. These results suggest a higher susceptibility to cavitation in pedunculate oak seedlings than in red oak seedlings. In mature forest trees, Cochard et al. (1992) found a reverse trend: red oak was more vulnerable to cavitation in the vessels of petioles and twigs. Nevertheless, compared to another species (*Q. petraea*), Ponton et al. (2002) found that water use efficiency of pedunculate oak seedlings was significantly lower, after a moderate drought in full sun, and they concluded that it could account for a lower survival.

Cold storage induced desiccation and increased soluble carbohydrate content in the roots, resulting probably from starch hydrolysis. The increase in root metabolic solute contents (soluble carbohydrates and inositol for both species; proline for red oak only) could perhaps be explained by an osmotic response to desiccation stress during cold storage, but this type of response to desiccation has not been reported previously. These metabolic changes appear to be very similar to those induced by drought after planting. During drought stress, osmotic adjustment occurs probably in an attempt to maintain cell turgor (Cyr et al. 1990; Meier et al. 1992; Tan et al. 1992; Clifford et al. 1998). Soluble carbohydrate accumulation probably plays a role in this adjustment (Tan et al. 1992; Tholakalabavi et al. 1997; Clifford et al. 1998). Thomas and Gausling (2000) noticed that osmotic adjustment (occurring as a physiological acclimation process to achieve dehydration tolerance) and alterations in biomass compartmentation (resulting in a decreased leaf/fine root ratio) seem to be the most important processes of acclimation to drought stress at the leaf level in *Q. petraea* and *Q. robur*.

Cyclitol (Nguyen and Lamant 1988; Tholakalabavi et al. 1997; Clifford et al. 1998), proline and other amino acids (Cyr et al. 1990; Meier et al. 1992; Tan et al. 1992; Larher et al. 1993; Tholakalabavi et al. 1997; Clifford et al. 1998) also rise during drought. For Lahrer et al. (1993), proline accumulation in a whole plant might result from non-structural carbohydrate accumulation.

The variation in metabolic solutes between freshly lifted and treated seedlings involved two factors: cold storage and desiccation. Girard et al. (1997) showed that fast desiccation (<12 days at 8°C) led to an increase in soluble carbohydrates in roots of *Q. rubra* (+33%), whereas Ritchie (1982) in *Pseudotsuga menziesii* and Jiang et al. (1994) in *Picea glauca* showed a decrease in root soluble carbohydrate due to cold storage. In red oak seedlings lifted in October or November and stored in bags until March, root soluble carbohydrate content decreased, whereas when they were lifted in December or January (as in our experiment) or February, root soluble carbohydrate content did not vary (at about 45 mg g^{-1}) (Girard 1996). Thus, it is suggested that the increase in root soluble carbohydrate content in treated seedlings is also mainly due to desiccation and not to cold storage.

After treatment, red oak seedlings survived better than pedunculate oak seedlings, and also had a higher root content in soluble carbohydrates, proline and inositol. As referred to drought stress, these metabolic changes may explain the better tolerance of red oak to water stress. Some references are available on comparisons within a given species, but only one was found between species (Ain-Lhout et al. 2001). Tan et al. (1992) showed that faster growing progenies of *Picea mariana* that had been subjected to moderate moisture stress (18% PEG) accumulated more soluble carbohydrate than slower growing progenies (respectively, 750 μmol g^{-1} and 550 μmol g^{-1}). Clifford et al. (1998) considered carbohydrates (mainly glucose and fructose), cyclitol (mainly scyllo-inositol) and proline important in conferring drought tolerance to *Ziziphus mauritiana*. After 13 days of drought stress, glucose and fructose contents quadrupled (reaching respectively 130 and 90 μmol g^{-1}), and scyllo-inositol content increased by 52% (reaching 38 μmol g^{-1}). Nguyen and Lamant (1988) noticed an increase of myo-inositol in the roots of a drought resistant population of *Pinus pinaster*. When the water potential of solution reached −0.5 MPa, the root myo-inositol content of one provenance was three times higher (15 μmol g^{-1}) than that of another provenance (5 μmol g^{-1}). Arndt et al. (2001) observed that in *Ziziphus rotundifolia* 2-year-old trees exposed to a gradual drought stress, soluble carbohydrates and especially sucrose accumulated in roots. For red oak in this experiment, the larger increase in the root content of soluble carbohydrates and other compounds which are involved in osmotic protection (proline for stabilisation of membranes and proteins, cyclitol for DNA protection, Clifford et al. 1998) could favour root formation and seedling survival. Rai (2002) found that an increase in proline correlates with drought resistance, but the constitutive content of proline was higher in susceptible cultivar. Accumulation of proline in dehydrated plants is caused by both the activation of its biosynthesis and by inactivation of its degradation (Yoshiba et al. 1997). In our experiment, there was a constitutive content of proline in pedunculate oak roots which did not increase after treatment, whereas a high increase in proline was noticed in red oak roots, due to treatment: this effect could also account for a better survival in red oak than in pedunculate oak. Moreover, Ain-Lhout et al. (2001) showed that the role of proline accumulation in two compared Mediterranean shrub species, rather than an osmotic agent (carbohydrates played the major role), is related to a protective action in the event of severe stress.

A wide range of metabolic solutes have been identified using ^{13}C NMR spectroscopy. Exposure to drying-out in the cold store affected seedling water status and biochemical status. The results indicate that red oak is more tolerant to cold storage desiccation than pedunculate oak, probably because it has a better osmotic protection mechanism and is better able to uptake and allocate solute compounds within the plant. Moreover, treatment induced

proline accumulation only in red oak roots, thus probably alleviating the damage caused by dehydration.

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References

Ain-Lhout F, Zunzunegui M, Diaz Barradas MC, Tirado R, Clavijo A, Garcia Novo F (2001) Comparison of proline accumulation in two Mediterranean shrubs subjected to natural and experimental water deficit. Plant Soil 230:175–183

Arndt SK, Clifford SC, Wanek W, Jones HG, Popp M (2001) Physiological and morphological adaptations of the fruit tree *Ziziphus rotundifolia* in response to progressive drought stress. Tree Physiol 21:705–715

Bates RM, Niemiera AX (1997) Effect of cold storage and pre-transplant desiccation on root growth potential and bud break of bare-root Washington hawthorn and Norway maple. J Environ Hortic 15:69–72

Bergmeyer HU, Bernt E (1974) Determination of glucose with glucose oxidase and peroxidase. In: Bergmeyer (ed) Methods of enzymatic food analysis using single reagents. Academic, New York, pp 1176–1179

Clifford SC, Arndt SK, Corlett JE, Sangeeta J, Narendra S, Popp M, Jones HG (1998) The role of solute accumulation, osmotic adjustment and changes in cell wall elasticity in drought tolerance in *Ziziphus mauritiana* (Lamk.). J Exp Bot 49:967–977

Cochard H, Bréda N, Granier A, Aussenac G (1992) Vulnerability to air embolism of three European oak species (*Quercus petraea* (Matt) Liebl, *Q. pubescens* Willd, *Q. robur* L). Ann Sci For 49:225–233

Cyr DR, Buxton GF, Webb DP, Dumbroff EB (1990) Accumulation of free amino acids in the shoots and roots of three northern conifers during drought. Tree Physiol 6:293–303

Deans JD, Lundberg C, Tabbush PM, Cannell MGR, Sheppard LJ, Murray MB (1990) The influence of desiccation, rough handling and cold storage on the quality and establishment of Sitka spruce planting stock. Forestry 63:129–141

Feret PP, Kreh RE, Mulligan C (1985) Effects of air drying on survival, height, and root growth potential of loblolly pine seedlings. South J Appl For 9:125–128

Garriou D (2000) Contraintes écophysiologiques liées à la transplantation des plants forestiers: effets de l'exposition à l'air entre l'arrachage et la plantation. Thèse de l'Université de Bourgogne, Dijon

Garriou D, Girard S, Guehl JM, Généré B (2000) Effect of desiccation during cold storage on planting stock quality and field performance in forest species. Ann For Sci 57:101–111

Généré B, Garriou D (1999) Stock quality and field performance of Douglas fir seedlings under varying degrees of water stress. Ann For Sci 56:501–510

Girard S (1996) Déterminants écophysiologiques de la crise de transplantation de plants d'espèces forestières résineuse (*Pinus nigra* ssp *laricio* Poir. var Corsicana) et feuillue (*Quercus rubra* L.). Effets du stockage des plants. Thèse de l'Université de Nancy I

Girard S, Clément A, Boulet-Gercourt B, Guehl JM (1997) Effects of exposure to air on stress in red oak seedlings. Ann Sci For 54:395–401

Grossnickle SC (1988) Planting stress in newly planted jack pine and white spruce. 1. Factors influencing water uptake. Tree Physiol 4:71–83

Haase DL, Rose R (1993) Soil moisture stress induces transplant shock in stored and unstored 2+0 douglas-fir seedlings of varying root volumes. For Sci 39:275–294

Insley H (1979) Damage to broadleaved seedlings by desiccation. Arboriculture research note 8/79/ARB. Forestry Commission, Alice Holt

Jiang Y, Zwiazek JJ, MacDonald SE (1994) Effects of prolonged cold storage on carbohydrate and protein content and field performance of white spruce bare-root seedlings. Can J For Res 24:1369–1375

Lahrer F, Leport L, Petrivalsky M, Chappart M (1993) Effectors for the osmoinduced proline response in higher plants. Plant Physiol Biochem 31:911–922

Lindqvist H (2001) Effects of different lifting dates and different lengths of cold storage on plant vitality of silver birch and common oak. Sci Hortic 88:147–161

McKay HM (1992) Electrolyte leakage from fine roots of conifer seedlings: a rapid index of plant vitality following cold storage. Can J For Res 22:1371–1377

McKay HM, White IMS (1997) Fine root electrolyte leakage and moisture content: indices of Sitka spruce and Douglas-fir seedling performance after desiccation. N For 13:139–162

McKay HM, Jinks RL, McEvoy C (1999) The effect of desiccation and rough-handling on the survival and early growth of ash, beech, birch and oak seedlings. Ann For Sci 56:341–402

Meier CE, Newton RJ, Puryear JD, Sen S (1992) Physiological responses of loblolly pine (*Pinus taeda* L.) seedlings to drought stress: osmotic adjustment and tissue elasticity. J Plant Physiol 140:754–760

Nguyen A, Lamant A (1988) Pinitol and myo inositol accumulation in water stressed seedlings of maritime pine. Phytochemistry 27:3423–3427

Omarzad O (1998) Etude, par RMN du ^{13}C, du métabolisme cellullaire de lignées normale et habituées de betterave sucrière (*Beta vulgaris* L. *altissima*). Thèse de l'Université d'Orléans

Omarzad O, Pichon R, Kervarec N, Hagège D (1998) NMR natural abundance estimation of ^{13}C-metabolic solutes in normal and habituated sugarbeet cell lines. Protoplasma 202:145–152

Ponton S, Dupouey JL, Bréda N, Dreyer E (2002) Comparison of water-use efficiency of seedlings from two sympatric oak species: genotype × environment interactions. Tree Physiol 22:413–422

Radoglou K, Raftoyannis Y (2001) Effects of desiccation and freezing on vitality and field performance of broadleaved tree species. Ann For Sci 58:59–68

Rai VK (2002) Role of amino acids in plant responses to stresses. Biol Plant 45:481–487

Roitsch T (1999) Source-sink regulation by sugars and stress. Curr Opin Plant Biol 2:198–206

Ritchie GA (1982) Carbohydrate reserves and root growth potential in Douglas-fir seedlings before and after cold storage. Can J For Res 12:905–912

Sands R (1984) Transplanting stress in radiata pine. Aust For Res 14:67–72

Sharpe AL, Mason WL (1992) Some methods of cold storage can seriously affect root growth potential and root moisture content and subsequent forest performance of Sitka spruce and Douglas fir transplants. Forestry 65:463–472

Tabbush PM (1987) Effect of desiccation on water status and forest performance of bare-rooted Sitka spruce and Douglas fir transplants. Forestry 60:31–43

Tan WX, Blake TJ, Boyle TJB, Tan W (1992) Drought tolerance in faster and slower growing black spruce (*Picea mariana*) progenies: II. Osmotic adjustment and changes of soluble carbohydrates and amino acids under osmotic stress. Physiol Plant 85:645–651

Tholakalabavi A, Zwiazek JJ, Thorpe TA (1997) Osmotically stressed poplar cell cultures: anthocyanin accumulation, deaminase activity, and solute composition. J Plant Physiol 151:489–496

Thomas FM, Gausling T (2000) Morphological and physiological responses of oak seedlings (*Quercus petraea* and *Q. robur*) to moderate drought. Ann For Sci 57:325–333

Yoshiba Y, Kiyosue T, Nakashima K, Yamaguchi-Shinozaki K, Shinozaki K (1997) Regulation of levels of proline as an osmolyte in plants under water stress. Plant Cell Physiol 38:1095–1102