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Effects of drought stress on the evergreen *Quercus ilex* L., the deciduous *Q. robur* L. and their hybrid *Q.* × *turneri* Willd.

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Abstract

Five-year-old trees of deciduous *Quercus robur* L., evergreen *Q. ilex* L., and their semideciduous hybrid, *Q.* × *turneri* Willd. (var. *pseudoturneri*), growing in pots, were subjected to drought stress by withholding water for 18–22 days, until leaf water potentials decreased below –2 MPa. Gas-exchange rates, oxygen evolution, and modulated chlorophyll (Chl) fluorescence measurements revealed that by strong stomata closure and declining photosynthetic capacity down to approximately 50%, all three taxa responded with strongly reduced photosynthesis rates. In *Q. robur*, photochemical quenching of the drought-stressed plants was much lower than in nonstressed controls. Dissection of the occurring events in the photosynthetic electron transport chain by fast Chl fluorescence induction analysis with the JIP-test were discussed.

Additional key words: chlorophyll fluorescence; gas exchange; oak; OJIP-test; *Quercus*.

Introduction

Climate change exerts fundamental challenges on Central European forestry strategies. Trees to be harvested in 100 years must be planted today, taking into consideration both present-day climatic circumstances such as cold winters and future expected, increased frequencies of heat waves and drought periods (Schär *et al.* 2004). One possible attempt to prepare for future climatic changes is the plantation of "foreign" tree species in Central European woodlands; it should be species from other continents, exposed to hotter and dryer summers in their natural habitat, or it should be species from the Mediterranean, assuming that future Central European climate becomes more or less similar to the actual Northern Mediterranean climate (*e.g.* Schmiedinger *et al.* 2009). In this context, we studied the behaviour of Central European and Mediterranean oak taxa of forestry importance under adverse climate conditions. While holm oak, *Q. ilex* L., has been the object of several studies with respect to its response to Mediterranean summer drought (*e.g.* Filella *et al.* 1998, Scarascia-Mugnozza *et al.* 1996, Ogaya and Penuelas 2003, Peña-Rojas *et al.* 2004), much less attention has been paid to the behaviour of pedunculate oak (*Q. robur* L.) under drought stress (DS). At typical *Q. robur* stands, this has not been a major issue of interest to Central European forestry in the last century. Epron and Dreyer (1993) comparing drought effects on *Q. petraea* (Matt.) Liebl. and *Q. robur* in a field experiment in Southern France reported lower leaf water potentials in *Q. petraea*, but identical photosynthetic rates in both species. Grassi and Magnani (2005) compared the photosynthetic performances of ash (*Fraxinus oxyphylla*) and pedunculate oak in an Italian stand in years with

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Abbreviations: Chl – chlorophyll; C_i – intercellular CO_2 mole fraction; DS – drought stress; ET – electron transport; F_v – maximal variable fluorescence; F_v/F_m – maximum quantum yield of primary photochemistry; g_s – stomatal conductance of water vapour; LWC – leaf water content; NPQ – nonphotochemical quenching; PAM – pulse-amplitude-modulated; PAR – photosynthetically active radiation; P_{max} – photosynthetic capacity (capacity of the photosynthetic system, if photorespiration and stomatal effects are avoided due to high CO_2 concentration); P_N – net photosynthetic rate; PI_{abs} – performance index on absorption basis; PS – photosystem; Q – plastochinon; q_I – photoinhibitory quenching; q_P – photochemical quenching; RC – reaction centre; V_I – relative variable fluorescence at I-step (30ms); V_J – relative variable fluorescence at I-step (30ms); V_K – relative variable fluorescence at K-step (300 µs); V_{IP} – IP-phase (1-V_I); Φ_{D0} – quantum yield of energy dissipation; Φ_{E0} – quantum yield of electron transport; Φ_{P0} – maximum quantum yield of primary photochemistry; ψ_{leaf} – predawn leaf water potential; ψ_0 – probability that a trapped exciton moves an electron into the electron transport chain beyond Q_A^-.

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different soil water availability and found only minor differences in the responses of both species to moderate and severe DS. Comparative studies of evergreen and deciduous, co-occurring oak species have been performed to assess the relative contributions of stomatal and mesophyll limitations of photosynthesis (Mediavilla *et al.* 2002, Mediavilla and Escudero 2004), photosystem II (PSII) activity (Méthy *et al.* 1996), and differences in hydraulic properties (Nardini *et al.* 1999).

Chl *a* fluorescence signals from PSII have been widely used to assess plant "fitness" (Percival 2005, Tsimilli-Michael and Strasser 2008, Koller *et al.* 2013). In the past, mainly parameters studied with the pulse-amplitude-modulated (PAM) method under equilibrium photosynthesis conditions were used (quenching coefficients of the decay of Chl fluorescence from its maximum value, F_m, *cf.* Schreiber *et al.* 1986, Krause and Weis 1991), in the last decade, by newly developed, easy-to-use instrumentation, the analysis of the fast Chl fluorescence induction curve from F_0 to F_m has come into focus (JIP or OJIP-test, Strasser *et al.* 2000, 2004). While the latter measuring technique focuses on electron transport reactions within PSII and the electron transport chain in the dark-adapted state, data observed with the first method include information about the performance of the photosynthetic dark reactions and on energy dissipation under photosynthetic conditions. The JIP-test has been successfully applied to describe DS in various plant species (barley: Oukarroum *et al.* 2007, 2009; *Q. ilex* field study: Bussotti 2004, *Sorghum* and barley: Jedmowski *et al.* 2012). In the present contribution, we compared the effects of experimentally applied, severe DS over two weeks on photosynthesis and Chl fluorescence parameters of *Q. robur*, *Q. ilex,* and their semi-deciduous hybrid, *Q.* × *turneri* Willd., to assess whether different response strategies between the species with different leaf life traits can be observed and to study, which parameters are informative with respect to the description of DS.

Materials and methods

Plant material, drought stress, and sampling dates: In the Botanical Garden of the University of Frankfurt, Germany, young (5-year-old), about 2 m high plants of *Q. robur* L. (provenance: D-81705), *Q. ilex* ssp *ilex* L. (provenance: F-QIL 702), and 2.5 to 3 m high *Q.* × *turneri* Willd. var. *pseudoturneri* were grown in plastic pots of 45 L (*Q. ilex*, *Q. robur*) and 60 L (*Q.* × *turneri*), respectively, filled with loamy soil (sand + silt: 86–89%, clay: 11–14%), and watered every other day until the beginning of the DS experiment. Volumetric soil water content was monitored with a *Theta probe ML2x* (*Delta-T Devices*, Cambridge, UK) for DS plants. Plants were grown over the spring and summer period in a foliar greenhouse (*Nitsch & Sohn GmbH & Co*, Kreuztal-Eichen, Germany). During the summer, maximum midday light intensities in the greenhouse were 1,430 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR). Light intensity and temperature within the greenhouse were monitored continuously with an *iMetos sm SMT280* climate station *(Pessl Instruments*, Weiz, Austria). The DS experiments took place after full leaf expansion and before the onset of natural leaf senescence, between June 9 and August 30. Water was completely withheld from a subset of the pots for a period of 18–22 d (depending on taxon), after which the plants were rewatered and monitored to ensure that the stress was not fatal (data not shown). Measurements were taken after 18–22 d of DS. Predawn leaf water potentials (ψ_{leaf}) were recorded with a Scholander pressure chamber (*SKPM 1400 pressure chamber*, *SKYE Instruments*, Powys, Wales, UK) on one branchlet per tree. For each taxon, six plants went into the experiment, three of which (stressed group) were subjected to the DS treatment and three (control group) were well watered throughout the experiment.

Gas-exchange measurements: Gas-exchange rates were measured before onset of the drought treatment on all plants and after 18–22 d of the DS experiment in both groups of stressed and nonstressed, control plants on randomly selected, south-exposed leaves in the morning (09:00 to 12:00 h) with the *GFS 3000* gas-exchange system (*Walz*, Effeltrich, Germany) and a Clark oxygen electrode in an *LD 2/3* setup (*Hansatech*, King´s Lynn, UK). The *GFS-3000* measurements were carried out at 400 mg kg^{-1}(CO_2), 25°C, 900 µmol(quantum) m^{-2} s^{-1} PAR, emitted by the *GFS 3040L* light source. Net photosynthetic rate (P_N), stomatal conductance to water vapour (g_S), and intercellular CO_2 mole fraction (C_i) were calculated by the internal software of *GFS 3000* according to the equation system of von Caemmerer and Farquhar (1981). Values of leaves smaller than cuvette size (8 cm²) were recalculated considering actual leaf area. For the measurements with the oxygen electrode to assess the maximum photosynthetic capacity (P_{max}) at saturated CO_2 concentration, leaf discs (13 to 32 mm in diameter, depending on leaf size) were excised with a cork borer. O_2 production was measured at 25°C, 900 µmol(quantum) m^{-2} s^{-1} PAR (emitted by the *LH36/2R* light source) under 2% O_2, 4.5% CO_2 in N_2.

Chl fluorescence analyses: Two different approaches were used to gather information on the fast (µs to s) rise and the slow (s to min) decay of Chl fluorescence. Transients of the fast Chl induction (OJIP) were measured on five south-exposed leaves per tree with a *Pocket-PEA* (*Hansatech*, King´s Lynn, UK); first fluorescence signal was measured at 50 µs and analysed by the means of the JIP-test, which is used to translate the fluorescence measurements into different phenomenological and biophysical expressions that quantify PSII status (Strasser and Strasser 1995). For full dark adaptation, measurements were performed at predawn before sunrise. Induction curves were processed with the *PEA Plus 1.0.0.1* and the *Biolyzer 3.0* softwares (1999–2001, by Ronald M. Rodriguez, *Bioenergetics Lab* Geneva, Switzerland, according to Strasser *et al.* 2000, 2004, 2010). For explanation of JIP-test parameters, *see* Table 1.

Transients of the slower Chl fluorescence decay were measured with a *PAM 101* (*Walz*, Germany) setup under steady-state photosynthesis conditions, *i.e.* 900 µmol m^{-2} s^{-1} PAR from a *KL 1500* (*Schott*, Mainz, Germany) light source, 5,000 µmol m^{-2} s^{-1} saturating pulses of 2 s according to Schreiber *et al.* (1986) and Brüggemann (1992) in a measuring gas consisting of 2% O_2, 4.5% CO_2 in N_2. A setup similar to the one used for the measurement of O_2 production was used, except that the leaf disc cuvette was tailormade for optimum distance between the leaf disc and the fiber optic and transients were recorded with a chart recorder (*Servogor 220, BBC Goertz,* Austria). The ground fluorescence level, F_0, was recorded for 3 min (after 15 min of dark adaptation of the sample in the cuvette) at low measuring light provided by *PAM 101* (light intensity < 0.5 µmol m^{-2} s^{-1}). Following a saturating flash of light for determination of maximum fluorescence level, F_m, the fluorescence decay was recorded for 15 min to a steady state, F_t. Then, F_m' was recorded after another saturating flash at steady state. The quenching parameters – photochemical quenching [$q_P = (F_m' - F_t)/(F_m' - F_0')$] and nonphotochemical quenching [$NPQ = (F_m/F_m') - 1$] – were calculated with [$F_0' = F_0/(F_v/F_m + F_0/F_m')$] (Oxborough and Baker 1997)]. Actinic light was switched off to analyze the relaxation kinetics. Six saturating light pulses with 3-min intervals enabled the calculation of photoinhibitory quenching (q_I) after Quick and Stitt (1989).

Statistical analysis: In order to check the observed changes in the fluorescence and gas-exchange parameters by DS treatments for their statistical significance, the obtained data were analyzed using the *GraphPad Prism 5.04* software (*GraphPad Software Inc*., La Jolla, USA). Calculations were done using the unpaired *t*-test.

Results

Climate data and development of DS: During the summer, mean temperatures in the open sided, foliar greenhouse ranged between 15 and 25°C with minima not below 10°C. Hot diurnal maxima of 35–40°C occurred several times for 1–2 days, promoting strong evaporation and use of the water body in the pots in all three taxa. In each drought experiment, one such heat period occurred. Soil dried from approx. 26.40% (v) to 5.23 ± 0.80% (v) in *Q. robur*, 6.10 ± 0.70% (v) in *Q. ilex*, and 5.73 ± 1.61% (v) in *Q.* × *turneri*. While predawn Ψ_{leaf} in controls stayed above –0.3 MPa, the withholding of water led to the development of moderate DS after 7 d (*i.e.* Ψ_{leaf} of –0.6 to –2 MPa) in *Q. robur* and *Q.* × *turneri*, while *Q. ilex* maintained values around –0.2 MPa during the first week, possibly due to lower initial transpiration rates [0.6 ± 0.4 mmol m^{-2} s^{-1} as compared with *Q. robur* (2.5 ± 1.1 mmol m^{-2} s^{-1}) or *Q.* × *turneri* (1.1 ± 0.4 mmol m^{-2} s^{-1})] and concomitantly, slower consumption of the water available in the pots. Severe DS was recorded in all three taxa after 18–22 d [*i.e.* Ψ_{leaf} below –2 MPa, except for one replicate of *Q. ilex* with ψ_{leaf} of –1.39 MPa, soil water content below 7% (v)]. Due to technical limitations, maximum negative Ψ_{leaf} beyond –2 MPa could not always be measured accurately in the DS trees. All *Q. robur* and *Q. ilex* plants analyzed in this study recovered from DS after rewatering and developed normal P_N rates one month after the stress treatment (data not shown). The *Q.* × *turneri* plants, however, kept their stomata closed and showed the same low P_N and P_{max} values as they did at the end of the DS treatment for at least one month after DS, but they also retained their leaves (data not shown).

Photosynthetic responses of *Quercus* spp. to DS: The reaction of both *in situ* gas-exchange rates and P_{max} (Fig. 1) to the withholding of water proved that after 18–22 d the trees suffered from severe DS. *In situ*, photosynthetic rates decreased to 40% or less of the initial rates, while stomata closed nearly completely. C_i increased in *Q. robur, Q.* × *turneri* and in *Q. ilex* upon DS, indicating also nonstomatal limitation of photosynthesis. When measured under saturating CO_2, photosynthetic rates decreased by approx. 50% under DS in the parental taxa and even more in the hybrid (Fig. 1*D,E,F*). Data for nonstressed, control plants, measured on the same day as the stressed plants, were presented to account for possible influences of the light and temperature regimes on the day of measurement.

Effects of DS on Chl fluorescence induction curves: DS led to changes of the OJIP transient when compared with controls (Fig. 2*A,B,C*). These changes differed between the taxa and had an effect on double normalization (Fig. 2*D,E,F*), which was used for most JIP-test parameter calculations (Table 1). In *Q. robur,* F_0 and F_m levels did not change upon DS, but DS led to an increase of the fluorescence level, F_I, at ~30 ms. In *Q. ilex*, the differences in the fast Chl fluorescence induction curve were due to a decrease in the F_m levels in one of the DS replicates, whereas the transients measured on *Q.* × *turneri* showed an increase in F_0 levels under DS. Double normalization $[V(t) = (F_t - F_0)/(F_m - F_0)]$ recalculates every fluorescence value per transient to a value between zero (F_0) and one (F_m). This procedure enables the comparison of the relative positions of the intermediate I- and J-step in different samples. The calculations of a multitude of JIP-test parameters were based on this double normalization (Fig. 3, Table 1). The JIP-test parameter, $V_{OJ300\mu s}$, quantifying the relative position of V_K (at 300 µs) to V_J (at 2 ms) increased under DS in *Q. ilex* and *Q.* × *turneri*, while ΔV_{IP} [= 1 – V_I (at 30 ms)] decreased. With respect to the quantum yields of the (partially competing) energy transduction processes, no significant changes occurred in DS plants of *Q. robur* compared with the controls. Changes of F_v led to small decreases of F_v/F_m in *Q.* × *turneri* and *Q. ilex*, but not in *Q. robur*, as measured by the frequently used F_v/F_m (= Φ_{P0}) parameter. In *Q. ilex* and *Q.* × *turneri*, the quantum yield of electron transport (Φ_{E0}) decreased and the quantum yield of dissipation (Φ_{D0}) increased. In *Q. robur*, the performance index, PI_{abs}, decreased to about 85% of the control value. In the other taxa, effects of DS on the density of photoactive reaction center (expressed as RC/ABS), F_v/F_m and on the efficiency of electron transport beyond Q_A^- (Ψ_0) contributed to further declining PI_{abs} values to about 50% in *Q. ilex* and 19% in the hybrid. When comparing the DS effects on the spider plots of the Chl fluorescence parameters between the three taxa, it became evident that the directions of changes were similar in *Q. ilex* and *Q.* × *turneri*, but the amplitudes differed.

When analyzing the Chl fluorescence parameters obtained under steady-state conditions in saturating CO_2 and low O_2, DS plants of *Q. robur* showed a strong decrease of q_P, as compared with nonstressed control plants measured on the same day (Fig. 4*D*). In *Q.* × *turneri* and *Q. ilex*, these differences were smaller (Fig. 4*E,F*). NPQ was higher in DS plants of *Q. robur* than in nonstressed control plants of the same age, but in the other two taxa, no significant differences were observed (Fig. 4*G,H,I*). However, the susceptibility towards photoinhibition (q_I) increased in all three taxa in DS plants as compared with nonstressed controls of the same age (Fig. 4*J,K,L*).

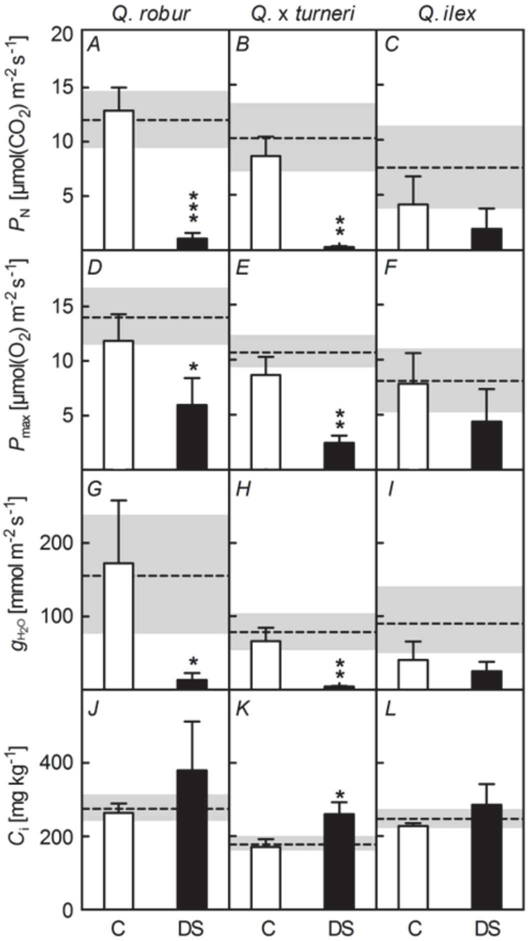

Fig. 1. Gas-exchange parameters affected by drought stress in *Q. robur* (*A,D,G,J*), *Q.* × *turneri* (*B,E,H,K*), and *Q. ilex* (*C,F,I,L*). *A,B,C*: Net photosynthetic rate (P_N); *D,E,F*: photosynthetic capacity (P_{max}) under 900 µmol(quantum) m^{-2} s^{-1}, 2% O_2, 4.5 % CO_2; *G,H,I*: stomatal conductance (g_s), and *J,K,L*: intercellular CO_2 mole fraction (C_i) under ambient gas conditions and 900 µmol(quantum) m^{-2} s^{-1}. Columns show means ± SD of drought stressed (DS, black, $n = 3$) and control (C, white, $n = 3$) plants after 18–22 d. *Broken line* and *shaded area* behind the columns show mean ± SD of control plants before treatment ($n = 6$). Where indicated, parameters of DS plants differ significantly from those of control plants at day 18–22 with * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ (unpaired *t*-test).

Discussion

In the field, in its natural Mediterranean environment, *Q. ilex* is often exposed to severe summer drought events, which result in strong to complete depression of photosynthetic CO_2 uptake (Filella *et al.* 1998, Ogaya and Peñuelas 2003) and downregulation of PSII activity (Bussotti 2004). However, with the onset of autumn rainfall, this decrease in photosynthetic activity is fully reversible (Ogaya and Peñuelas 2003), and *Q. ilex* may use favorable weather conditions in the winter for photosynthetic productivity (Gratani *et al.* 2000). In contrast, *Q. robur* revealed an irreversible decline of photosynthetic activity under prolonged DS (*i.e.* 50 days) with soil water potentials below –1.5 MPa, despite complete recovery of g_s after autumn precipitation (Grassi and Magnani 2005).

As a general feature of evergreen oaks inhabiting xeric sites, a “drought-avoidance” strategy has been identified, which is characterized by low leaf area per shoot volume ratios, low Huber values (ratio of sapwood area to leaf area, Cavender-Bares and Holbrook 2001,

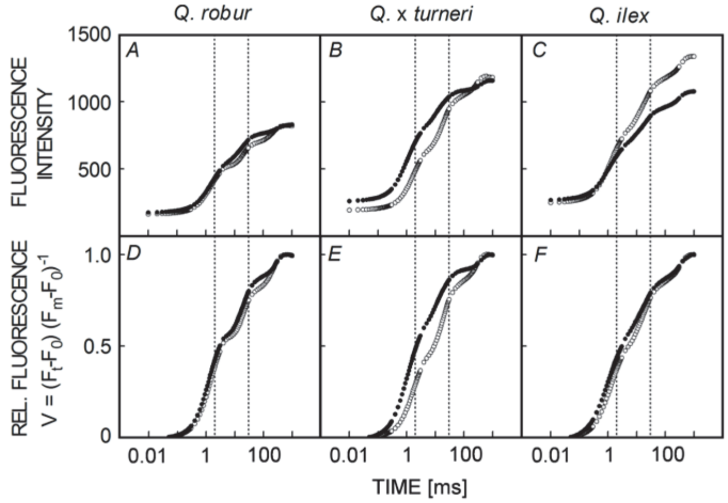

Fig. 2. Chl *a* fluorescence OJIP transients affected by drought stress in *Q. robur* (*A,D*), *Q.* × *turneri* (*B,E*), and *Q. ilex* (*C,F*). *A,B,C*: averaged (n = 15) Chl fluorescence induction curves. *D,E,F*: Double normalized induction curves V = $(F_t - F_0)\ (F_m - F_0)^{-1}$, averaged ($n$ = 15). J- and I-steps are indicated by *vertical broken lines*. Black dots – drought stress, white dots – control.

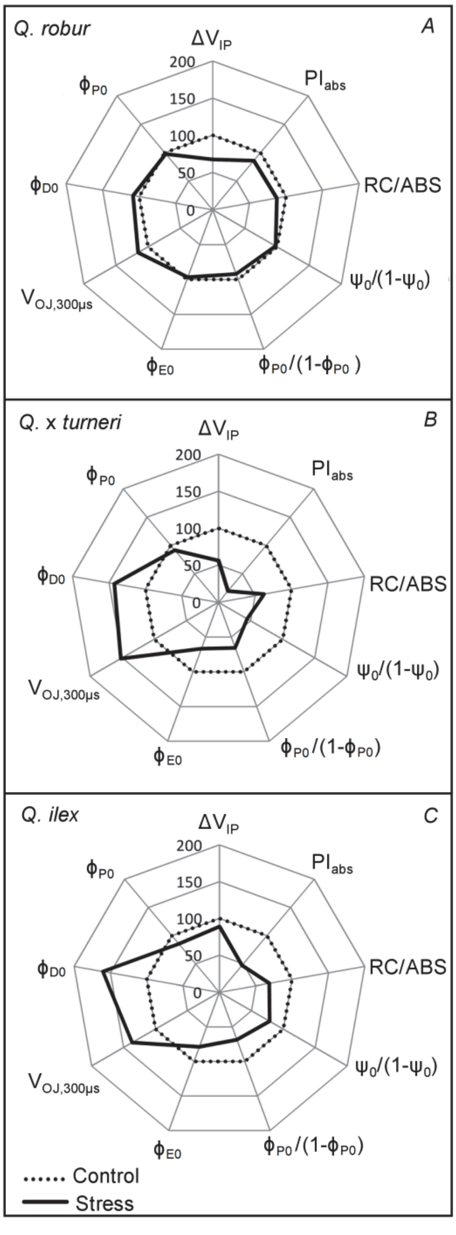

Fig. 3. Spider plots of normalized, mean relative Chl fluorescence JIP-test parameters (n = 15) extracted from the induction curves. For symbols and calculations *cf.* Table 2. *Broken line* at 100%: control plants; *solid line*: drought-stressed plants to control plants [%].

Huber 1928), strong stomatal control, moderately negative Ψ_{leaf}, and relatively high leaf water content (LWC). Co-occurring deciduous species, on the other hand, usually inhabiting less xeric sites in the same area, tend to a drought-tolerant behaviour, *i.e.* they survive drought periods despite stronger water loss, evident in extremely low Ψ_{leaf} and LWC, and they reveal a less conservative water management with less efficient control of stomatal water loss, especially under conditions of soil drying and concomitant decline of root hydraulic conductance (Nardini *et al.* 1999, Mediavilla and Escudero 2004).

In our experimental system, all three oak taxa revealed the expected strong decreases of morning P_N in response to the drought treatments. In the control group of *Q. ilex*, P_N was decreased compared with the measurements prior of the treatment, resulting in a lower numerical difference of DS on P_N between control and stress group. The additionally observed, lower mean values of g_s in the well watered group lead to the conclusion that this observation was due to a stomatal instead of a nonstomatal limitation as seen in decreased values of P_{max} in the stress group (Fig. 1). In the DS plants of all taxa, nearly complete stomata closure could explain the reductions of P_N (Fig. 1*A,B,C*). In addition, nonstomatal limitation was observed under saturating CO_2 (Fig. 1*D,E,F*). Epron and Dreyer (1993) reported abrupt declines in g_s upon DS in the deciduous taxa *Q. petraea* (Matt) Liebl, *Q. rubra* L., and *Q. cerris* L, with much more gradual declines in P_N. In their study, predawn ψ_{leaf} below –2 MPa led to P_N below 5 μmol(CO_2) $m^{-2}\ s^{-1}$ and g_s below 50 mmol(H_2O) $m^{-2}\ s^{-1}$, which is in accordance with the results presented above (Fig. 1). Despite closed stomata and decreased maximal photosynthetic capacity under elevated CO_2, all three species avoided severe,

Table 1. Explanation and calculation of Chl fast induction curve JIP-test parameters used in this study.

Fluorescence parameters	
$F_0 = F_{50\mu s}$	Ground fluorescence level, fluorescence intensity at 50 µs
F_J	Fluorescence intensity at 2 ms
F_I	Fluorescence intensity at 30 ms
F_m	Maximal fluorescence intensity
M_0	Initial slope of the induction curve. $M_0 = 4\,(F_{300\mu s} - F_0)\,(F_m - F_0)^{-1}$
V_J	Relative variable fluorescence at 2 ms. $V_J = (F_{2ms} - F_0)\,(F_m - F_0)^{-1}$
$V_{OJ300\mu s}$	Relative variable fluorescence of the OJ-phase at 300 µs. $V_{OJ,300\mu s} = (F_{300\mu s} - F_0)\,(F_{2ms} - F_0)^{-1}$
ΔV_{IP}	Relative amplitude of I-P phase. $\Delta V_{IP} = 1 - V_I = (F_m - F_I)\,(F_m - F_0)^{-1}$
Performance index and quantum efficiencies	
PI_{abs}	Performance index. $PI_{abs} = RC/ABS\,[\phi_{P0}/(1 - \phi_{P0})]\,[\psi_o/(1 - \psi_o)]$
RC/ABS	Q_A reducing RCs per PSII antenna Chl. $RC/ABS = (V_J\,\phi_{P0})\,M_0^{-1}$
ϕ_{P0}	Quantum yield of primary photochemistry. It expresses the probability that an absorbed photon leads to a reduction of Q_A. $\phi_{P0} = (F_m - F_0)/F_m$
ψ_o	It expresses the efficiency by which a trapped exciton, having triggered the reduction of Q_A to Q_A^-, can move an electron further than $Q_A -$ into the electron transport chain. $\psi_o = 1 - V_J$
ϕ_{E0}	Quantum yield of electron transport. It expresses the probability that an absorbed photon leads to an electron transport further than Q_A.. $\phi_{E0} = ET_0/ABS = (F_m - F_J)/F_m$
ϕ_{D0}	Quantum yield of dissipation. It expresses the probability that the energy of an absorbed photon is dissipated as heat. $\phi_{D0} = 1 - \phi_{P0}$

long lasting photoinhibition (*cf.* also Epron and Dryer 1993) and showed only moderate declines of ϕ_{P0} in *Q. ilex* and *Q.* × *turneri* (Fig. 3*A,B,C*).

Manes *et al.* (2006) compared the response of *Q. ilex* to those of the deciduous species *Q. frainetto* Ten. and *Q. cerris* under a severe, experimental 20-d DS. They reported that, presumably because of its better water conserving strategy during the initial phase of DS, *Q. ilex* maintained its stomata partially open for a longer period than the deciduous species, enabling the plants to retain (low) positive photosynthetic rates for the whole stress period, while P_N in the deciduous species showed a fast and complete decrease. The lower initial g_s of *Q. ilex*, as compared to *Q. frainetto* and *Q. cerris*, in the cited study, was similar to our findings in *Q. robur* and *Q. ilex*. In agreement with these authors, we observed positive P_N in drought-stressed *Q. ilex*. Although *Q. robur* also maintained positive P_N, the relative decrease of P_N was much stronger in the deciduous species (and also in the hybrid). Grassi and Magnani (2005) pointed out the necessity to consider possible increases in diffusive resistance for CO_2 within *Q. robur* leaves under DS, leading to (much) lower CO_2 concentrations in the chloroplast than in the intercellular space. This could explain that the increased C_i did not compensate the loss on photosynthetic rate by stomata closure, although P_{max} was only a half of that in DS plants (Fig. 1).

The values of F_v/F_m (ϕ_{P0}) are within the range of 0.75–0.85 in well watered controls (Björkman and Demmig 1987). In *Q. robur*, F_v/F_m stayed unaffected upon DS, while in *Q.* × *turneri* and *Q. ilex*, a slight decrease to the level of controls could be noted. The F_v/F_m values measured with the PAM setup (Fig. 4*A–C*) and the *Pocket-PEA* (Fig. 3*A,B,C*) resembled each other, despite different measuring techniques. In *Q. ilex*, the decrease of F_v/F_m (Fig. 2*C*) resulted mostly from a decline of F_m in one of the DS replicates and less or not at all from an increase in ground fluorescence, presumably as a consequence of the inactivation of PSII RCs (Krause 1988). Among others, Méthy *et al.* (1996) and Epron and Dreyer (1993) observed only a small sensitivity of F_v/F_m to moderate DS in different European *Quercus* species, and F_v/F_m declined only at predawn water potentials beyond –4 MPa. Due to technical problems with the pressure chamber above –2 MPa, it can be only stated that a plant reached ψ_{leaf} below –2 MPa or not. In contrast to F_v/F_m, the PAM measurement derived quenching parameters, q_P, NPQ, and q_I revealed strong differences between control and DS plants in *Q. robur*. The decreased P_{max} upon DS no longer allowed a high oxidation state of the electron transport chain and led to an over-reduction of the primary electron acceptor (decrease in q_P). The lower q_P values in *Q. ilex* and *Q.* × *turneri* in well watered plants compared with *Q. robur* might be a consequence of the lower P_{max} rates in these taxa under control conditions. Concerning the NPQ values obtained in the light-adapted state (and not to be confused with dissipation parameters observed by the OJIP measurements in the dark-adapted state), the data showed that the heat dissipation processes [in general mediated by the xanthophylls, viola- and antheraxanthin (Demmig-Adams and Adams 1996, Gilmore 1997)] were

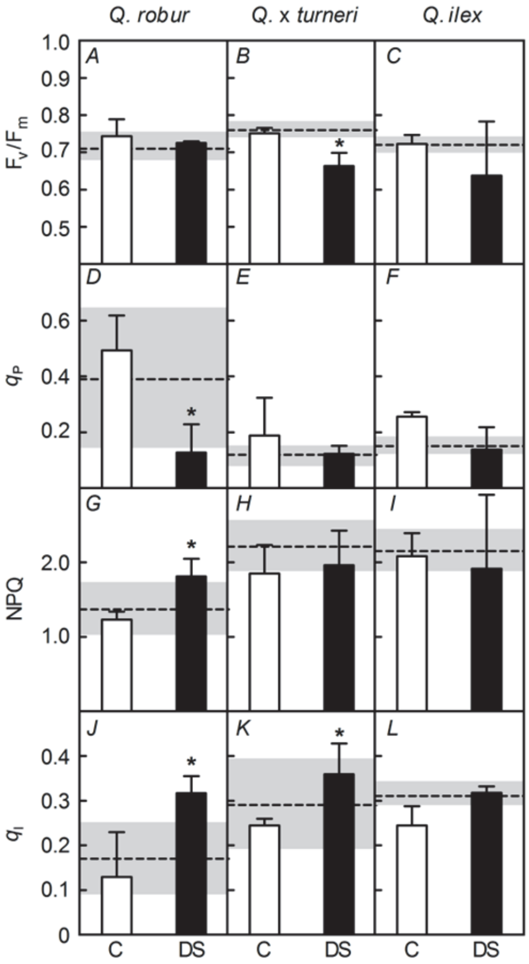

Fig. 4: Chl *a* fluorescence parameters affected by drought stress in *Q. robur* (*A,D,G,J*), *Q.* × *turneri* (*B,E,H,K*), and *Q. ilex* (*C,F,I,L*). *A,B,C*: maximum quantum efficiency of PSII (F_v/F_m), *D,E,F*: photochemical- (q_P), *G,H,I*: nonphotochemical- (NPQ), and *J,K,L*: photoinhibitory quenching (q_I) under 900 μmol(quantum) m^{-2} s^{-1}, 2% O_2, 4.5 % CO_2. Columns show means ± SD of drought-stressed (DS, black, $n = 3$) and control (C, white, $n = 3$) plants after 18–22 d. *Broken line* and *shaded area* behind the columns show mean ± SD of plants before treatment ($n = 6$). Where indicated, parameters of DS plants differ significantly from those of control plants at day 18–22 with * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ (unpaired *t*-test).

well functioning under the given DS, as shown by Haldimann *et al.* (2008) for *Q. pubescens* at water potentials of –2.50 to –3.34 MPa.

As a consequence of decreasing ϕ_{P0} (F_v/F_m), average ϕ_{D0} values in *Q.* × *turneri* and *Q. ilex* increased upon DS. The spider plots function as a tool to facilitate the visualization of changes between many parameters with different units. However, differences in amplitude may be the result of normalization and it should be interpreted with caution, since the range of change is not equal in all parameters. This can be shown by the quantum yields, ϕ_{P0} and ϕ_{D0}, which are connected by $\phi_{D0} = 1 - \phi_{P0}$. A change in ϕ_{P0} from 0.8 to 0.7 (–12.5%) resulted in a change of ϕ_{D0} from 0.2 to 0.3 (+50%). Still, a comparison is valid and the visualization is a useful tool to assess differences of the reaction to a treatment. An increase of $V_{OJ,300\mu s}$ values and the initial rise of the transient upon DS could be noted for all taxa, which is associated with a K-step, and it has been also observed by van Heerden *et al.* (2007) in two drought-treated, desert shrub species (*Augea capensis* and *Zygophyllum prismatocarpum*). This K-step reflects damage to the water splitting system under heat stress (Strasser 1997). Alternatively, the increasing slope of the initial induction curve (which is mathematically expressed in the $V_{OJ300\mu s}$ value) may also result from an increase in effective antenna size, which was reflected in the decline of RC/ABS, *i.e.* the measure for photoactive RC densities in *Q. ilex* and *Q.* × *turneri* (Fig. 3*B,C*), which is one of three components of the PI_{abs} calculations. In *Q. ilex* and *Q.* × *turneri*, this might reflect that an increased percentage of "silent" RCs (Strasser *et al.* 2004) accumulated, which are not involved in electron transport, but dissipate light energy independently from the xanthophyll cycle. Thus, PSII electron transport rates were adjusted to the demand, preventing overexcitation of the electron transport chain under the light intensities in the foliar greenhouse. This hypothesis would explain why the (already high) NPQ of control plants showed no further increase under DS (Fig. 4*H,I*). In *Q. robur*, in contrast, the unaffected photoactive RC density under DS, together with the strongly decreased P_{max} enforced the strong decrease in q_P and the increase in NPQ under actinic light. Additionally and/or alternatively, dissociation of PSII antennae from the RC and emission of absorbed light through heat dissipation by the antenna carotenoids would be an option to release electron pressure in the electron transport chain. From *Q. pubescens*, it is known that severe DS decreases Chl (*a*+*b*) concentrations, but this was not accompanied by reductions in light-harvesting complex II (LHCII) (Haldimann *et al.* 2008). Instead, it lead to increased carotenoid/Chl and xanthophyll/Chl ratios, presumably being a key factor in the protection of the photosynthetic apparatus against photooxidative damage through heat dissipation, since antioxidant levels (α-tocopherol, ascorbate pool) did not increase (Galle *et al.* 2007). However, a decrease of variable fluorescence through an increase of F_0 and/or a decrease of F_m, leading to declining F_v/F_m (ϕ_{P0}) values, cannot be simply due to changes in Chl content. Dinc *et al.* (2012) showed that the Chl *a*/*b* ratio is a more important determinant of F_m than the Chl content. An inactivation of PSI through oxygen radical buildup by DS and the concomitant decline of the PSI/PSII ratio results in a specific decline of ΔV_{IP} (Oukarroum *et al.* 2009) through an increase of V_I. Similar to the observations in *Q. robur* and *Q.* × *turneri*, a decrease of ΔV_{IP} upon DS has also been observed in *Hordeum vulgare* and *Cicer arietinum* (Oukarroum *et al.* 2009), *Sorghum bicolor* (Jedmowski *et al.* 2012) or *Augea capensis* and *Zygophyllum prismatocarpum* (van Heerden *et al.* 2007).

A decrease of PI_{abs} was observed for all three oak taxa upon DS. PI_{abs} may uncover changes in the behaviour of PSII, before changes in F_v/F_m (ϕ_{P0}) can be noted. The very strong decrease of PI_{abs} in *Q.* × *turneri* was due to significant decreases in all contributing partial steps, with the strongest influence of $\psi_0/(1-\psi_0)$, while all components of PI_{abs} contributed in a similar manner to its decline in *Q. ilex*. We might conclude that in the DS *Q.* × *turneri* plants, the relative contribution of the reactions behind Q_A^- reoxidation were affected stronger than in the parental species, as it was revealed by the patterns of the normalized Chl fluorescence induction curves (Fig. 2*E*).

We can state that DS resulted in different modifications of the photosynthetic apparatus in *Q. robur* on one hand and *Q. ilex* and *Q.* × *turneri* on the other hand, which could be quantified by gas exchange, fast Chl fluorescence rise, and slow Chl fluorescence decay parameters. Each of these methods provided different information. The analysis of the OJIP transient yielded additional insight into the acclimation processes to the traditionally used gas exchange and fluorescence parameters. A future study, correlating differentially decreased leaf water potentials to OJIP transients, may yield information on the differential functionality of PSII upon DS in evergreen and deciduous *Quercus* taxa.

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