Frost-acclimation of photosynthesis in overwintering Mediterranean holm oak, grown in Central Europe

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Abstract

As a consequence of global change, forestry in Central Europe has to expect and be prepared for an increase of hot and dry summers in the near future. In two model plantations of the Mediterranean holm oak (*Quercus ilex* L.) in Central Europe (Rhine-Main basin) we tested its potential as a future forestry tree for drought-threatened stands by studying its overwintering strategy under harsh winter conditions. During prolonged frost periods, chronic photoinhibition was developed, which lasted until the end of the frost period. Nearly all plants survived minimum temperatures of -16 to -18°C and their photosynthetic apparatus recovered completely during late winter. A detailed study of the temperature dependence of chlorophyll (chl) fluorescence parameters of the OJIP test revealed statistically significant correlations between minimum temperature and maximum quantum yield of primary photochemistry (Fv/Fm), absorption rate/reaction centre (ABS/RC), dissipation rate/reaction centre (DI_0/RC) and electron transport rate/reaction centre (ET_0/RC) as well as with the deepoxidation state (DES) of the xanthophyll pigments. The DES correlated with Fv/Fm, ABS/RC, DI_0/RC and ET_0/RC. It is concluded, that from the point of view of the winter hardiness of the photosynthetic apparatus, *Q. ilex* should be further investigated as a potential future forestry tree also for very dry and warm stands in Central Europe under the scenarios of climate change.

Introduction

In the course of Global Climate Change, it is expected that in the long run southern European tree species will increasingly be favoured in the warm and dry areas in Central Europe over the nowadays dominating beech.[1] In forestry, this will lead to a slow change in tree species composition towards an increasing abundance of evergreen species.[2,3] However, natural migration rates of South European species may not be sufficient to fill gaps obtained by local dieback of local tree species.[4] Therefore, forestry researchers are currently evaluating and testing various *foreign* tree species for their potential as future forestry species.[5] Planting North American tree species like (deciduous) *Quercus rubra* and (evergreen) *Pseudotsuga menziesii* to minimize future Climate Change-prone risks in economic forestry might alter the existing ecosystems significantly. The alternative concept of *human-aided migration* of South European tree species and/or ecotypes into already warm and dry Central European stands can be regarded as a measure, which might enable a smooth transformation of existing, but threatened forest communities to mixed, future-suited forest systems.[6] In this context, we evaluate deciduous and evergreen *Quercus* species from the Mediterranean for their potential as forestry trees,[7] including their potential for winter survival under the actual still harsher winter conditions as compared to the Mediterranean basin.

Evergreen tree species from arctic/alpine biomes are known to acclimate to severe winter conditions by a mechanism called *chronic photoinhibition*, which is characterized by a decrease of predawn maximum quantum yield of primary photochemistry, accompanied by high deepoxidation states of the xanthophyll pigments (*sustained high AZ/VAZ ratios*).[8] It is interpreted as a means to allow for harmless heat dissipation even at early morning, when a combination of low temperatures and light otherwise would lead to photooxidative damage. This behaviour has also punctually been documented in field studies of the evergreen *Quercus ilex* L. in Spain, where leaf samples have been analysed at single dates during winter in connection with moderate frost events (-4 to -8°C).[9-11] However, northern ecotypes of the species may tolerate much lower winter temperatures down to approx. -20°C.[12] *Q. ilex* is successfully cultivated in parks in warmer places in central Europe and Britain and is known to colonize the Atlantic coast up to Brittany. It has therefore been suggested, that *Q. ilex* together with other (deciduous) Mediterranean oak species may play a future role in forestation experiments in Central Europe with respect to expected climate change effects on existing oak stands, especially on already dry soil conditions.[7]

The analysis of the fast induction curve of chl fluorescence from PS II (so-called OJIP-test),[13] allows the dissection of different steps of exciton/electron movements in the initial phase of the electron transport chain. It has been used to study stress physiology of trees[14-16] and has recently also been introduced for the assessment of frost tolerance of winter cereals.[17,18] To the authors' knowledge, so far no thorough study of the effects of chronic photoinhibition in evergreen broadleaf plants on the OJIP parameters has been published.

The aim of the present study was to analyse the direct effect of low, subzero temperatures under Central European field conditions on *Q. ilex* PS II functionality not on a punctual base, but throughout the winter period. It is known, that in the Mediterranean basin, *Q. ilex* uses the (usually) mild winter conditions for continuous photosynthetic activity, whenever allowed by climatic conditions.[10-19] We hypothesize that *Q. ilex* may tolerate not only punctual frost episodes like in the Mediterranean basin, but also severe and long-lasting subzero temperatures in the field by the so-called chronic photoinhibition mechanism without inducing further damage. To study this, we analysed the performance of *Q. ilex* PS II in experimental plantations under two different frost and one frost-free regime throughout the unusually severe winter 2009/10 in the Rhine-Main valley with the OJIP test.

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Materials and Methods

Plant material and experimental sites

Two-year old plants of *Q. ilex* ssp. *ilex* (certified seed material F-QIL702 Provence), collected from a population close to the border of Provence/Languedoc and supplied by Darmstädter Forstbaumschule (Darmstadt, Germany) were sown in 2004 and planted at the Botanical Garden of the University of Frankfurt (50° 07.564' N, 8° 39.495' E, site denomination: FR) in 2006 on a fully sunny-exposed site in common garden soil. These plants were exposed to full sunlight until approximately 5 pm in May. 86 out of 88 plants survived all winters until 2009 without damage. In October 2009, at age 5 yo, 4 of them (ca. 1-1.5 m high) were transplanted with their root system as intact as possible into a foliar greenhouse erected on the same soil, which was kept frost-free during winter (site denomination: FRFF) and 6 of them were transplanted into an experimental forest site in the vicinity of Rüsselsheim Germany (49° 57.232' N, 8° 24.914' E, site denomination: RU). All transplanted plants were irrigated regularly until onset of autumn/winter precipitation to prevent drought stress from root damage due to the transplantation process.

The plants at FRFF were irrigated once or twice weekly throughout the winter.

The Rüsselsheim site is a sparse Pinus sylvestris forest (ca. 60 yo) on an inland sand dune, dominated by *Rubus fruticosus* aggr. and with natural rejuvenation of *Quercus robur, Q. rubra* (from an adjacent plantation), *Prunus serrotina*, *Crataegus monogyna*, *Robinia pseudoacacia* and *Betula pendula*. The average ground water table lies at -5.5 m below ground level. At the RU and at the FRFF site, the average PFDs reaching the plants were approximately 60-70% of full sunlight by partial shading by the pines and by the absorbance of the foliar greenhouse, respectively. Three randomly chosen plants of average size were identified from the population left at the Frankfurt frost-exposed site and monitored throughout the winter. Thus, data sets of FR plants rely on 3, those on FRFF on 4 and those on RU on 6 biological replicates. In general, per biological replicate and measuring date, 10 (fluorescence) or 2 (AZ/VAZ) technical replicates (individual leaves) were analysed. Data for global radiation were obtained from Deutscher Wetterdienst, Offenbach, Germany, for the closest by measuring station Geisenheim (ca. 30 km west of RU and ca. 45 km west of FR).

Data for temperature were either recorded at FR or taken from Deutscher Wetterdienst, Offenbach, Germany, for Frankfurt Airport (ca. 8 km east of RU).

Chlorophyll fluorescence measurements

On the evening before each measuring day, twigs of *Q. ilex* were covered in black fabric to prevent light acclimation of the leaves during the following dusk. On the next morning at predawn, measurements were started by attaching darkening leaf clips to the leaves prior to withdrawing the fabric. Chlorophyll fluorescence induction curves were measured with a Pocket PEA (HansaTech, Germany) and a saturating pulse of 3500 μmol quanta m^{-2} s^{-1} red light (650 nm). Data were analysed using the PEA plus program (HansaTech, Germany) in the mode, which uses the extrapolated F$_0$ value (not the F_0=*F(50 μs)* mode). The OJIP-values of chl fluorescence were obtained at 0 (extrapolated), 2, 300 and 900 ms, respectively. The initial phase of the fluorescence induction curve was represented by the data extracted at 50 μs, 100 μs, 300 μs, 2 ms, 30 ms, given in Tables 1-3.

Representative induction curves were normalized as done *e.g.* by Van Heerden *et al.*[20] and others as $F_{normalized}=[F(t)-F_0]/(F_M-F_0)$ and the shape of the F$_{normalized}$ *vs.* log t curves was analysed. It turned out that induction curves with (F$_M$-F$_0$) <600 units did not express the J and I steps in a satisfying manner; hence, they were regarded as *fault* datasets and excluded from further analysis. Such data sets occurred occasionally in frost-exposed plants (5-13 % of all measurements in FR and RU) between

Table 1. Mean values of *raw* fluorescence intensities of the Pocket-PEA measurements at T0-T5 and for the maximum fluorescence (FM) for all data points in Figures 3-4 obtained at the Rüsselsheim site (RU), at the Frankfurt site outdoors (FR) and at the Frankfurt site under frost-free conditions (FRFF); average SDs were 17-28% of the means.

site	date	F0 (extrapolated)	F1 (0.05 ms)	F2 (0.1 ms)	F3 (0.3 ms)	F4 (2 ms)	F5 (30 ms)	FM (900 ms)
RU	19.11.2009	4885	5062	5235	5964	10159	18399	25635
	23.12.2009	4571	4656	4750	5086	6941	9641	12160
	05.01.2010	4004	4062	4125	4345	5496	6809	7583
	10.01.2010	2957	5008	5058	5241	6090	7120	7817
	27.01.2010	4160	4200	4243	4390	5142	6054	6577
	02.02.2010	4456	4513	4571	4771	5680	6777	7632
	18.02.2010	3649	4061	4094	4192	4701	5463	5854
	26.02.2010	3976	4036	4103	4333	5481	7173	7942
	25.03.2010	3640	3718	3796	4089	5594	7624	8657
	29.04.2010	3335	3454	3577	4027	6067	10391	13606
FR	26.11.2009	5077	5294	5509	6420	11426	20017	27924
	13.01.2010	4454	4530	4606	4898	6371	8389	9715
	03.02.2010	3953	4017	4087	4326	5504	7287	8688
	17.02.2010	3566	3593	3624	3722	4174	4858	5299
	25.02.2010	3891	3956	4021	4262	5545	7729	8883
	11.03.2010	3373	3397	3423	3502	3873	4519	4938
	28.04.2010	3909	4051	4191	4771	7953	13807	19378
FRFF	26.11.2009	5233	5443	5648	6397	11663	21758	30471
	13.01.2010	3800	3912	4022	4480	7898	15049	22052
	03.02.2010	4553	4707	4868	5493	9240	17420	24022
	17.02.2010	4856	5023	5181	5878	9994	17389	22727
	25.02.2010	4503	4651	4804	5440	9877	18688	26333
	11.03.2010	3551	3625	3711	4004	5819	10457	15701
	28.04.2010	4929	5106	5283	5975	9243	17959	23805

January and March 2010, in particular in three out of the six trees planted in the frost-exposed site at RU. In the latter group, the frequency of such *fault* data sets increased up to 40% by late March 2010 in two of these trees, which turned out to be damaged by the winter conditions and were hence completely excluded from further analysis. The following parameters derived from the OJIP test are used in this contribution:[13,21,22] Fv/Fm (maximum quantum yield of primary photochemistry in the dark-adapted state), ABS/RC [absorbance per reaction centres (RC)], S_m [*i.e.* normalized area, $_0\int^{t_{FM}} (F_M-F_t)\,dt/(F_M-F_0)$], which is regarded as a measure of the energy needed to close all reaction centres during the OJIP phase, and which can be interpreted as the electron acceptor capacities of the Q_A, Q_B and PQ pools,[21] TR_0/RC (maximum trapping rate of active RC), DI_0/RC (effective energy dissipation of active RC), ET_0/RC (electron transport rate of active RC) and ET_0/TR_0 (the probability to feed electrons residing on Q_A^- into the electron chain beyond PSII).

Xanthophyll cycle pigments

Leaf discs of 14 mm diameter were collected in the dark (pre-dawn and night measurements) or under the prevailing natural illumination (day measurements in Figure 1C and G) from the same twigs, on which PEA measurements were performed and immediately frozen in liquid N_2. Pigment extraction in acetone in the presence of Na-ascorbate and analysis by HPLC were performed as described by De las Rivas *et al.*[23] in the presence of 0.7% (v/v) triethylamine in both elution solvents (J. Abadia, CSIC Zaragoza, personal communication) and xanthophyll pigments were calculated according to Färber *et al.*[24] The deepoxidation state of the xanthophyll pigments viola (V) - anthera (A) - and zeaxanthin (Z) was calculated as AZ/VAZ= ([A]+[Z])/([V][A]+[Z]), with [V], [A] and

Table 2. Mean values of *raw* fluorescence intensities of the Pocket-PEA measurements at T0-T5 and for the maximum fluorescence (FM) for all data points in Figures 1-2 at the Frankfurt site outdoors (FR) and at the Frankfurt site under frost-free conditions (FRFF); Measuring Date: 14.01.2010.

site	time	F0 (extrapolated)	F1 (0.05 ms)	F2 (0.1 ms)	F3 (0.3 ms)	F4 (2 ms)	F5 (30 ms)	FM (900 ms)
FR	09:00	3959	4027	4093	4334	5525	7255	8351
	12:00	3544	3628	3673	3801	4555	5922	6998
	15:00	3502	3528	3562	3658	4193	5094	5717
	18:00	3287	3320	3359	3484	4278	5466	6198
	21:00	3726	3784	3848	4076	5447	7263	8391
	00:00	5281	5405	5525	6014	8493	10932	12800
	03:00	3871	3946	4025	4305	5950	8049	9531
	06:00	3911	3988	4070	4365	6096	8242	9701
	09:00	4094	4173	4254	4557	6362	8616	10127
FRFF	09:00	4427	4578	4738	5346	9621	17463	24981
	12:00	3849	3949	4055	4487	7695	14450	20469
	15:00	4073	4203	4327	4863	8862	15660	22020
	18:00	3550	3646	3746	4141	7680	13726	19930
	21:00	4119	4255	4387	4969	10065	17216	23851
	00:00	5139	5370	5590	6611	14130	22363	29670
	03:00	3673	3782	3890	4348	8490	15312	21393
	06:00	4103	4238	4373	4960	10086	17723	24217
	09:00	4243	4396	4546	5191	10509	18113	24783

Table 3. Mean values of *raw* fluorescence intensities of the Pocket-PEA measurements at T0-T5 and for the maximum fluorescence (FM) for all data points in Figures 1-2 obtained at the Frankfurt site outdoors (FR) and at the Frankfurt site under frost-free conditions (FRFF); Measuring Date 25.05.2010.

site	time	F0 (extrapolated)	F1 (0.05 ms)	F2 (0.1 ms)	F3 (0.3 ms)	F4 (2 ms)	F5 (30 ms)	FM (900 ms)
FR	09:00	3343	3456	3573	4014	6597	12442	17643
	12:00	3455	3586	3721	4212	6948	12545	17631
	15:00	3508	3655	3798	4357	7354	12693	17573
	18:00	3423	3563	3702	4273	7759	13168	17272
	21:00	3209	3341	3467	4014	7956	14219	17844
	00:00	3997	4203	4402	5307	11590	18990	23124
	03:00	3560	3729	3899	4658	10263	16642	20532
	06:00	3189	3322	3458	4021	7990	13857	17476
	09:00	3770	3909	4048	4595	7750	13387	17925
FRFF	09:00	3982	4130	4268	4842	8101	15665	20669
	12:00	4095	4245	4403	5011	8353	14142	20044
	15:00	4238	4394	4559	5175	8193	12301	17975
	18:00	3987	4121	4267	4803	8161	15422	19951
	21:00	3637	3761	3896	4446	8686	15795	19668
	00:00	4398	4590	4769	5592	11389	19114	23086
	03:00	4124	4287	4442	5148	10643	18431	22594
	06:00	3947	4080	4204	4744	8861	16638	21281
	09:00	4232	4369	4509	5052	8257	15929	21216

[Z] being the respective relative molar concentrations of the xanthophylls in the leaf extracts.[8]

Statistical analysis

Correlations between chl fluorescence parameters and T_{min} or AZ/VAZ were calculated with the inherent statistic software of the GraphPad PRISM® software. The Spearman25 rank correlation analysis was chosen, since it takes into account whether the compared data sets follow a monotonous function (*i.e.* including the possibility of threshold values of one parameter, *i.e.* temperature, after which the dependent parameter, *i.e.* AZ/VAZ or a fluorescence parameter, shows a strong change). It does not assume linear correlations, which are the basis of the Pearson correlation analysis, since no a priori no linearity between the studied parameters needs to exist. The correlation coefficients in Table 4 contain the respective levels of significance [*i.e.* <0.05 (*) and <0.01 (**)].

Results

Diurnal courses of electron flow and energy dissipation

To assess, whether chronic photoinhibition occurred during winter frost periods, the diurnal and nocturnal patterns of chl fluorescence parameters and of AZ/VAZ were monitored during a frost period (T_{min} of -7 to -1°C) in January 2010 under cloudy sky, to prevent strong dynamic photoinhibition obscuring chronic effects. Fv/Fm of the frost-exposed plants (Figure 1B) showed clear chronic and - on top of it - moderate dynamic photoinhibition, which was accompanied by a slight diurnal rise and nocturnal decrease of the AZ/VAZ ratio, starting from already very high predawn values (Figure 1C). In contrast, in spring, under non-frost conditions, practically no diurnal course of Fv/Fm was observed, while sun-exposed leaves revealed the typical diurnal pattern of AZ/VAZ (Figure 1F, G). Dissection of the initial rise of the fluorescence induction curve showed that energy dissipation (DI_0/RC) revealed a similar pattern as the AZ/VAZ curves, with very high values in plants kept outdoor in winter (Figure 1D).

The dissection of the initial rise of the fluorescence induction curve furthermore revealed increased ABS/RC, TR_0/RC and ET_0/RC values for frost-exposed leaves (Figure 2 A-D) and slight diurnal patterns of these parameters under spring conditions (Figure 2 E-H). Concerning the normalized area parameter S_m, plants exposed to frost outside showed a lower level than those sheltered in the frost-free greenhouse. However, when compared to plants measured in spring, the plants from the frost-free greenhouse revealed an increased level of S_m (Figure 2C,G).

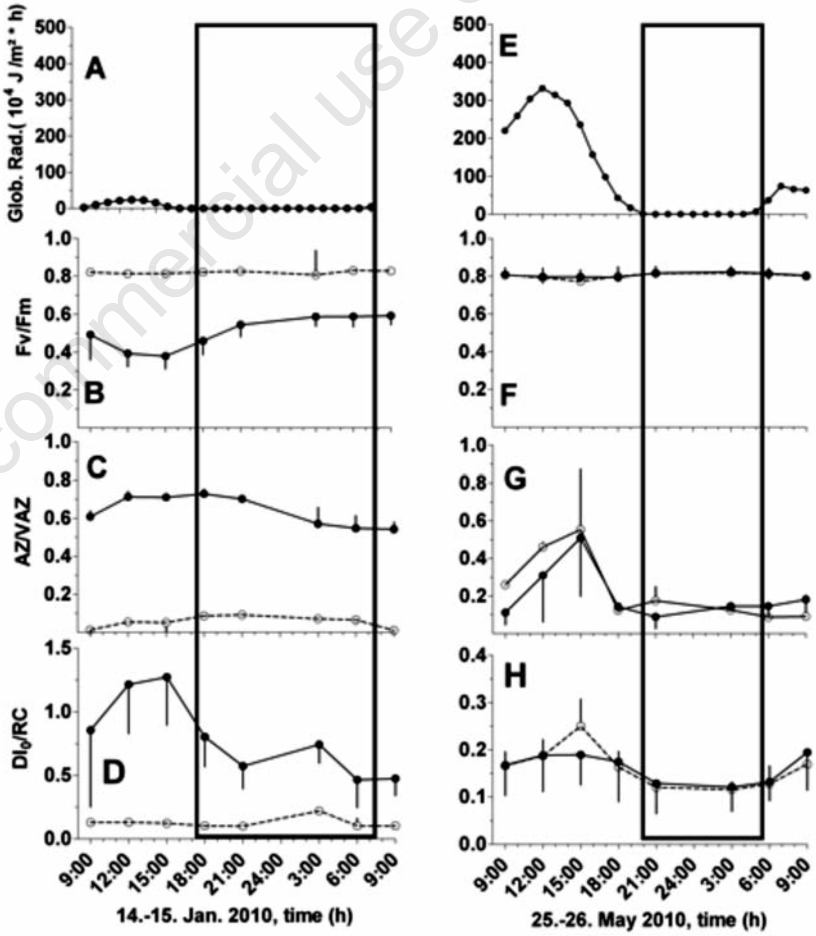

Figure 1. Diurnal courses of global radiation at Geisenheim, chl fluorescence parameters and AZ/VAZ in *Q. ilex* growing outdoors (●, FR) or in a frost-free foliar greenhouse (○, FRFF) at Frankfurt on 14.01.2010 (1A-D, T=-3° to +1°C, heavily clouded) and on 25.05.2010 (1E-H, T=+13° to +25°C, very sunny). Night periods are denoted by the black rectangles. Means±SD; n=10 (AZ/VAZ: n=2) leaves (technical replicates) per three trees (biological replicates) analysed on each location. A, anthera; Z, zeaxanthin; V, viola; FR, Frankfurt; FRFF, Frankfurt, frost-free.

Semi-annual courses of electron flow and energy dissipation

During winter 2009/10, very low outdoor temperatures (-16 to -18°C) were observed several times between mid-December and the end of February in the vicinity of the forest plot at Rüsselsheim (Figure 3A). This is close to the lethal temperature for *Quercus ilex* (*i.e.* -22°C for the northernmost Italian ecotype from Lake Garda).[12] In the Botanical Garden in Frankfurt, the city climate provided less severe temperature minima (-14 to -16°C) and less frost days. In the frost-free greenhouse, the heating assured temperature fluctuations only between +10 and +4°C, frost did not occur. While, in the greenhouse and in the Botanical Garden, winter survival of aboveground parts of *Q. ilex* was 100% (also for all of the *Q. ilex* plants not included in the measurements depicted here), at the forest site in Rüsselsheim 2 out of 6 trees lost their aboveground viable parts due to the preceding frost after 01.04.2010, and one of them died back completely, while the other resprouted from the roots in May/June. The two damaged trees were excluded from the evaluation after 01.04.2010. As evident from Figure 3C, the trees outdoor, both at the forest and the city site gradually developed severe chronic photoinhibition during winter after the first harsh frost days and remained photoinhibited until mid March, with only a transient relief in late February, when the weather was milder for a week. At the RU site, photoinhibition was more severe with predawn Fv/Fm decreasing below 0.3, except for March 11th, when the predawn measurements at FR revealed strong effects of the immediately preceding high-light and frost period, and no measurements were performed in RU in that particular week. The photoinhibition phase was accompanied by a reversible strong increase of predawn AZ/VAZ (Figure 3D). After the first strong frost period at mid-december, and anti-parallel to the development of the Fv/Fm ratio, energy dissipation increased, more so in the RU than in the FR site.

The dissection of the initial induction curve of chl fluorescence (Figure 4) also revealed a strong increase of ABS/RC (Figure 4B) and a moderate reversible increase of the trapping rate (TR_0/RC, Figure 4C) during the frost period, while the electron transport rate beyond Q_A (ET_0/RC) was hardly affected (Figure 4E). In frost-free kept plants, increased values of up to 80 arbitrary units for S_m were observed during the winter months, while the plants exposed to frost showed a tendency to decreased values, down to 40 arbitrary units, as had been the case in the analysis of the diurnal course in Figure 2. The probability of electron transport (ET_0/TR_0, Figure 4F) slightly decreased in the trees in the forest and in the frost-exposed trees in the city, as compared to frost-free control trees.

The analyses of the correlations between the fluorescence parameters and minimum night temperatures or AZ/VAZ are shown in Table 4. Significant correlations (Spearman) were observed between Fv/Fm, AZ/VAZ, DI_0/RC, ABS/RC and ET_0/TR_0 with T_{min} and between Fv/Fm, DI_0/RC, ABS/RC and ET_0/TR_0 with AZ/VAZ.

Discussion

Diurnal courses of energy dissipation and electron flow

As reported for various evergreen species,[8] the chronic winter photoinhibition (Figure 1B) was accompanied by a high sustained AZ/VAZ ratio, allowing for harmless energy dissipation (Figure 1C). On top, a minor diurnal oscillation was visible, antiparallel with the dynamic oscillation of Fv/Fm. Notably, this phenomenon was also strongly temperature (*i.e.* frost-) dependent and not an inherent characteristic of winter acclimated *Q. ilex*, since the frost-free plants showed no such increase of AZ/VAZ.

Apparently, the sustained high AZ/VAZ ratios allowed very efficient energy dissipation in the frost-exposed plants (Figure 1D), while, under spring conditions, the diurnal pattern of light induced Violaxanthin deepoxidation (Figure 1G) was reflected only in a slight diurnal pattern of energy dissipation efficiency (Figure 1H).

Under frost conditions, the dynamic photoinhibition imposed on the plants outdoors resulted in much higher ABS/RC values during daytime (Figure 2A), indicating either an increase of light absorbance (per leaf area) during daytime (*e.g.* by increasing antenna size) and/or a decrease of the concentration of active reaction centres. If compared to the diurnal fluctuation in unstressed leaves during late may (Figure 2E), it becomes evident, that the natural diurnal course of the ABS/RC value by diurnal fluctuations in antenna size[26] influences this parameter to a much lower extent. It is therefore concluded, that the high ABS/RC values around 2.0 during daytime in Figure 2A are the result of a large amount of *silent centres* under frost conditions.[21] The diurnal fluctuation of ABS/RC in unstressed leaves was accompanied by small parallel increases in light trapping (TR_0/RC), normalized area (S_m) and electron transport beyond Q_A (ET_0/RC) during daytime (Figure 2F-H). Under winter conditions, however, these oscillations are diminished or absent (Figure 2B-2D) and the frost-treated plants revealed higher values of TR_0/RC and ET_0/RC and lower S_m values than the frost-free treated ones. S_m, which can be interpreted as a measure of the capacity of the plastoquinone pool, had apparently increased in the winter-acclimated, but frost free plants as compared to plants measured in spring (Figure 4). Under frost conditions, this effect was obviously lost. We suggest, that the differences in TR_0/RC and

Table 4. Spearman correlation coefficients (r_s) and significance levels of the xanthophyll deepoxidation state AZ/VAZ and the fluorescence parameters Fv/Fm, DI0/RC, ABS /RC and ET0/TR0 with Tmin and AZ/VAZ. Correlations between TR_0/RC, ET_0/RC and S_m with T_{min} or AZ/VAZ were not significant.

vs	Fv/Fm	AZ/VAZ	DI0/RC	ABS/RC	ET0/TR0
T_{min}	0.65^{**}	-0.77^{**}	-0.68^{**}	-0.65^{**}	0.69^{**}
AZ/VAZ	-0.73^{*}	-----	0.78^{**}	0.80^{**}	-0.72^{**}

$^{*}P < 0.05$, $^{**}P < 0.01$. A, anthera; Z, zeaxanthin; V, viola; Fv/Fm, maximum quantum yield of primary photochemistry; DI0, dissipation rate; RC, reaction centre; ABS, absorption rate; ET0, electron transport rate; TR0: trapping rate.

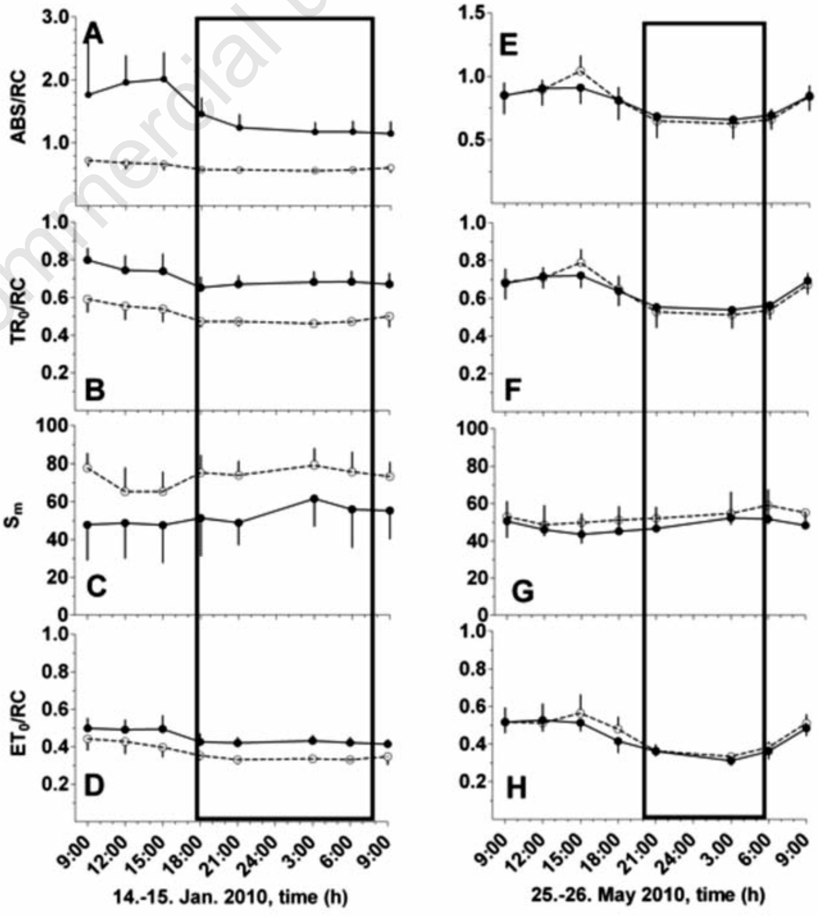

Figure 2. Diurnal courses of chl fluorescence parameters in *Q. ilex* growing outdoors (●, FR) or in a frost-free foliar greenhouse (○, FRFF) at Frankfurt on 14.01.2010 (A-D) and on 25.05.2010 (E-H). FR, Frankfurt; FRFF, Frankfurt, frost-free (see also Figure 1).

ET$_0$/RC could be the effect of a lower electron pressure in the frost-treated plants due to a decrease of active (electron transporting) reaction centres as a consequence of both chronic and dynamic photoinhibition.

It is - in our eyes - notable that in the frost-free, but winter acclimated plants, the diurnal oscillation of the fluorescence parameters is diminished as compared to the plants in late May.

One possible explanation could be that the diurnal change in antenna size, reported by Liker and Garab[26] relies on de novo synthesis of LHC proteins, which may be depressed under the lower winter temperatures.

Semi-annual courses of energy dissipation and electron flow

The unusually cold winter 2009/10 in Germany killed one out of six *Q. ilex* plants in the forest plantation and damaged another one severely. In the slightly milder city climate of Frankfurt, *Q. ilex* plants from the same batch remained unaffected. Larcher[12,27] described the frost sensitivity of *Q. ilex* in his studies on the northernmost Italian population from Lake Garda and showed that -17°C is 50% lethal to the buds and -26 to -28°C are 50% lethal to the xylem and the sprout cambium, respectively. When outdoor winter temperatures decreased beyond -5°C, reversible extracellular freezing occurred in the *Q. ilex* leaves, and Fv/Fm values decreased strongly as a consequence of cytoplasm dehydration.[27]

He therefore considered *Q. ilex* a frost-tolerant species, in contrast to other (frost-avoiding) Mediterranean sclerophyllous plant species, which avoid leaf freezing by supercooling and are irreversibly damaged, once the temperature falls below this critical minimum threshold.

Presumably independent from this direct effect, several studies with punctual sampling during winter[7,9-11] reported the occurrence of chronic photoinhibition at field sites in the Iberian Peninsula and in Central European city climate, when exposed to severe frosts and the same phenomenon is reported here. In the present study, the amount of sampling dates allowed a correlation of the data for maximum quantum yield of primary photochemistry of photosystem II in the dark-adapted state with the actual, preceding night temperatures and clearly indicated a significant correlation between these parameters in the range of -15 to +10°C (Table 4). The same held true for the correlations between minimum temperature and predawn AZ/VAZ and energy dissipation of active RC (Table 4). It has been shown previously[8,28] that winter-induced increase of AZ/VAZ in overwintering, evergreen plant species is closely related to a decrease in Fv/Fm. We could corroborate this correlation statistically over the winter period and extend it to the correlation between the parameters pre-dawn AZ/VAZ and DI$_0$/RC. The latter finding enhances the interpretation given in Adams *et al.*,[8] that *sustained high AZ/VAZ ratios* - independent of irradiation - may function as protective mechanisms in overwintering evergreens to prevent PS damage by excessive light in the early morning at subzero temperatures.

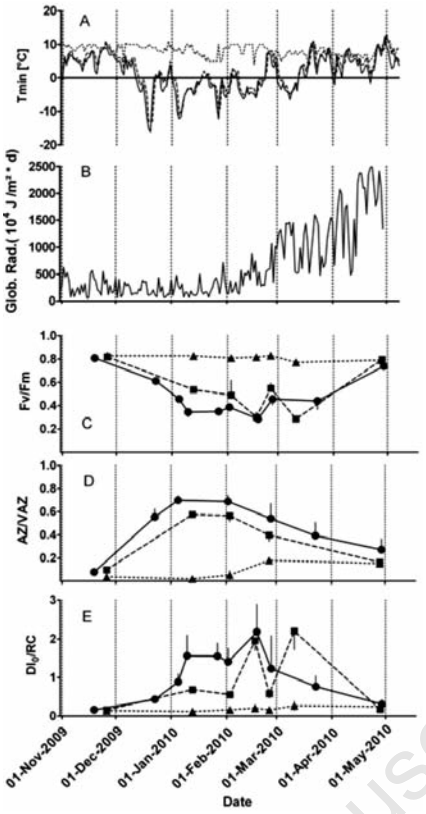

Figure 3. Semi-annual courses in winter of T$_{min}$ (3A), global radiation at Geisenheim (3B) and of maximum quantum yield of primary photochemistry (3C), AZ/VAZ (3D) and of the dissipation rate DI$_0$/RC (3E) in *Q. ilex* growing outdoors at RU (●), FR (■) and FRFF (▲). Daily minimum temperatures (3A) given for RU (____), FR (------) and FRFF (…..). Means±SD, n=10 (Fv/Fm) and 2 (AZ/VAZ) leaves per tree, respectively. Numbers of trees analysed were 6 in RU, 3 in FR and 4 in FRFF. A, anthera; V, viola; Z, zeaxanthin; FR, Frankfurt; FRFF, Frankfurt, frost-free; RU, Rüsselsheim; Fv/Fm, maximum quantum yield of primary photochemistry.

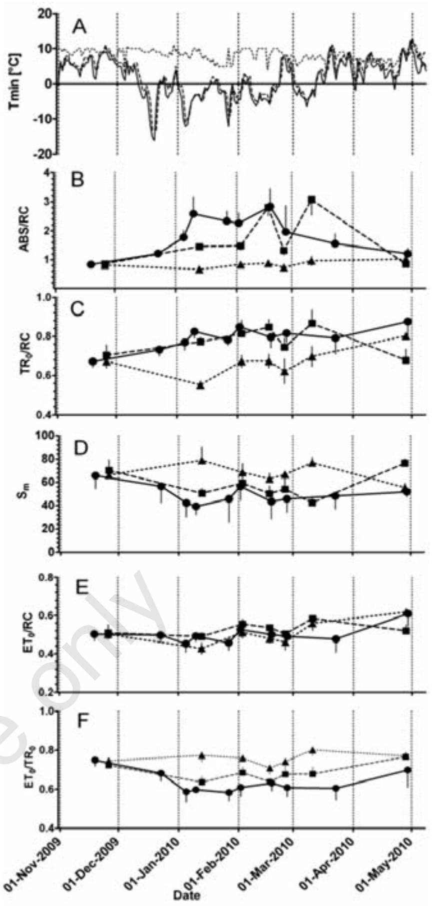

Figure 4. Semi-annual courses in winter of T$_{min}$ (4A) and of the OJIP-parameters ABS/RC (4B), trapping efficiency TR$_0$/RC (4C), S$_m$ (4D), electron transport efficiency ET$_0$/RC (4E), and electron transport probability ET$_0$/TR$_0$ (4F) in *Q. ilex* growing outdoors at RU (●), FR (■) and FRFF (▲). Daily minimum temperatures (3A) given for RU (____), FR (------) and FRFF (…..). Means±SD, n=10 leaves per tree. Numbers of trees analysed were 6 in RU, 3 in FR and 4 in FRFF. ABS, absorption rate; RC, reaction centre; TR$_0$, trapping rate; ET$_0$, electron transport rate; RU, Rüsselsheim; FR, Frankfurt; FRFF, Frankfurt, frost-free.

The analysis of the exciton/electron flow components showed, that the chronic photoinhibition period between mid December 2009 and mid-March 2010, which was modulated by the warm period at the end of February 2010 and the high light / frost period at begin of March (cf. data points for FR on March 11th), was accompanied by a reversible strong increase of the ABS/RC and DI$_0$/RC ratios. Energy dissipation under these conditions is mainly due to AZ/VAZ-mediated heat emission from the antennae, a minor amount only due to fluorescence.[8] Lehner and Lütz[29] found decreased Fv/Fm and increased ABS/RC values at a single date in February in two *Pinus spp.* from the alpine timberline. Unfortunately, at that time they did not calculate the dissipation flux from their data, but it can reasonably be assumed, that in these *Pinus spp.* similar processes as in *Q. ilex* occurred, *i.e.* a AZ/VAZ-mediated downregulation of PS II, which lead to a decrease in active reaction centres and an increase in heat dissipation capacity - independent of a light-driven pH gradient. Trapping (TR_0/RC) and electron transport (ET_0/RC) flows showed only minor differences between the frost-exposed and the frost-free plants. Similar results were reported for *Pinus cembra* under severe frost at one representative winter date.[30]

Since the fluorescence kinetic-derived parameters only take the active reaction centres into account,[21] and since the ABS/RC and DI_0/RC parameters reacted quite fast to the intermittent warming by the end of February, we conclude that their increase was not the (unlikely) result of a frost-associated increase of absorbance capacity. Instead, we suggest that a decrease of available active reaction centres due to downregulation and inactivation of the majority of the reaction centres occurred (Figure 4D).[8,31] The analysis of the electron transport probability (ET_0/TR_0) showed a moderate depression of the latter during the period of chronic photoinhibition, consistent with the rationale brought forward by Force *et al.*[22] on the expected effects of photoinhibition. From their experiments with isolated intact and broken chloroplasts and photoinhibited leaves, they concluded that decreases in the ET_0/TR_0 fluorescence parameter are indicative of impaired Q_A^- reoxidation, presumably the result of impaired binding of Q_B to the D1 protein.[32] Hence, while the frost effects on energy dissipation (DI_0/RC) and on predawn AZ/VAZ were large, whereas the effect on electron transport probability (ET_0/TR_0) was significant, but small, we probably see two more or less independent sides of chronic photoinhibition on the photosynthetic apparatus and the observed significant correlation between AZ/VAZ and ET_0/TR_0 may well be correlative, but not causal. It might therefore well be worthwhile to compare the correlations between DI_0/RC, ET_0/TR_0 and AZ/VAZ in both chronically and dynamically photoinhibited *Q. ilex* leaves in future experiments.

As already found during the diurnal measurements in Figure 2, S_m increased throughout winter in FRFF plants, but not in the frost-exposed plants (Figure 4), leading to a nearly twofold difference between FRFF and RU plants on some sampling days during the frost period (Jan14 Mar). Griffith *et al.*[33] and Gray *et al.*[34] have shown, that the total plastoquinone (PQ) pool in winter rye thylakoids acclimates to shifts in electron pressure (*i.e.* it can increase twofold under high excitation pressure). During leaf senescence, the photosynthetically active PQ pool size decreases significantly within one or two weeks.[35] Since chloroplasts can contain at least three populations of PQ [*i.e.* a fast reducible pool in granal thylakoids, a slowly reducible PQ pool in stromal thylakoids, and a (usually non-reducible) storage pool in plastoglobuli,[36] it can be hypothesized, that acclimation processes of the fast reducible PQ pool size towards experienced redox pressure can occur within limited time, *i.e.* a few days. S_m might then be regarded as a *memory index* of the preceding redox poise of the PQ pool. In our case, from January to March, the redox poise in the FRFF plants may have been higher than in the field sites, because the FR and RU plants showed a 10-fold or more increased amount of energy dissipation as heat instead of electron transport into the ET chain due to their chronic photoinhibition (Figure 3E).

The intermittent increase of S_m at the FR site in late April (in late May, their S_m values equalled those of the FRFF plants again, Figure 2) possibly was due to a specific high light and low morning temperature situation in the unshaded experimental field on the days preceding the measurements. However, to substantiate the interpretation of the S_m data as a *memory index* of the preceding redox pressure on the electron transport chain, additional experiments would be necessary, *e.g.* by differential shading and independent assessment of the redox state of the PQ pool during the days preceding the pre-dawn OJIP measurements.

Conclusions

In summary, we present conclusive evidence that the Mediterranean broadleaf evergreen species *Q. ilex* shows the competence for similar photoprotective behaviour with continuously increasing AZ/VAZ and downregulation of PS II under severe, long-lasting Central European frost periods as it is known from species from the alpine timberline. We suggest that *Q. ilex* may, from this point of view, have the potential to be considered a potential forestry tree species for future forestation experiments in warm, dry Central European stands, *e.g.* in mixed plantations with other Central and South European drought tolerant species to minimize the economic risks of Climate Change. However, since part of the plants at the Rüsselsheim experimental site was damaged by the severe frost in the winter of 2009/10, direct frost effects on the stems (*i.e.* on meristematic tissue and on water transport properties) may be limiting the frost tolerance of the aboveground parts of *Q. ilex* and only a few degrees difference in minimum temperature between the city climate and the forest climate may be crucial for (aboveground) survival. It should be recalled - in this context - that *Q. ilex* trees in parks in the city climate in Frankfurt have survived and been well growing for 60 or more years.[7] Since *Q. ilex* is a diffuse porous species,[37] which uses the same vessels for several years, embolism damage due to xylem freezing in young plants with low stem diameter at temperatures of -18°C or lower may strongly influence the survival rates, independent of damage to the photosynthetic apparatus.[12,38,39] Repair mechanisms of such frost-induced embolism events[38] may not be sufficient in individual plants and these effects as well as possible ecotype gradients of frost tolerance within the species depending on geographical provenience should be taken into account and analysed in future experiments.

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