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Chilling-induced photoinhibition in two oak species: Are evergreen leaves inherently better protected than deciduous leaves?

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Abstract

We compared the sensitivity to cold stress, in terms of photosynthetic capacity and changes in chlorophyll fluorescence of photosystem 2 (PS2), of an evergreen and a deciduous oak species, which co-occur in the southeastern United States. We predicted that the evergreen species, *Quercus virginiana*, which must endure winter, is likely to have an inherently greater capacity for energy dissipation and to be less susceptible to chilling stress than the deciduous species, *Quercus michauxii*. Short-term cold stress in both species lead to greater than 50 % reduction in maximum photosynthetic rates, 60-70 % reduction in electron transport, and irreversible quenching of PS2 fluorescence. The kinetics of recovery in the dark after exposure to 1 h high irradiance (1000 μmol m^{-2} s^{-1}) and chilling (5 °C) showed that the evergreen *Q. virginiana* exhibited more protective q_E and less irreversible quenching (q_I) than the deciduous *Q. michauxii*. The large q_E which we observed in *Q. virginiana* suggests that the capacity for photoprotection at low temperatures is not induced by a long-term acclimation to cold but preexists in evergreen leaves. This capacity may contribute to the ability of this species to maintain leaves during the winter.

Additional key words: chlorophyll fluorescence; evergreen and deciduous trees; leaf habit; non-photochemical quenching; photoprotection; *Quercus michauxii; Quercus virginiana.*

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Abbreviations: Chl: chlorophyll; ETR: electron transport rate; F_0, F_s: initial and steady state levels of Chl fluorescence; F_m and F_m': maximum levels of Chl fluorescence in a dark adapted leaf and in an irradiated leaf; HI: high irradiance; LT: low temperature; P_N: net photosynthetic rate; PS: photosystem; q_E: energetical quenching; q_T: state II transition; q_I: irreversible quenching; q_N: non-photochemical quenching.

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Introduction

Quercus virginiana Miller and *Quercus michauxii* Nutall are two oak species which co-occur in the southeastern United States but differ in leaf habit (evergreen *vs.* deciduous). Both species are white oaks (*Quercus*, section *Quercus*) and both occupy the same mesic, fertile habitats. However, *Q. virginiana* maintains its leaves and continues to assimilate carbon during the winter months, making it likely to have better photoprotection of its photosynthetic apparatus at low temperatures than the deciduous *Q. michauxii.*

Photoinhibition occurs under low temperatures and high irradiance (Ögren *et al.* 1984, Ball 1994). In mid-autumn in the southeastern US it is common for seedlings of these two oak species to experience both high irradiance and cold temperatures (0-10 °C) during the early morning hours after sunrise, conditions likely to cause photoinhibition. Photoinhibition of photosynthesis is related to the absorption of radiant energy by the pigment antennae in excess to what can be dissipated photochemically (Osmond 1981, Öquist *et al.* 1987, Hodgson and Raison 1991) resulting in damages to the PS2 reaction centers (Björkman 1987, LeGouallec *et al.* 1991, Solhaug and Haugen 1998). Low temperatures impose rate limitations on enzymes involved in photosynthesis and inhibit the alternative ways of energy dissipation such as photorespiration, superoxide dismutase activity, and the xanthophyll cycle (Öquist *et al.* 1987, Pfündel and Bilger 1994). The extent of photodamage, however, depends upon the potentiality of protective mechanisms, such as energetical quenching (q_E), which is associated with zeaxanthin formation (Somersalo and Krause 1990, Schindler and Lichtenthaler 1994, Demmig-Adams *et al.* 1995).

In a study of two broad-leaved evergreen species, energy dissipation activity developed more slowly at lower leaf temperatures, but the final steady state level was greater at these lower temperatures (Adams and Demmig-Adams 1995). The rate at which energy dissipation activity increased was similar to that of de-epoxidation of violaxanthin to antheraxanthin and zeaxanthin at different temperatures (see also Adams and Demmig-Adams 1994). In five evergreen species large amounts of zeaxanthin and antheraxanthin were retained during winter cold stress, which was associated with a large non-photochemical quenching of PS2 Chl fluorescence (Verhoeven *et al.* 1996). This retention ability induced a protection of PS2, the efficiency of which increased rapidly when evergreen plants were returned to higher temperatures. Nevertheless, this increase in PS2 efficiency has not yet been clearly identified as the relaxation of classical q_E quenching. Relative to annual and deciduous species, leaves of evergreen species exhibit significantly reduced midday photosynthetic rate (Gamon *et al.* 1997). This reduction is associated with a reduced radiation efficiency, corresponding to an increased level of the protective xanthophyll, zeaxanthin. It has not been shown, however, whether there are inherent differences in photoprotective capacity at low temperatures in evergreen and deciduous species in the absence of cold acclimation.

The purpose of this study was to determine whether there are inherent differences in the sensitivity of *Q. michauxii* and *Q. virginiana* to photoinhibition during short-

term chilling (LT) and high irradiance (HI), as indicated by changes in photosynthetic capacity and changes in Chl fluorescence of PS2. We tested the following hypothesis: *Q. virginiana* is inherently more tolerant of cold stress under HI than *Q. michauxii*. Specifically, *Q. virginiana* will exhibit a lower reduction in maximum photosynthetic rates and electron transport rates at cold temperatures relative to warm temperatures and a faster recovery of PS2 after exposure to HI and LT than *Q. michauxii*.

Materials and methods

Plants: Fifteen seedlings of *Q. michauxii* and *Q. virginiana* were grown from seeds (collected in Gainesville, Florida) in 20 000 cm^3 containers in a mixture of peat, perlite, vermiculite, and sand in a greenhouse in Orsay, France. Plants were grown under natural irradiance, and daylength was extended with artificial light sources to maintain a constant photoperiod of about 12 h. The daytime temperature in the greenhouse was maintained at about 24 °C. Measurements were started, when the plants were approximately 7 months old, on the newest fully expanded leaves. Plants were watered regularly. Leaf temperatures in all experiments were controlled using the leaf temperature control in the sensor head of the *LI 6400* photosynthesis system (*Li-cor*, Lincoln, NE, USA) in combination with an air conditioning and heating unit inside of an enclosed, walk-in chamber.

Experiment 1: Accelerated diurnal cycles at chilling temperatures: We measured the effects of LT on linear electron transport rates (ETR) and photosynthesis. We made continuous measurements of ETR and CO_2 assimilation rates (P_N) during an artificial diurnal cycle of irradiation lasting 4 h, which reached a maximum irradiance of 1000 μmol m^{-2} s^{-1}. In the control treatment, four seedlings of each species were dark adapted overnight at 24 °C. Two or three F_v/F_m measurements were taken in the dark prior to the onset of irradiation. Irradiation, provided by a slide projector and filtered by a $CuSO_4$ solution, was increased step-wise following a Gaussian curve with 32 steps total. Each irradiation interval was maintained for 400 s for a total photoperiod of about 210 min. In this way, responses of each plant to both increasing and decreasing irradiance was observed. Plants were then allowed to recover in the dark for about one hour. After several days, these manipulations were repeated on the same plants at cold temperatures. Plants were dark adapted overnight at 8 °C and the diurnal cycles were carried out at 5 °C followed by a dark period of 1 h at 5 °C. Finally, the same cold temperature manipulations were repeated again, but plants were allowed to recover at warm temperatures (24 °C). During the treatment periods, Chl fluorescence parameters, F_0 or F_s, and simultaneous P_N measurements were taken every 30 s on attached leaves. F_m or F_m' measurements were taken every 30 min. In order to increase the sample size for measurements of radiant energy-saturated P_N and ETR, these parameters were measured on six additional seedlings of each species after exposure to HI (1000 μmol m^{-2} s^{-1}) for 15 min at warm (24 °C) or cold (5 °C) temperature. Prior to these measurements, plants were dark adapted overnight at ambient or cold temperature (20 or 8 °C), and initial F_v/F_m was

measured. Plants were then pre-adapted to ambient irradiance during the morning (*ca.* 500 μmol m^{-2} s^{-1}) to induce electron flow and photosynthesis prior to saturating irradiance. Using irradiance curves, we verified that the P_N was saturated at 1000 μmol m^{-2} s^{-1} for both species.

Experiment 2: Relaxation kinetics after exposure to HI and chilling temperatures: Chl fluorescence parameters were monitored during a series of manipulations of irradiance and temperature in order to observe the relaxation kinetics of non-photochemical quenching (q_N) following simultaneous HI and LT (Fig. 3). Plants were first acclimated to HI for 1 h at 5 °C. They were then left in the dark for 15 min and returned to HI for 10 min. During their return to HI, the temperature was raised to 24 °C. Finally, the plants were returned to darkness at 24 °C. Throughout the experiment, F_0 or F_s was monitored continuously. F_m or F_m' was monitored every 10 min, although more frequently during initial Chl fluorescence recovery period in the dark. Kinetics of the relaxation of q_N were analyzed in terms of multi-exponential components (cf. Horton and Hague 1988, Walters and Horton 1991).

Absorption, Chl fluorescence, and photosynthesis measurements: Absorption measurements were made on representative leaves of each species to use in calculations of electron transport rate. We used an integrating sphere *RSA-HP-53* (*Labsphere*, North Sutton, NH, USA) attached to a spectrophotometer (*Hewlett Packard 8453*, Les Ulis, France).

Chl fluorescence was measured at a distance of 1 m from the leaf using a Frequency Induced Pulse Amplitude Modulated Fluorometer (FIPAM) (Moya, patent pending). With this apparatus, F_0 and F_s were obtained using 3 μs pulses of an excitation laser diode (635 nm, 15 mW) with a frequency of 0.6 Hz. F_m and F_m' were measured by switching this frequency to 106 KHz. The FIPAM also measured photosynthetically active radiation (PAR) and leaf temperature. The rate of linear electron flow was calculated using the following equation: $ETR = (\Delta F/F_m') \times PAR \times 0.8 \times 0.5$, where the term 0.8 is the fraction of incident quanta absorbed by oak leaves.

Photosynthesis measurements in Exp. 1 were made with a *Li-Cor 6400* portable photosynthesis system (*Li-Cor*, Lincoln, NE, USA). Measurements were logged automatically after a signal from the FIPAM so that the photosynthesis and Chl fluorescence measurements would be made simultaneously.

Results

Exp. 1: Variations of Chl fluorescence during the accelerated diurnal cycle at 24 and 5 °C are shown for *Q. virginiana* and *Q. michauxii* (Fig. 1). In both the evergreen and deciduous species, the quantum efficiency of PS2 and of linear electron transport, measured by the $\Delta F/F_m'$ during a diurnal cycle of irradiation, decreased considerably in the cold even at low irradiance. However, dark adapted F_v/F_m, therefore the potential quantum yield of PS2, decreased only slightly after chilling in the dark. The LT treatment decreased capacities of P_N and ETR by a factor of

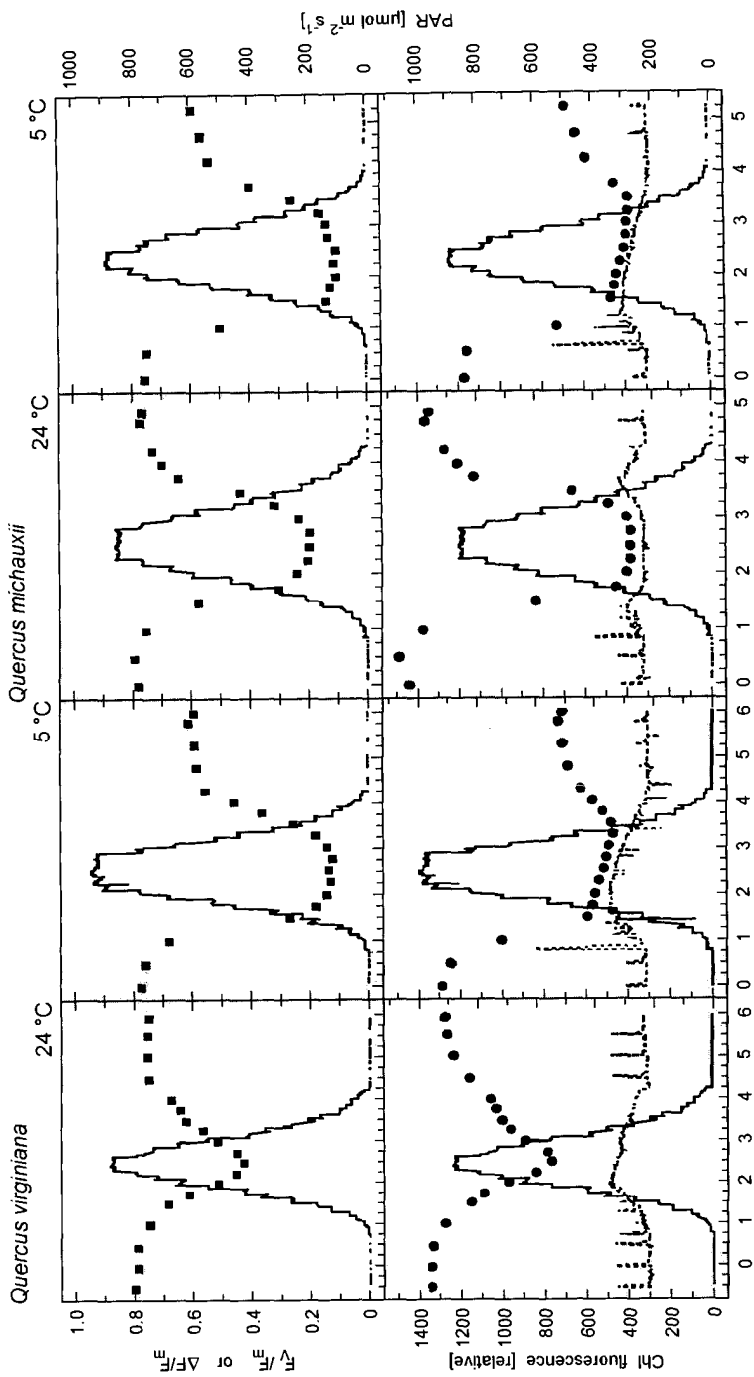

Fig. 1. The response of chlorophyll (Chl) fluorescence parameters (F_v/F_m or $\Delta F/F_m'$, F_m or F_m', and F_0 or F_s) to accelerated diurnal cycles of irradiation at 24 and 5 °C on attached leaves of *Q. virginiana* and *Q. michauxii*. Variable Chl fluorescence ratios, F_v/F_m (dark adapted leaf) or $\Delta F/F_m'$ (irradiated leaf) are shown in the upper panels (■). In the lower panels, *black circles* show maximum fluorescence: F_m (dark adapted leaf) or F_m' (irradiated leaf); *dotted lines* show initial or steady state Chl fluorescence, F_0 or F_s. Solid lines in all panels show increasing and decreasing PAR values throughout the diurnal cycle, and shaded regions indicate when the leaf was in darkness.

approximately 2.5 for both species (Fig. 2). Mean values for the ratio of warm to cold ETR (or P_N) under saturating irradiance suggest that *Q. michauxii* was somewhat more affected by LT than *Q. virginiana*. Regardless of the temperature or the species, about 45 % of the total electron transport between PS2 and PS1 was consistently partitioned to CO_2 fixation.

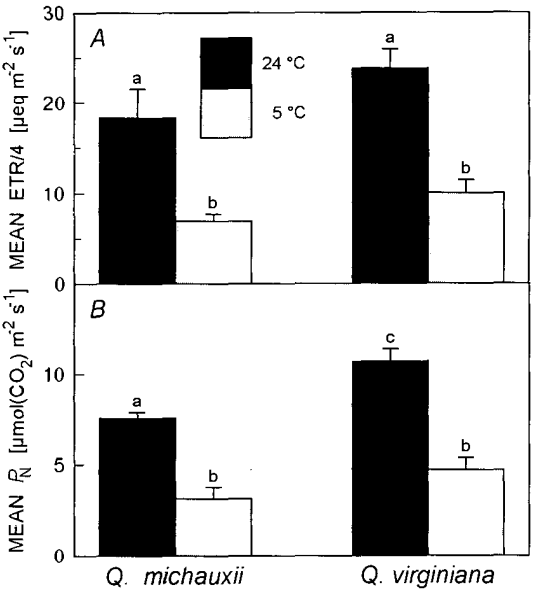

Fig. 2. Mean electron transport rates (ETR) (*A*) and light-saturated net photosynthetic rates, P_N (*B*) for *Quercus virginiana* and *Quercus michauxii* at ambient and chilling temperatures. In (*A*), μeq. are divided by 4 in order to compare to CO_2 assimilation. Error bars are +1 SE. Means are different if $p<0.05$.

Chl fluorescence measurements at LT during the diurnal cycles showed not only that PS2 efficiency of both species was reduced in the light, but that after a diurnal cycle, when the plants were again in darkness, they did not return to their initial quantum yield ($F_v/F_m = 0.8$). A quenching of F_m was apparent which was irreversible at cold temperatures but partially reversible after a transition to warm temperatures (24 °C) (values not shown).

Exp. 2: In representative plants of both species (Fig. 3), a similar amplitude of total q_N was reached after 1 h of HI at 5 °C (see Table 1). Greater photoprotection in leaves of *Q. virginiana* was apparent from the kinetics of reversion of F_m at 24 °C (Fig. 3, Table 1). These kinetics are best decomposed into two exponential processes: a rapid one ($t_{1/2}$ 20 s) and a slow one ($t_{1/2}$ several min) with a plateau. The rapid phase corresponds to a quenching of the q_E type and the slow phase to a quenching of the q_T type, *i.e.*, a state 2 transition (Horton and Hague 1988). Finally, the plateau corresponds to a very slow, essentially irreversible phase of the q_I type, which has been frequently interpreted as photoinhibition (Horton and Hague 1988). The kinetic

analysis of the reversion of F_m at 24 °C after a cold treatment in HI showed that in *Q. michauxii* less q_E was formed, and consequently, more q_I developed in this species due to diminished q_E which serves a protective function (Table 1).

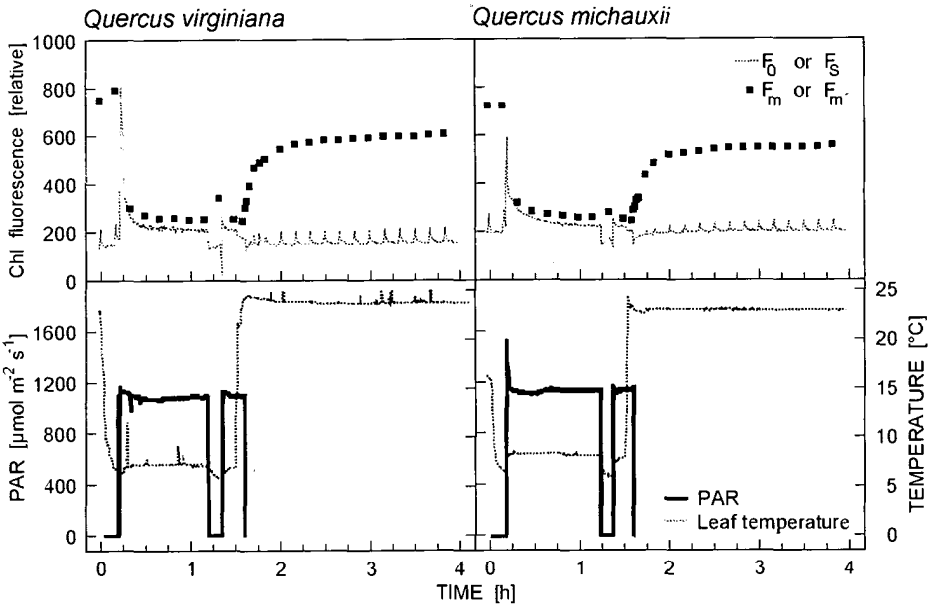

Fig. 3. Variations in chlorophyll (Chl) fluorescence in response to temperature and PAR in the evergreen oak, *Quercus virginiana* (*left*) and the deciduous oak, *Quercus michauxii* (*right*). Plants were acclimated to high irradiance (PAR = 1000 µmol m^{-2} s^{-1}) for 1 h at 5 °C. They were then left in the dark for 15 min and returned to high irradiance for 10 min. The temperature was raised to 24 °C, and plants were returned to darkness to allow relaxation of q_N. *Top panels* show Chl fluorescence parameters F_0 or F_s (*dotted line*) and F_m or F_m' (■), and *bottom panels* show PAR (*solid line*) and leaf temperature (*dotted line*) during the experiment.

Table 1. A comparison of the kinetics of relaxation of the non-photochemical quenching (q_N) of chlorophyll fluorescence for the evergreen and deciduous oak species. Total q_N was calculated according to Stern-Volmer: (dark-adapted F_m/steady-state F_m after 1 h light) - 1. A is the relative amplitude of components of q_N relaxation (mean and SD). A_0 corresponds to the irreversible component. $T_{1/2}$ are half lives of the components of q_N relaxation.

Plant		Total q_N	A_0 [%]	A_{slow} [%]	A_{fast} [%]	$T_{1/2\ slow}$ [min]	$T_{1/2\ fast}$ [s]
Q. virginiana	mean	2.17	11.4	33.1	55.5	11.15	18.7
	SD	0.17	2.4	8.8	8.3	7.52	14.2
Q. michauxii	mean	2.08	18.8	56.9	24.0	4.33	15.5
	SD	0.11	6.3	3.6	6.0	0.70	2.3

Discussion

Exposure to simultaneous HI and LT, a condition which is common in temperate regions during winter, poses a problem of excess excitation energy for plants which can lead to damages in the reaction centers associated with photoinhibition (*e.g.*, Bilger and Björkman 1991, Björkman and Demmig-Adams 1994, Briantais 1996). Previous studies have shown a retention or increase of xanthophyll cycle pigments in winter-acclimated evergreen plants (Adams and Demmig-Adams 1994, Ottander *et al.* 1995, Verhoeven *et al.* 1996) as well as an increase in the extent of de-epoxidation of violaxanthin to antheraxanthin and zeaxanthin during the winter compared with the summer (Adams and Demmig-Adams 1995). The conversion of violaxanthin to zeaxanthin is thought to protect the photosynthetic apparatus against the damaging effects of singlet state oxygen by dissipating excess excitation energy, thus diminishing the formation of singlet oxygen (Adams *et al.* 1990, Bilger and Björkman 1994; but see Schindler and Lichtenthaler 1996).

It is not clear, however, whether high energy dissipation activity in cold-stressed evergreen plants is due to an inherent potential to protect against the risk of photodamage or whether it is the result of cold acclimation. Cold acclimation is thought to lead to temperature adjustment of the photosynthetic apparatus resulting in less over-excitation of PS2 (Somersalo and Krause 1989, Wijk and Hasselt 1990). Long-term cold acclimation increases anti-oxidant and carotenoid contents as well as active-oxygen scavengers (Schöner and Krause 1990), protective mechanisms which would reduce the risk of photoinhibition (Wijk and Hasselt 1990).

We predicted that evergreen species, which must endure winter, are likely to have a greater inherent capacity for energy dissipation and to be less susceptible to chilling stress than deciduous species, which drop their leaves prior to winter. Our results show that both the evergreen and deciduous oak species, which were not cold acclimated, experienced reduced carbon assimilation and electron transport rates at cold temperatures under saturating irradiance (Fig. 2). However, the evergreen *Q. virginiana* exhibited significantly more protective q_E ($p<0.008$) and less irreversible quenching (q_I) at LT than the deciduous *Q. michauxii* (Table 1). The large q_E which we observed in *Q. virginiana* suggests that the capacity for photoprotection at LT is not induced by an acclimation to cold but preexists in evergreen leaves.

We also observed a significantly greater q_T in *Q. michauxii* relative to *Q. virginiana* ($p<0.015$; Table 1). This quenching mechanism can be interpreted as a redistribution of excitation energy which may happen by a change in the extent of direct energy transfer (spillover) from PS2 to PS1 or by a change in the fraction of incident radiation initially absorbed by PS1 by direct movement of light-harvesting complexes from PS2 to PS1 (Canaani 1990, Briantais 1996). Taylor and Craig (1971) indicated that the spillover effect could be due to a HI- and LT-induced de-stacking of the thylakoid grana. Both of these q_T mechanisms effectively decrease the incoming radiant energy flux to PS2.

In summary, we demonstrate here that two closely related species, one deciduous and one evergreen, which were both grown under warm conditions, showed differences in their potential for protective q_E of PS2 Chl fluorescence when exposed

to HI and short-term chilling. The higher potential for q_E in the evergreen leaves of *Q. virginiana* was not the result of cold temperature acclimation. Thus, we conclude that *Q. virginiana* is inherently more protected against photoinhibitory damage at LT than *Q. michauxii*, a factor which may contribute to the former's ability to maintain leaves during the winter.

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