

Chilling and freezing stress in live oaks (*Quercus* section *Virentes*): intra- and inter-specific variation in PS II sensitivity corresponds to latitude of origin

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Abstract Sensitivity to cold and freezing differs between populations within two species of live oaks (*Quercus* section *Virentes* Nixon) corresponding to the climates from which they originate. Two populations of *Quercus virginiana* (originating from North Carolina and north central Florida) and two populations of the sister species, *Q. oleoides*, (originating from Belize and Costa Rica) were grown under controlled climate regimes simulating tropical and temperate conditions. Three experiments were conducted in order to test for differentiation in cold and freezing tolerance between the two species and between the two populations within each species. In the first experiment, divergences in response to cold were tested for by examining photosystem II (PS II) photosynthetic yield ($\Delta F/F_m'$) and non-photochemical quenching (NPQ) of plants in both growing conditions after short-term exposure to three temperatures (6, 15 and 30°C) under moderate light (400 $\mu\text{mol m}^{-2} \text{s}^{-1}$). Without cold acclimation (tropical treatment), the North Carolina population showed the highest photosynthetic yield in response to chilling temperatures (6°C). Both ecotypes of both species showed maximum $\Delta F/F_m'$ and minimum NPQ at their daytime growth temperatures (30°C and 15°C for the tropical and temperate treatments, respectively). Under the temperate treatment where plants were allowed to acclimate to cold, the *Q. virginiana* populations showed greater NPQ under chilling temperatures than *Q. oleoides* populations, suggesting enhanced mechanisms of photoprotective energy dissipation in the more temperate species. In the second

and third experiments, inter- and intra-specific differentiation in response to freezing was tested for by examining dark-adapted F_v/F_m before and after overnight freezing cycles. Without cold acclimation, the extent of post-freezing declines in F_v/F_m were dependent on the minimum freezing temperature (0, -2, -5 or -10°C) for both populations in both species. The most marked declines in F_v/F_m occurred after freezing at -10°C, measured 24 h after freezing. These declines were continuous and irreversible over the time period. The North Carolina population, however, which represents the northern range limit of *Q. virginiana*, showed significantly less decline in F_v/F_m than the north central Florida population, which in turn showed a lower decline in F_v/F_m than the two *Q. oleoides* populations from Belize and Costa Rica. In contrast, after exposure to three months of chilling temperatures (temperate treatment), the two *Q. virginiana* populations showed no decline in F_v/F_m after freezing at -10°C, while the two *Q. oleoides* populations showed declines in F_v/F_m reaching 0.2 and 0.1 for Costa Rica and Belize, respectively. Under warm growth conditions, the two species showed different F_0 dynamics directly after freezing. The two *Q. oleoides* populations showed an initial rise in F_0 30 min after freezing, followed by a subsequent decrease, while the *Q. virginiana* populations showed a continuous decrease in F_0 after freezing. The North Carolina population of *Q. virginiana* showed a tendency toward deciduousness in response to winter temperatures, dropping 58% of its leaves over the three month winter period compared to only 6% in the tropical treatment. In contrast, the Florida population dropped 38% of its leaves during winter. The two populations of the tropical *Q. oleoides* showed no change in leaf drop during the 3-months winter (10% and 12%) relative to their leaf drop over the same timecourse in the tropical treatment. These results indicate

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important ecotypic differences in sensitivity to freezing and cold stress between the two populations of *Q. virginiana* as well as between the two species, corresponding to their climates of origin.

Keywords Chilling and freezing sensitivity · Chlorophyll fluorescence · Irreversible decline in $F_v/F_m - F_0$ · Non-photochemical quenching · Population and species-level variation · Ecotypes · Climatic gradient · Live oaks

Abbreviations

F_0	Fluorescence level when leaf is in a non-energized (dark) state, all non-photochemical processes are at a minimum and plastoquinone electron acceptor pool (Q_a) of PS II is fully oxidized
F_m	Maximum fluorescence level in a dark leaf when Q_a is transiently fully-reduced and non-photochemical processes are at a minimum
F_m'	Fluorescence level in an illuminated leaf when Q_a is transiently fully-reduced
F_v	Maximum variable chlorophyll fluorescence ($F_m - F_0$) in a dark leaf when all non-photochemical processes are at a minimum
F_s	Steady state fluorescence in an illuminated leaf
ΔF	Variable fluorescence in an illuminated leaf ($F_m' - F_s$)
F_v/F_m	Maximum photochemical efficiency of photosystem II in a dark leaf when non-photochemical processes are at a minimum
$\Delta F/F_m'$	Photochemical efficiency of photosystem II in an illuminated leaf
NPQ	Non-photochemical quenching of excited chlorophyll calculated as $(F_m - F_m')/F_m'$ (Stern-Volmer relationship)
PAR	Photosynthetically-active radiation (between wavelengths 400 nm and 700 nm)
PS I	Photosystem I
PS II	Photosystem II

Introduction

Freezing stress is often considered to limit the northern distribution (in the Northern Hemisphere) of many plant species because an increasing number of physiological mechanisms and cold-induced transformations are required to prevent lethal freezing of living tissues as winter becomes more severe at higher latitudes (Guy 2003; Cavender-Bares 2005). Freezing stress is most immediate for above ground tissues, particularly leaves, which have low thermal mass and are directly exposed to air temperatures. The risk of

lethal freezing inside living cells is thus higher for leaves than for more protected organs (Burke et al. 1976; Wisniewski et al. 1991; Ball et al. 2004). Chilling temperatures above freezing also pose a stress for leaves; as enzymatic processes decline with temperature, the capacity of the photosynthetic apparatus to dissipate absorbed energy through photochemistry is reduced. In the absence of mechanisms to increase dissipation of excess light energy at low temperatures, photodamage can result (e.g., Somersalo and Krause 1990; Öquist and Huner 1991; Holly et al. 1994; Adams et al. 1995; Ball et al. 1997; Hovenden and Warren 1998; Cavender-Bares et al. 1999; Verhoeven et al. 1999b; Atkin et al. 2000; Blennow and Lindkvist 2000; Oliveira and Peñuelas 2001).

Among woody angiosperms, the most common strategy for surviving freezing is deciduousness, accompanied by winter dormancy. In species, such as live oaks that maintain leaves year-round, however, protection of photosynthetic processes in leaves is likely to be critical for tolerance to cold and freezing stress (Ball et al. 1991; Garcia-Plazaola et al. 1997; Garcia-Plazaola et al. 1999; Cavender-Bares and Bazzaz 2004). Previous studies have shown that cold-acclimation involves a series of morphological and biochemical changes in leaves and other organs that increase freezing tolerance of plants adapted to temperate climates (e.g., Strand and Öquist 1988; Somersalo and Krause 1989; Öquist and Huner 1991; Rajashekar and Lafta 1996; Savitch et al. 1997; Logan et al. 1998b; Bertrand et al. 1999; Schrader and Sauter 2002; Ball et al. 2004). Increased thermal dissipation of light absorbed by photosystem II (PS II) due to increased carotenoid pools and activity of the xanthophyll cycle is known to be an important factor in acclimation of many plants, including oak species, to low temperatures under conditions of high light (Adams and Demmig-Adams 1995; Koroleva et al. 1995; Garcia-Plazaola et al. 1999; Corcuera et al. 2004; Corcuera et al. 2005). Sensitivity to freezing is known to vary across the seasons, due to cold acclimation, and even closely related species respond differently to seasonal changes in temperature (Cavender-Bares et al. 2005). Species also differ in their inherent sensitivity to chilling and freezing stress in the absence of cold acclimation (Cavender-Bares et al. 1999; Feild and Brodribb 2001).

Intraspecific variation in the cold sensitivity of plants has previously been documented, particularly in species that occur across a broad geographic range (e.g., Aho 1994; Tretiach et al. 1997; Boorse et al. 1998b; Nardini et al. 2000; Nilsson 2001; Rehfeldt et al. 2002; Rehfeldt 2004). Several of these studies demonstrated variation in cold tolerance in woody plants that corresponds predictably to the habitat of origin, providing evidence in support of local adaptation (e.g., Nilsson and Walfridsson 1995; Rehfeldt et al. 2002; Rehfeldt 2004). In particular, Aranda et al.

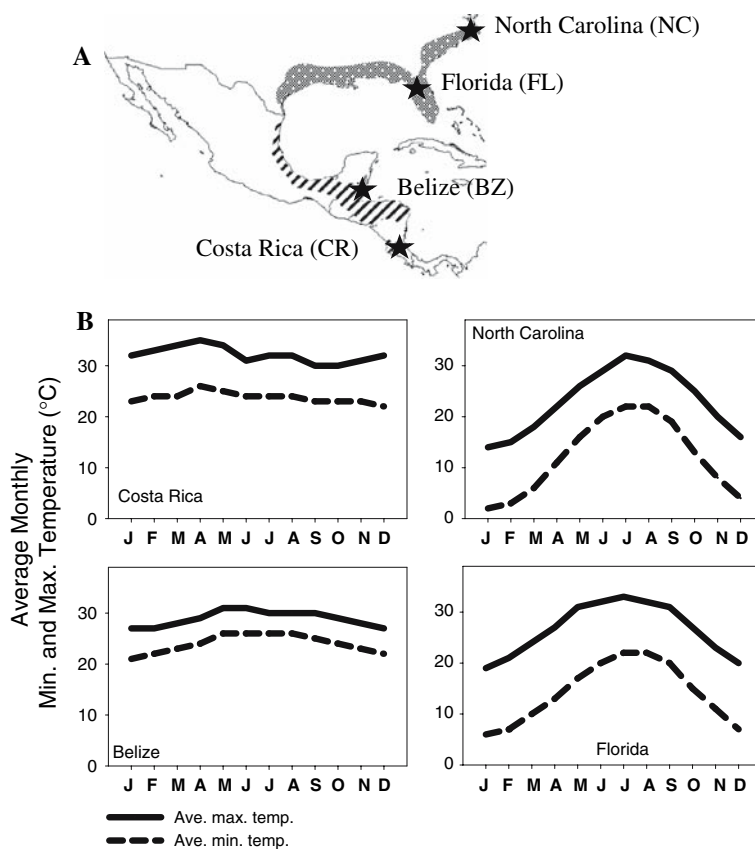
(2005) found that among populations of cork oak (*Quercus suber*), those originating from warmer sites showed the largest declines in F_v/F_m in response to cold stress. For ten populations originating in various locations throughout Spain, they found a correlation between mean annual temperature at the population source and the mean population F_v/F_m recorded in the coldest-month normalized by the F_v/F_m spring measurement (Aranda et al. 2005). In the present study, I investigate inter- and intra-specific variation in PS II sensitivity to chilling and freezing stress in two American evergreen oaks, *Q. virginiana* and *Q. oleoides*. I address the question of whether variation between populations and between species in sensitivity to chilling and freezing stress corresponds to the climate of origin, indicating support for local adaptation. I also examine changes in sensitivity to chilling and freezing in plants grown under contrasting climates to determine whether there is evidence in support of adaptive plasticity.

Q. virginiana and *Q. oleoides* are the two most broadly distributed members of a small, monophyletic lineage of interfertile live oaks (*Quercus* series *Virentes* Nixon) which falls within the white oak group (Nixon and Muller 1997). *Q. virginiana* extends from the outer banks of southern Virginia and North Carolina into northern Mexico, and *Q. oleoides* extends from northern Mexico to northwestern

Costa Rica. These species are known to hybridize in regions where their distributions overlap, and we have documented gene flow among populations in Texas and Mexico where their ranges are in contact (J. Cavender-Bares, unpublished). Therefore, the species boundary is likely to be weak and populations throughout the range of the complex may potentially represent demes within a cline.

Live oaks are of considerable conservation significance because of their important ecological roles in many coastal and lowland forests, and due to severe declines in population sizes as a result of over-harvesting, since European settlement (Spector and Putz 2006). They are also coveted shade trees and protected by law in some regions. At their northern range limit, the live oaks are a dominant species in the maritime forest, a vanishing ecosystem under threat both by coastal development and by sea level rise (e.g., Conner et al. 2005). Efforts to restore the maritime forest must consider potential differences in physiological tolerances among live oak ecotypes and the extent to which the source of propagules matters. Therefore, understanding ecotypic variation in sensitivity to temperature extremes is an important area of investigation. The more temperate distribution of *Q. virginiana* compared to the tropical-subtropical distribution of *Q. oleoides* (Fig. 1A) invites the possibility that the two species have diverged in their

Fig. 1 (A) Distributions of the two live oak species (*Quercus virginiana*, gray shading, and *Q. oleoides*, hashed marks) and collection sites of acorns (stars): coastal North Carolina and north Central Florida (*Q. virginiana*); central Belize and northwestern Costa Rica (*Q. oleoides*). (B) Average monthly maximum and minimum temperatures for cities within the four collection regions, including Liberia, Costa Rica; Belize City, Belize; Wilmington, North Carolina; and Gainesville, Florida. The monthly maximum and minimum temperatures in North Carolina and Costa Rica (upper panels) were used as the monthly set point temperatures for daytime and nighttime in the greenhouse for the temperate and tropical climate treatments, respectively



sensitivity to cold and freezing stress, despite their close phylogenetic relationship. Furthermore, the wide range of climatic variation encountered by the two species throughout their ranges (Fig. 1B), most pronounced in *Q. virginiana*, allows the opportunity to test the hypothesis that populations at different latitudes within species are differentially adapted to cold and freezing stress. Alternatively, populations within species could be equally tolerant of cold and freezing, due to ancestral acquisition of such traits, even though only some populations currently experience these stresses. In this case, apparent physiological differences among populations could be largely the result of acclimation to stress factors, i.e., phenotypic plasticity (e.g., Gordon 1992; Sultan 1995; Ackerly et al. 2000; Atkin et al. 2006). A previous study shows that stomatal traits and leaf hydraulic properties in the two live oak species show significant plasticity in response to drought (Cavender-Bares et al. 2007). Distinguishing between plasticity and genetically-based variation has important implications for understanding range limits and how plants respond to changing environments. Phenotypic plasticity may allow plants to maintain high fitness under changing conditions (Sultan 2001). Alternatively, maintenance of fitness under new environmental conditions may only come about through genetic change.

The goals of this study were to examine the extent to which live oak populations show evidence for selection in response to cold and freezing stress as assessed by chlorophyll fluorescence measurements. This article is part of a larger study examining differentiation among live oak ecotypes across a climatic gradient. The central questions that I address in this study are as follows:

- 1) Do species and populations within species originating from climates with different minimum temperatures show differences in PS II photosynthetic yield ($\Delta F/F_m'$) at chilling temperatures?
- 2) Does minimum temperature in the climate of origin predict freezing damage to PS II, as assessed by irreversible changes in maximum photochemical efficiency (F_v/F_m), in populations and species across a latitudinal gradient?
- 3) How does cold acclimation alter PS II responses to freezing in populations and species from different latitudes?

Methods and materials

Study species

Acorns were collected from two populations within each of the two live oak species (*Q. oleoides*: Costa Rica and

Belize; *Q. virginiana*: north central Florida and North Carolina) in fall 2002 (Fig. 1A) and synchronously planted in greenhouse facilities at the Franklinville Experimental Station (Franklinville, NY). In Costa Rica, acorns were collected from trees distributed in random locations throughout the Santa Rosa National Park in Guanacaste Province in northwestern Costa Rica. In Belize, acorns were collected in three locations near Burrell Boom northwest of Belize City and in three locations near or in Mountain Pine Ridge in southern Belize. Acorns were collected from five trees in Alachua County Florida in and around Gainesville, and in North Carolina acorns were collected from trees in Goose Creek State Park, in or near Wilmington and in New Bern, North Carolina. All of the necessary collection permits, export permits, phytosanitary certificates, and import permits were obtained (available upon request). Each population included five to seven maternal families and approximately 25 individuals per family per treatment. While the seeds were collected from randomly selected trees within each population, the number of maternal families per population was relatively low; these families may not adequately represent the entire population. Moreover, germination among families was unequal, and some families had very low numbers. A larger study with more populations and greater sampling within populations is currently underway.

The experimental design included two temperature treatments: (1) a tropical treatment, in which daytime temperature was maintained near 30–35°C, and nighttime temperatures between 22°C and 26°C; and (2) a temperate treatment, in which winter growth temperatures reached a minimum nighttime temperature of 4°C with a daytime temperature of 15°C, simulating the monthly average temperatures in Liberia, Costa Rica and Wilmington, North Carolina, respectively (Fig. 1B). Each temperature regime was replicated in 3 different rooms. Overnight freezing events were simulated using a controlled freezer box, where temperature was regulated at 1 min intervals to within 0.5°C for freezing sensitivity measurements following previously described methods (Cavender-Bares and Holbrook 2001; Cavender-Bares et al. 2005).

Experiment 1: PS II chlorophyll fluorescence after short-term chilling stress under cold-acclimated and non-acclimated conditions

During late January 2004 of the second year of growth, light-adapted chlorophyll fluorescence yield ($\Delta F/F_m'$) and non-photochemical quenching (NPQ) were measured at three controlled temperatures (6, 15, and 30°C) using a pulse amplitude modulation chlorophyll fluorometer (mini-PAM, Walz, Effeltrich, Germany) (Schreiber et al. 1993; Schreiber et al. 1994) after short-term exposure to each

temperature. Plants were measured under controlled levels of photosynthetically active radiation (PAR) of approximately $400 \mu\text{mol m}^{-2} \text{s}^{-1}$ using metal halide illumination. This moderate light level corresponded reasonably well with ambient light levels in the greenhouse at this time of year, achieved by a combination of natural light and metal halide illumination. Note, however, that ambient light levels in the greenhouse can be variable in time and space depending on external weather conditions, sun angle, and obstruction by roofing structures; peak PAR values reach approximately $1200 \mu\text{mol m}^{-2} \text{s}^{-1}$. In the temperate treatment, plants had been exposed to approximately two months of daytime temperatures at 15°C and $4\text{--}6^\circ\text{C}$ nighttime temperatures, while plants in the tropical treatment remained under warm temperatures as described in the previous section. Dark-adapted F_v/F_m was measured just prior to dawn on a subsample of seedlings from each population ($N \approx 40$) in both the temperate and tropical treatments at their normal nighttime growth temperatures ($\sim 25^\circ$ and $\sim 5^\circ\text{C}$, respectively). Leaves were marked where the measurement was made. After plants were exposed for several hours to their daytime growing temperatures (*ca.* 30°C and 15°C in the tropical and temperate treatments, respectively), the same plants were exposed for 15 min to chilling temperatures (6°C), intermediate temperatures (15°C) or warm temperatures (30°C) at midday (11–13 h). Measurement temperatures were achieved by moving plants into greenhouse rooms where the temperature was set to one of the three setpoints. Chlorophyll fluorescence yield was then measured on the same spot on the leaf as the predawn F_v/F_m measurements. The angle and height of the fiber optic probe above the leaf was consistent across all measurements. The chlorophyll fluorescence measurements at predawn were used to calculate non-photochemical quenching according to Stern-Volmer $(F_m - F_m')/F_m'$. On a subsample of plants exposed to short-term chilling temperatures, predawn F_v/F_m was remeasured the following day to determine whether the decline in yield at cold temperatures was fully reversible.

Experiment 2: Freezing cycles at decreasing minimum temperatures

In mid-February of 2005, approximately three months after the onset of cold temperatures in the temperate climate treatment in the third year of growth, freezing manipulations were conducted to determine differences in freezing sensitivity within and between the two species grown in both climate treatments. Dark-adapted chlorophyll fluorescence (F_v/F_m) was measured in situ to determine photosynthetic quantum yield on one leaf from 6 seedlings per population (from at least three families) in both species and in both climate treatments. Branchlets with at least five

leaves, including the measured leaf, were subsequently cut under water, placed in water-filled rose tubes in the dark and frozen in a temperature-controlled freezer box overnight at minimum temperatures of 0°C , -2°C , -5°C , or -10°C . Temperature inside the freezer box, measured by a probe, was controlled using a programmable chiller (Cole-Parmer Instrument Co., Vernon Hills, Illinois) with Dynalene heat-transfer fluid combined with a fan-coil unit in the freezer box. The freezing and thawing rate was less than 0.25°C per min. In order to allow recovery, frozen samples were placed in a dark chamber for 24 h at room temperature before remeasurement of dark-adapted F_v/F_m following Boorse et al. (1998a). The time allowed for recovery of F_v/F_m after stress varies with the species, the degree of stress, and light conditions. For example, Verhoeven et al. (1999a) found very long recovery periods for *Pinus ponderosa* (100 h) compared to *Malva neglecta* (5 h) in field plants exposed to winter stress and ambient light. In a field common garden study with 4 evergreen oak species, Cavender-Bares et al. (2005), found no recovery beyond 24 h after freezing at various temperatures under dark conditions. Given that plants in this experiment were not exposed to light during freezing, slow recovery of F_v/F_m due to non-photochemical quenching mechanisms would not be expected. Copper-constantan thermocouples, attached to a Campbell data logger, were placed on the leaves of each plant to verify homogeneity of freezing conditions.

Experiment 3: -10°C Freezing cycles

The results of the previous experiment indicated that freezing at -10°C was diagnostic in showing differentiation among populations in response to freezing. Freezing cycles at -10°C were subsequently repeated the following February (2006) with greater replication and to determine the dynamics of F_0 and F_v/F_m over time after freezing. A similar protocol was followed but only a minimum freezing temperature of -10°C was used (Fig. 2), dark-adapted F_v/F_m was measured at room temperature (25°C) prior to

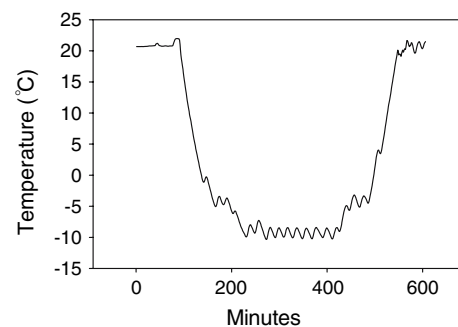


Fig. 2 Typical freezing cycle with a minimum temperature of -10°C , as measured via a thermocouple attached to each branchlet

freezing, and replication was increased to 17–20 individuals per population in each growth temperature treatment ($N = 144$). Samples were taken from all families per populations (ranging from 3 to 6) for which healthy branchlets were available without having to remove the apical meristem. In this experiment, samples were measured 30 min after the freezing cycle ended, 7 h afterwards and 14 h afterwards. Measurements beyond 14 h were not made, thus simplifying the experimental procedure but compromising the ability to detect any recovery that might have occurred after 14 h. As in Experiment 2, plants were not exposed to light during any part of the protocol making recovery from non-photochemical quenching mechanisms unlikely. In order to determine whether excising branchlets could affect recovery of chlorophyll fluorescence yield, a control procedure was also followed, in which excised branchlets (six per population per treatment) were placed in the freezer box maintained at room temperature (25°C).

Winter leaf loss

Numbers of leaves on approximately 35 individuals of each population (across all families) within each species and climate treatment ($N = 280$) were censused in November 2004 and recounted later in February 2005. Flushing did not occur during this period in either treatment. Missing leaves from each plant were calculated based on the number missing from the previous census and verified by fallen leaves in the pot and leaf scars on the branches.

Statistical analysis

The statistical treatment of plants from the four seed sources requires some consideration given the biological issues involved in how populations and species are defined. *Q. virginiana* and *Q. oleoides* are sister taxa known to introgress (Muller 1961; Nixon 1985; Nixon and Muller 1997). The species boundary is supported both morphologically (ibid) and by nuclear molecular data (Cavender-Bares et al., unpublished), indicating that grouping populations within species is one appropriate way to partition the variance. Therefore, I chose to conduct hierarchical ANOVAs treating family, nested within population and population nested within species as random factors. Species is treated as a main fixed factor. The disadvantage of nesting populations within species is that population means for all source locations cannot be directly compared. There is a real possibility that four populations actually represent demes within a continuous cline. Moreover, molecular evidence (Cavender-Bares et al., unpublished) indicates that the neutral genetic differentiation between the Costa Rica and Belize populations is, as great as the genetic differentiation between the two species. Therefore, alternative models for analysis could be

justified. Interpretation of the results should be considered in this context.

For the first experiment, a hierarchical ANOVA was conducted testing the effects of growth treatment and species (both treated as fixed factors), as well as population nested within species and family nested with population (both treated as random factors). Only significant interactions were included in the model. $\Delta F/F_m'$ and NPQ at each temperature were the dependent variables. For the second experiment, a hierarchical ANOVA was conducted to test the effects freezing temperature, species (fixed factors), population nested within species, and family nested with population (random factors), and their two-way interactions on the decline in F_v/F_m . Main effects were tested for separately in each growth temperature treatment. Decline in F_v/F_m , calculated as the difference in F_v/F_m before and after freezing, was arcsin transformed to meet the assumption of normality. For the third experiment, a hierarchical repeated measures (rm) ANOVA was conducted because each individual was measured at multiple time intervals after freezing at -10°C . The rm ANOVA tested, whether the temporal dynamics of F_0 or F_v/F_m differed among growth temperature treatments, species, populations nested within species, and families nested within populations. Growth temperature, species, individuals and time were main fixed effects; population (species) and family (population) were random factors. A significant species by time, population by time, or family by time interaction would indicate that species, populations, or families differed in the change in F_v/F_m (or F_0) over time. A significant treatment by time interaction would indicate that the change in F_v/F_m (or F_0) over time differed between the tropical and temperate treatments. Data Desk (Velleman 1997) was used for all of the statistical analyses.

Results

In all years (2004, 2005, and 2006) F_v/F_m measured in early February was significantly lower in the temperate treatment during winter than in the tropical treatment for both species and all populations. Slight differences in F_v/F_m between populations within species in the cold-winter treatment in 2004 (Fig. 3A) were not significant nor consistent in subsequent years but are important to show as references to compare the $\Delta F/F_m'$ and NPQ measurements.

Response of PS II to chilling temperatures under ambient light

PS II yield—In the tropical treatment with continuously warm temperatures, populations of both species showed the highest yield at warm temperatures (30°C) and declining

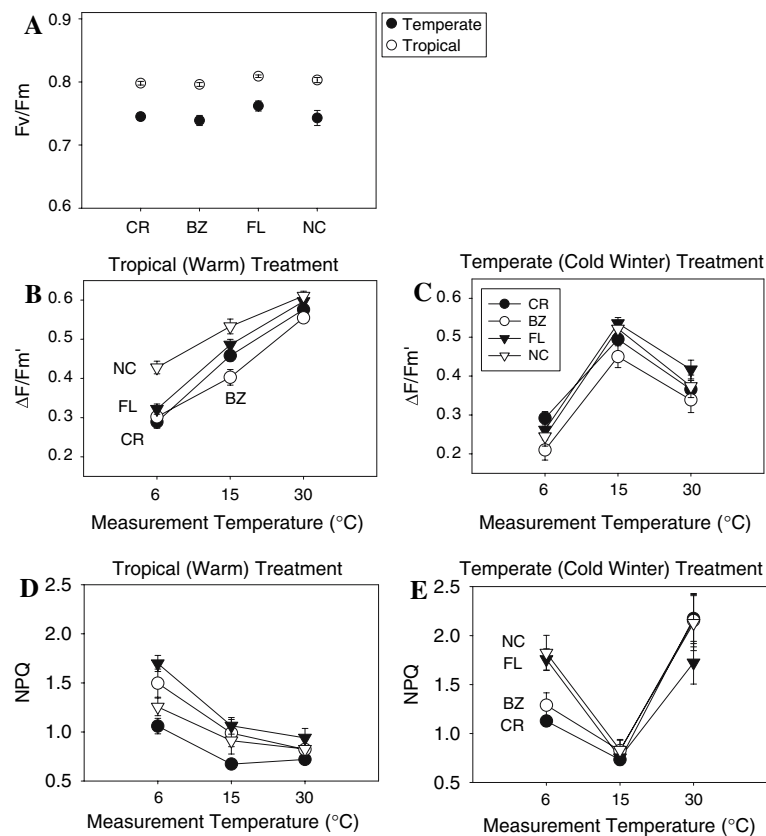


Fig. 3 (A) Dark F_v/F_m measured predawn in the temperate (cold winter) treatment (filled circles) and tropical (warm) treatment (open circles) for the two populations in each species in early February. (B–E) Effects of short-term chilling temperatures under ambient light. Upper panels (B–C) show chlorophyll fluorescence yield in light-exposed leaves at three measuring temperatures (6°, 15° and 30°C) for the two populations in each species grown under (B) tropical

conditions (30°C daytime growth temperatures) and (C) temperate conditions (15°C daytime growth temperatures) during late January under ambient light levels ($400 \mu\text{mol m}^{-2} \text{s}^{-1}$). Lower panels (D–E) show non-photochemical quenching (Stern-Volmer NPQ) from the same experiment. *Q. virginiana* is represented by triangles (open = North Carolina; closed = Florida), and *Q. oleoides* is represented by circles (open = Belize; closed = Costa Rica)

yields with declining temperature (Fig. 3B). After short-term exposure to chilling temperatures (6°C) the North Carolina population of *Q. virginiana* grown in the tropical temperature treatment showed significantly higher PS II yields than the Florida population and than both populations of *Q. oleoides* (Fig. 3B). This resulted in a significant effect of population on yield at 6°C ($P = 0.019$, Table 1). The lower yields in the Florida population reduced the mean yield for *Q. virginiana*. Hence, there was no significant difference between the two species. Families were not significantly different at 6°C or 30°C, but there was a significant effect of family on PS II yield at 15°C. Chlorophyll fluorescence yield at all three measurement temperatures depended significantly on the growth temperature (Table 1). Within the temperate (cold winter) treatment, neither species nor populations within species differed in their response to chilling temperatures (Fig. 3C). In the temperate treatment, the photosynthetic temperature optimum shifted, such that all populations again showed the highest chlorophyll fluorescence yield at

their daytime growth temperature (~15°C in Dec–Jan) and the lowest yield at chilling temperatures (6°C).

Non-photochemical quenching—In the tropical treatment, plants from both populations within both species showed high non-photochemical quenching at 6°C and lower values at 15° and 30°C. The Costa Rica population had lower NPQ at 6°C and 15°C, than the other three populations, but these other populations did not differ from each other. Both populations within both species showed similar values of NPQ at 30°C (Fig. 3D). Plants in the temperate treatment showed the lowest values of non-photochemical quenching at their growth temperatures (15°C), and there were no differences among species or populations within species at this temperature (Fig. 3E, Table 1). NPQ increased after short-term exposure to both chilling (6°C) and warm (30°C) temperatures. At chilling temperatures (6°C), *Q. virginiana* populations showed higher NPQ than *Q. oleoides* populations although this effect was only significant when the treatments were analyzed separately (not shown). At warm temperatures

Table 1 Results of ANOVA for the effects of growth temperature (temperate, 15°C daytime or tropical, 30°C daytime), species, population (species), and family (population) on dark chl fluorescence yield (F_v/F_m ; measured predawn at ambient growth temperatures), daytime chl fluorescence yield ($\Delta F/F_m'$) and non-photochemical quenching (NPQ)

Dependent variable	Source	df	SS	MS	F-ratio	Prob
F_v/F_m	Growth temperature	1	0.2166	0.2166	169.21	<0.0001
	Species	1	0.0055	0.0055	1.647	0.328
	Population	2	0.0067	0.0034	2.647	0.100
	Family	17	0.0216	0.0013	0.992	0.468
	Error	305	0.3903	0.0013		
	Total	326	0.6567			
$\Delta F/F_m'$ at 6°C	Growth temperature	1	0.429	0.429	31.186	<0.0001
	Species	1	0.096	0.096	1.201	0.387
	Population	2	0.159	0.080	5.061	0.019
	Family	17	0.268	0.016	1.145	0.310
	Error	305	4.196	0.014		
	Total	326	5.292			
$\Delta F/F_m'$ at 15°C	Growth temperature	1	0.066	0.066	5.639	0.018
	Species	1	0.208	0.208	10.807	0.081 ^a
	Population	2	0.039	0.019	0.659	0.530
	Family	17	0.497	0.029	2.497	0.001
	Error	305	3.571	0.012		
	Total	326	4.574			
$\Delta F/F_m'$ at 30°C	Growth temperature	1	3.362	3.362	252.42	<0.0001
	Species	1	0.050	0.050	38.8	0.025
	Population	2	0.003	0.001	0.097	0.908
	Family	17	0.228	0.013	1.007	0.450
	Error	305	4.063	0.013		
	Total	326	7.983			
NPQ at 6°C	Growth temperature	1	1.26499	1.26499	2.823	0.094^a
	Species	1	8.47179	8.47179	3.9416	0.186
	Population	2	4.29861	2.14931	4.7489	0.023
	Family	17	7.69407	0.452593	1.01	0.447
	Treatment*Species	1	2.2194	2.2194	4.9529	0.027
	Error	304	136.223	0.448101		
	Total	326	166.472			
NPQ at 15°C	Growth temperature	1	1.098	1.098	2.864	0.092^a
	Species	1	0.672	0.672	1.847	0.307
	Population	2	0.728	0.364	0.745	0.490
	Family	17	8.306	0.489	1.275	0.207
	Error	305	116.925	0.383		
	Total	326	129.58			
NPQ at 30°C	Growth temperature	1	115.695	115.695	96.045	<0.0001
	Species	1	0.036	0.036	0.199	0.699
	Population	2	0.363	0.181	0.207	0.815
	Family	17	14.911	0.877	0.728	0.773
	Error	305	367.4	1.205		
	Total	326	500.41			

$\Delta F/F_m'$ and NPQ were quantified at three measurement temperatures (6°C, 15°C and 30°C) with light levels of 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$. Significant effects ($P \leq 0.05$) are shown in bold, and marginally significant effects ($P < 0.1$) are indicated by^a

(30°C), all populations showed similarly high values, with Florida slightly lower than the other populations (Fig. 3E). The Costa Rica and Belize populations showed significantly higher NPQ at 30°C than at 6°C. There was no difference among families within populations for NPQ in any of the treatment combinations.

Decline in F_v/F_m after freezing at different minimum temperatures

Populations from the tropical *Q. oleoides* showed greater loss of photosynthetic function after freezing than populations from the temperate *Q. virginiana*. Photographs of warm-grown branchlets from both populations of each species, taken approximately 14 h after freezing at -10°C , demonstrate differences in leaf damage (Fig. 4). Plants in the tropical treatment that had been experiencing warm growth conditions showed increasing loss of photosynthetic function with decreasing minimum temperatures, as indicated by



Fig. 4 Photos of leaves and excised branchlets in water-filled rose tubes after freezing at -10°C for representative plants from the two populations of *Q. oleoides* (A) and from the two populations of *Q. virginiana* (B) grown in the temperate treatment

a decline in F_v/F_m (Fig. 5A). This pattern resulted in a significant effect of freezing temperature on decline in F_v/F_m (Table 2). The decline in F_v/F_m corresponded to latitude, with the two *Q. oleoides* populations (Belize and Costa Rica) showing a decline in F_v/F_m at higher temperatures than the two *Q. virginiana* populations (Florida and North Carolina). At -10°C , both populations in both species suffered declines in F_v/F_m with the North Carolina population showing only a minimal decline in F_v/F_m and the two *Q. oleoides* populations showing the largest declines. Plants in the Florida population of *Q. virginiana* showed an intermediate response at -10°C . Differences between populations within *Q. virginiana* in response to declining freezing temperatures resulted in a significant interaction of population by freezing temperature on the decline in F_v/F_m (Table 2A).

Q. virginiana populations (North Carolina and Florida) that were grown in the temperate treatment in which plants were acclimated to three months of winter temperatures between Dec.-Feb., showed no decline in F_v/F_m , even at -10°C , while *Q. oleoides* populations (Belize and Costa Rica) showed values of F_v/F_m that dropped below 0.2 at -10°C (Fig. 5B). Both species and population effects on decline in F_v/F_m were significant under the temperate treatment (Table 2). There was also a significant effect of freezing temperature on the decline in F_v/F_m in both the temperate growth treatment and in the tropical treatment. In the temperate treatment, there was a significant species by freezing temperature interaction, although there was no significant population by temperature interaction, in contrast to the tropical growth temperature treatment (Table 2).

Changes in PS II chlorophyll fluorescence parameters over time after freezing at -10°C

After freezing cycles reached a nadir of -10°C , F_v/F_m continued to decline as room temperatures were restored 30 min, 7 h and 14 h after the end of the freezing cycle (Fig. 6C, D). Under warm growth conditions, northern populations showed a greater decline in F_v/F_m than under conditions of cold acclimation as in the second experiment. *Q. oleoides* populations also showed a greater overall decline in F_v/F_m than *Q. virginiana* populations grown under both the temperate and tropical temperature treatments. However, there were differences between the two populations within *Q. virginiana*. In the temperate treatment, the North Carolina population showed significantly less decline in F_v/F_m 14 h after the freezing cycle than the Florida population (Fig. 6D). Repeated measures ANOVA for F_v/F_m indicated significant differences between species and between growth temperature treatments as well as species by time, population by time, and treatment by interactions (Table 3). There was no effect of family within populations. Values of F_0 showed

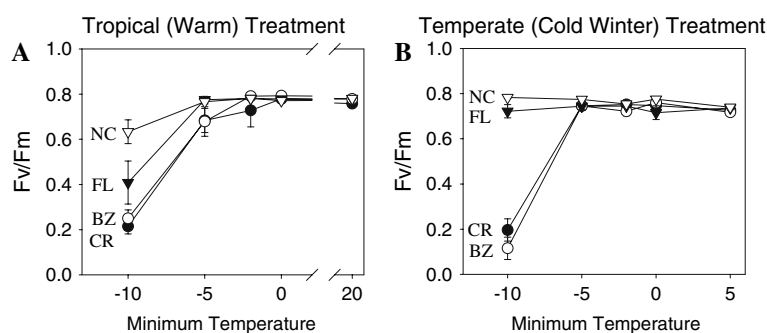


Fig. 5 Effects of minimum temperature (0°, -2°, -5°, and -10°C) on recovery of photochemical efficiency in dark leaves (**A**) in plants grown under warm conditions all year (tropical treatment), and (**B**) in plants acclimated to cold temperatures (temperate treatment). Dark F_v/F_m was measured on branches in water before and 24 h after a

dark freezing cycle with minimum temperatures of 0, -2, -5, and -10°C. Immediately after the freezing cycle, branches were placed in a dark cabinet at room temperature. Plants were measured in February 2005. Symbols are the same as in Fig. 3

Table 2 Results of ANOVA testing the effects of minimum freezing temperature (0, -2, -5, and -10°C), species, population (species), and family (population) on the decline in F_v/F_m after freezing for the

tropical treatment (A), in which plants had been experiencing warm growth conditions and for the temperate treatment (B), in which plants were cold acclimated

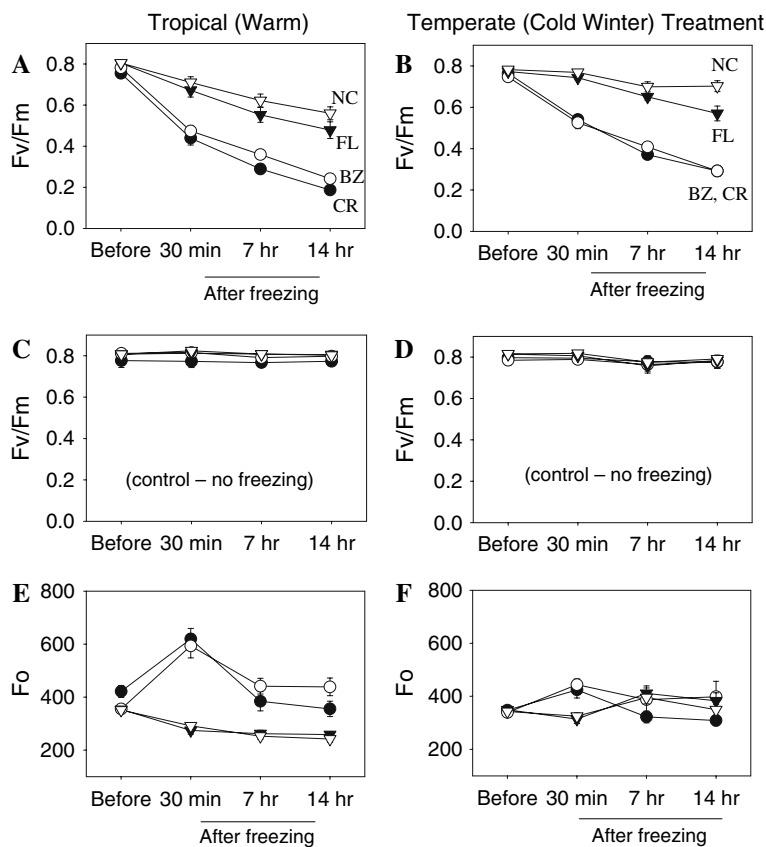
	Source	df	SS	MS	F-ratio	Prob
A.	Tropical treatment (warm all year)					
	Species	1	0.074	0.074	10.014	0.087 ^a
	Population	2	0.015	0.007	1.032	0.3884
	Family	11	0.078	0.007	0.578	0.8365
	Freezing temperature	3	1.500	0.500	14.918	0.0034
	Species*temperature	3	0.216	0.072	2.151	0.1951
	Population*temperature	6	0.201	0.034	3.880	0.008
	Family*temperature	23	0.199	0.009	0.701	0.8202
	Error	46	0.567	0.012		
Total	95	4.516				
B.	Temperate treatment (Cold winters)					
	Species	1	0.091	0.091	82.442	0.0119
	Population	2	0.002	0.001	0.459	0.6444
	Family	10	0.024	0.002	0.834	0.5986
	Freezing temperature	3	0.427	0.142	56.245	<0.0001
	Species*Temperature	3	0.365	0.122	48.152	0.0001
	Population*Temperature	6	0.015	0.003	0.799	0.5803
	Family*Temperature	24	0.076	0.003	1.094	0.3881
	Error	44	0.127	0.003		
Total	93	3.629				

Decline in F_v/F_m was calculated as the percent difference between F_v/F_m before and after freezing and was arcsin transformed. Significant effects ($P < 0.05$) are shown in bold. Marginally significant effects ($P < 0.01$) are indicated by^a

two different patterns that were associated with species-level differences (Fig. 6A,B). *Q. oleoides* populations grown under warm growth conditions showed an initial rise in F_0 30 min after the freezing cycle followed by a subsequent decline in F_0 . In contrast, *Q. virginiana* populations showed slight decreases in F_0 after freezing. Under winter growth

conditions in the temperate treatment, the same trend was apparent for the *Q. oleoides* populations, although considerably dampened. The two *Q. virginiana* populations, in contrast, showed a slight initial decrease in F_0 followed by a slight increase. Repeated measures ANOVA showed a strong species effect on F_0 , as well as a species by growth

Fig. 6 Upper panels: F_0 before and after freezing in plants grown under tropical (A) and temperate (B) conditions. Middle panels: Dark F_v/F_m before freezing and 30 min, 7 h and 14 h after freezing at -10°C in the two populations from each species grown under tropical (C) and temperate (D) conditions. Lower panels: F_v/F_m over the same time period for the same populations measured in a dark box maintained at 25°C in plants from the tropical (E) and temperate (F) treatments. Measurements were made in February 2006. Symbols are the same as in Fig. 3



temperature interaction and a species by time interaction (Table 3). There was also a significant population by time interaction as a result of differences between the Belize and Costa Rica populations in the changes in F_0 over time, although the general pattern and direction of response was similar for these two populations. Both family and the interaction of family and time were significant, indicating within population variation in F_0 over time after freezing.

In the control experiment, where branchlets excised under water were put in the freezer box set at 25°C , no change in F_v/F_m was found, even after 14 h. This indicates that the decline in F_v/F_m in the freezing treatments could not be attributed to desiccation or other artifacts of excising the branchlets (Fig. 6 E, F).

Winter leaf loss

In response to cold temperatures between November 2004 and February 2005, plants from the North Carolina population lost an average of 58% of their total leaves in the temperate treatment compared to only 6% in the tropical treatment (Fig. 7). Plants from the Florida population dropped an average of 38% of their leaves in the temperate treatment compared with 7% in the tropical treatment. In contrast, the Belize and Costa Rica populations lost only

10% and 12% of leaves, respectively, during the same period in the temperate treatment, which was not statistically different from their percentage leaf loss in the tropical treatment (Fig. 7).

Discussion

An important question in ecological research is the extent to which broadly distributed species are able to inhabit a wide range of climatic conditions as a result of wide amplitude in physiological tolerances, phenotypic plasticity or as a result of ecotypic differentiation. In this study, there is evidence for ecotypic differentiation in cold and freezing sensitivity among populations and species of live oaks across a latitudinal gradient. The correspondence of low temperature tolerance to latitude provides support for the hypothesis that at least one of the two broadly distributed live oak species is comprised of locally adapted populations. The study also shows that cold acclimation increases the freezing tolerance of the two populations within *Q. virginiana* that originate in climates that incur freezing stress, but not in the *Q. oleoides* populations that originate in tropical climates. These results are consistent with the idea that adaptive plasticity enhances freezing tolerance.

Table 3 Repeated measures ANOVA testing the effects of growth treatment (temperate or tropical), species, population (species), family (population), individual, and their interactions on changes in F_v/F_m and F_o over time after freezing

	Source	df	SS	MS	F-ratio	Prob
F_v/F_m	Growth treatment	1	0.239	0.239	4.147	0.179
	Species	1	3.031	3.031	36.512	0.026
	Population	2	0.166	0.083	3.634	0.053
	Family	14	0.320	0.023	0.724	0.747
	Individual	122	3.848	0.032	6.590	<0.0001
	Time	3	11.483	3.828	221.290	<0.0001
	Species*Treatment	1	0.030	0.030	0.522	0.545
	Population*Treatment	2	0.115	0.058	2.146	0.160
	Family*Treatment	12	0.322	0.027	0.851	0.598
	Treatment*Time	3	0.174	0.058	19.211	0.0018
	Species*Time	3	0.748	0.249	14.409	0.0038
	Population*Time	6	0.104	0.017	4.001	0.003
	Family*Time	42	0.182	0.004	0.903	0.646
	Species*Treatment*Time	3	0.042	0.014	4.693	0.051
	Population*Treatment*Time	6	0.018	0.003	0.664	0.679
	Family*Treatment*Time	36	0.163	0.005	0.949	0.557
	Error	366	1.752	0.005		
Total	623	28.620				
F_o	Growth treatment	1	1025	1025	0.037	0.866
	Species	1	807918	807918	6.470	0.126
	Population	2	249740	124870	2.299	0.137
	Family	14	760372	54312.3	1.836	0.041
	Individual	122	3.61 E + 06	29585.7	5.315	<0.0001
	Time	3	418120	139373	3.403	0.094 ^a
	Species*Treatment	1	415149	415149	14.898	0.061 ^a
	Population*Treatment	2	55731.5	27865.8	1.493	0.264
	Family*Treatment	12	223948	18662.3	0.631	0.813
	Treatment*Time	3	181899	60633	105.410	<0.0001
	Species*Time	3	376464	125488	3.064	0.113
	Population*Time	6	245763	40960.6	4.898	0.0007
	Family*Time	42	351243	8362.93	1.502	0.027
	Species*Treatment*Time	3	70067	23355.7	40.602	0.0002
	Population*Treatment*Time	6	3451.37	575.228	0.082	0.998
	Family*Treatment*Time	36	253633	7045.35	1.266	0.147
	Error	366	2.04 E + 06	5566.88		
Total	623	1.15 E + 07				

Significant effects ($P \leq 0.05$) are shown in bold, and marginally significant effects ($P < 0.1$) are indicated by^a

Chlorophyll fluorescence yield and NPQ under chilling temperatures and ambient light

Under warm growth conditions, the northern most population (North Carolina) showed the highest daytime chlorophyll fluorescence yield at chilling temperatures (6°C; Fig. 3B). This result is consistent with selection for greater photosynthetic function at lower temperatures. Interest-

ingly, the North Carolina population did not show the same advantage under conditions of cold acclimation. Rather, the cold acclimated plants from the North Carolina population demonstrated reduced photosynthetic yield at chilling temperatures under ambient light conditions, and did not differ from any of the other populations. There are at least two explanations for this response. The first is that chlorophyll fluorescence was quenched by photoprotective

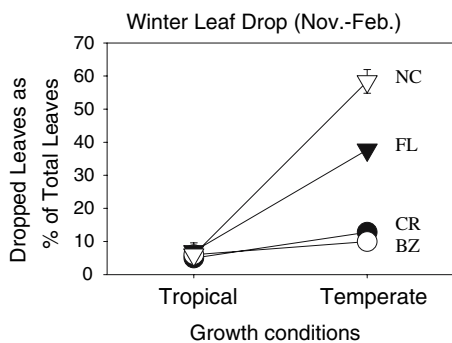


Fig. 7 Percentage of leaf loss during the interval between November and February 2005 in the tropical and temperate growth treatments. Symbols are the same as in the previous figures

mechanisms involving the up-regulation of pigments in the xanthophyll cycle (Adams and Demmig-Adams 1994; Adams and Demmig-Adams 1995; Adams et al. 1995; Garcia-Plazaola et al. 1997; Logan et al. 1998a; Verhoveen et al. 1999a, b; Oliveira and Peñuelas 2000). The data support this explanation, because NPQ increased significantly in the North Carolina population under conditions of cold acclimation compared to warm growth conditions (Fig. 3D,E). Note that the NPQ values reported in Fig. 3E may be slight underestimates, as the predawn F_v/F_m values for these plants were somewhat reduced (Adams and Demmig-Adams 2004). If these values do represent underestimates, however, NPQ would actually be even higher than it is reported for the cold treatment. A second explanation is that chlorophyll is degraded during cold-induced leaf senescence resulting in reduced photosynthetic yield (e.g., Adams et al. 1990; Tobias et al. 1995; Rosenthal and Camm 1997; Cavender-Bares et al. 2000; Lee et al. 2003; Pruzinska et al. 2005; Hortensteiner 2006; Yasumura et al. 2006). The significant increase in leaf drop of cold-exposed plants in the North Carolina population provide support for this explanation, although chlorophyll fluorescence measurements were only made on intact, healthy green leaves. Without xanthophyll pigment data, it is not possible to interpret the underlying basis for differences in NPQ among populations, or specifically, to determine whether increases in NPQ were the result of increased pool sizes of xanthophyll pigments. This is the subject of current investigation.

Consistent with other studies (e.g., Huner et al. 1993; Yamori et al. 2005; Hacker and Neuner 2006), the maximum daytime photosynthetic yield corresponded to growing conditions in all populations (Fig. 3B, C). Plants grown under daytime temperatures of 30°C had their maximum yield at 30°C, while plants acclimated to daytime temperatures of 15°C had their maximum yield at 15°C. However, after cold acclimation, the two populations of *Q. virginiana* (North Carolina and Florida) showed higher values of

non-photochemical quenching in response to chilling than the two populations of *Q. oleoides* (Fig. 3E).

Declines in F_v/F_m after dark freezing cycles

Loss of photochemical efficiency of PS II, as assessed by F_v/F_m , was dependent on minimum freezing temperature with increasing losses as temperatures declined, consistent with previous studies (e.g., Boorse et al. 1998a; Feild and Brodrribb 2001; Cavender-Bares et al. 2005). Under warm growth conditions, the two species, as well as the two populations within *Q. virginiana* were significantly differentiated both in terms of the temperature at which F_v/F_m declined, and in particular, in terms of the final F_v/F_m values obtained after freezing at -10°C (Fig. 5, Table 2). After freezing at -10°C , the North Carolina population of *Q. virginiana* suffered the least decline in F_v/F_m (after 24 h of recovery in the dark at room temperature), reaching a mean of 0.62, the Florida population reached 0.4 and the Costa Rica and Belize populations of *Q. oleoides* suffered the largest decline in F_v/F_m approaching a ratio of 0.2. At -5°C the two species showed significant differences: *Q. virginiana* showed no decline in F_v/F_m but F_v/F_m ratios of *Q. oleoides* approached 0.6. At -2°C , only the Costa Rica population showed a slight decrease in F_v/F_m . The loss of photosynthetic efficiency corresponded to minimum temperatures in the climate of origin for each population. These results are consistent with other studies showing ecotypic differentiation in cold and freezing tolerance (Billington and Pelham 1991; Ducouso et al. 1996; Benowicz et al. 2000; Rehfeldt et al. 2002; Rehfeldt 2004; Aranda et al. 2005), and provide support for the local adaptation hypothesis. Nevertheless, reciprocal transplant experiments in the field are necessary for definitive determination of local adaptation (e.g., Claussen et al. 1940; Dudley 1996; Etterson 2004).

Cold acclimation significantly ameliorated the effects of freezing on PS II photochemical efficiency in the *Q. virginiana* populations consistent with prior observations of the effects of cold-acclimation on freezing responses (e.g., Strand and Öquist 1988; Somersalo and Krause 1989; Warren et al. 1998; Zhu et al. 2002; Cavender-Bares et al. 2005). After exposure to prolonged cold temperatures, neither of the *Q. virginiana* populations suffered losses in F_v/F_m , even at -10°C (Fig. 5B). In *Q. oleoides*, however, cold acclimation only resulted in amelioration of the slight loss in photochemical efficiency at -5°C and at -2°C (Fig. 5A,B). At -10°C there was no amelioration effect of cold acclimation for the two *Q. oleoides* populations, and the decline in F_v/F_m in the temperate treatment was as large as or even larger than in the tropical treatment. Final F_v/F_m ratios were less than 0.2 after 24 h dark recovery at room temperature.

Continued decline in F_v/F_m with time after freezing at -10°C

The freezing experiments were conducted in the dark to exclude the possibility that a decline in F_v/F_m could result from light-induced down-regulation of PS II efficiency due, for example, to photoprotective non-photochemical quenching (Adams and Demmig-Adams 1995; Demmig-Adams et al. 1995; Lovelock et al. 1995; Verhoeven et al. 1999a) or increased energy transfer to PS I (Horton and Hague 1988; Lazar 2006). Dynamics of fluorescence parameters were observed over time after freezing cycles with minimum temperatures of -10°C the subsequent year. In all cases, initial declines in F_v/F_m ratios, measured 30 min after the end of the freezing cycle, were followed by continued losses in PS II photochemical efficiency for 14 h after freezing (Fig. 6A,B). Absence of longer-term recovery could not be verified because measurements were not continued after 14 h but this possibility seems unlikely for two reasons. First, spot measurements after 24 h showed continued declines in F_v/F_m , and second, photoprotective energy dissipation would not be expected under dark freezing conditions. As in the second experiment (Fig. 5), the tropical species, *Q. oleoides*, showed greater losses in F_v/F_m than the temperate species, *Q. virginiana* (Fig. 6). Within *Q. oleoides*, significant differentiation between the North Carolina and Florida populations in the loss of photochemical efficiency was apparent after 14 h, with the North Carolina population showing lower sensitivity to freezing than the Florida population (Fig. 6B, C).

Dynamics of F_0 after freezing

Dynamics of F_0 can often help interpret changes in F_v/F_m (e.g., Butler and Kitajima 1975; Ball et al. 1995; Lazar 2006). In this study, *Q. oleoides* populations showed an initial increase in F_0 after freezing followed by a decrease (Fig. 6A,B), particularly in plants grown under tropical conditions. In contrast, *Q. virginiana* populations showed a slight but continuous decline in F_0 in plants from the tropical treatment (Fig. 6A) and a slight decrease followed by a subtle increase and then decrease in cold acclimated plants (Fig. 6B). Generally, an increase in F_0 accompanied by a decrease in F_v/F_m has been interpreted as genuine photodamage, for example, as a result of the action of reactive oxygen species (Krause 1988; Hanelt et al. 1992; Guo et al. 2006). A decrease in F_0 accompanied by a decrease in F_v/F_m has generally been interpreted as downregulation of PS II efficiency due to photoprotective energy dissipation (Krause 1988). The interpretation of photoprotective energy dissipation may apply in cases, where simultaneous light and cold stress are imposed. In this experiment, however, where plants were not exposed

to light during freezing, such mechanisms should not have been engaged. Rather, continued decline in F_v/F_m may be interpreted as freezing damage related to membrane damage (including the tonoplast), leading to partial cell death (Boorse et al. 1998a). During this process, PS II reaction centers are likely to have become damaged. In the *Q. oleoides* populations grown under warm conditions, charge separation may still have been possible 30 min after freezing, while the acceptor side was already blocked, resulting in a temporary increase in F_0 . Subsequently, however, PS II units may have become completely degraded, leading to a general decrease of fluorescence yield and decreased F_0 . Additional experimentation, including analysis of light harvesting pigments, is necessary to understand more fully the mechanisms of F_v/F_m decline after freezing. Nevertheless, this study demonstrates that measurement of PS II quantum efficiency using chlorophyll fluorescence is a useful method for screening ecotypic variation in freezing tolerance. Similar methods have been proposed by Boorse et al. (1998a, b) and have been used in comparative studies of cold resistance in plants (e.g., Méthy and Trabaud 1990; Öquist and Huner 1991; Oliveira and Peñuelas 2000).

Winter leaf loss

Inter- and intraspecific variation in PS II sensitivity to cold and freezing stress was accompanied by parallel variation in leaf loss in response to cold temperatures. Only *Q. virginiana* populations showed significant leaf loss in response to winter temperatures compared to background leaf loss under warm growth conditions (Fig. 7). Within *Q. virginiana*, the North Carolina populations showed significantly higher leaf loss compared to the north central Florida population in response to cold. In both populations, leaf loss occurred almost exclusively in older leaf cohorts produced prior to the current year of growth. These results indicate that *Q. virginiana*, particularly the North Carolina population, undergoes leaf senescence in response to cold temperatures. This may be a strategy for recovering photosynthate and nutrients that coincides with greater investment in cold and freezing resistance in the remaining leaves. It is possible that this represents an intermediate state in the evolutionary transition from evergreenness to deciduousness that accompanies the range shift from tropical to temperate climates.

In summary, this study demonstrates ecotypic and species-level variation in cold and freezing sensitivity, as assessed by chlorophyll fluorescence. The degree of loss of photochemical efficiency after freezing and the minimum freezing temperature at which this loss occurs is predicted by the minimum temperature in the climate of origin at both the species and population level. Freezing resistance is

enhanced by cold acclimation in the *Q. virginiana* populations that originate from climates where freezing occurs with high probability, but not in the *Q. oleoides* populations that originate in the tropics. Within *Q. virginiana*, the acclimation effect is stronger in the North Carolina population than in the north central Florida population, corresponding to latitude. The contrasting degree of freezing resistance between the two populations that results from cold acclimation may be interpreted as adaptive plasticity (sensu Ackerly et al. 2000). The presence of a *Q. virginiana* population in North Carolina during the last glacial maximum is unlikely given the colder climate and presence of the Laurentide ice extending as far south as Connecticut (cf. Davis 1981; Delcourt and Delcourt 1987; McLachlan et al. 2005). Most likely, the North Carolina population would have colonized its current location subsequent to the glacial retreat. As a result, ecotypic differentiation between the North Carolina and north central Florida populations with respect to chilling and freezing sensitivity may be interpreted as evolving relatively recently. Taken together, these data support the hypothesis that live oaks are able to inhabit a range of climatic conditions both as a result of plasticity as well as relatively recent adaptive divergence among populations.

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References

- Ackerly DD, Dudley SA, Sultan SE, Schmitt J, Coleman JS, Linder CR, Sandquist DR, Geber MA, Evans AS, Dawson TE, Lechowicz MJ (2000) The evolution of plant ecophysiological traits: recent advances and future directions. *BioScience* 50:979–995
- Adams WW, Demmig-Adams B (1994) Carotenoid composition and down regulation of photosystem II in three conifer species during the winter. *Physiol Plant* 92:451–458
- Adams WW, Demmig-Adams B (1995) The xanthophyll cycle and sustained thermal energy dissipation activity in *Vinca minor* and *Euonymus kiautschovicus* in winter. *Plant Cell Environ* 18:117–127
- Adams WW, Demmig-Adams B (2004) Chlorophyll fluorescence as a tool to monitor plant response to the environment. In: Papa-georgiou GC, Govindjee (eds) Chlorophyll a fluorescence: a signature of photosynthesis. Advances in photosynthesis and respiration, vol 19. Springer, Dordrecht, pp 583–564
- Adams WW, Winter K, Schreiber U, Schramel P (1990) Photosynthesis and chlorophyll fluorescence characteristics in relationship to changes in pigment and element composition of leaves of *Platanus occidentalis* L. during autumnal leaf senescence. *Plant Physiol* 93:1184–1190
- Adams WW, Demmig-Adams B, Verhoeven AS, Barker DH (1995) Photoinhibition during winter stress—involvement of sustained xanthophyll cycle-dependent energy-dissipation. *Aust J Plant Physiol* 22:261–276
- Aho M-L (1994) Autumn frost hardening of one-year-old *Pinus sylvestris* (L.) seedlings: effect of origin and parent trees. *Scand J For Res* 9:17–24
- Aranda I, Castro L, Alia R, Pardos JA, Gil L (2005) Low temperature during winter elicits differential responses among populations of the Mediterranean evergreen cork oak (*Quercus suber*). *Tree Physiol* 25:1085–1090
- Atkin OK, Holly C, Ball MC (2000) Acclimation of snow gum (*Eucalyptus pauciflora*) leaf respiration to seasonal and diurnal variations in temperature: the importance of changes in the capacity and temperature sensitivity of respiration. *Plant Cell Environ* 23:15–26
- Atkin OK, Loveys BR, Atkinson LJ, Pons TL (2006) Phenotypic plasticity and growth temperature: understanding interspecific variability. *J Exp Bot* 57:267–281
- Ball MC, Hodges VS, Laughlin GP (1991) Cold-induced photoinhibition limits regeneration of snow gum at tree-line. *Funct Ecol* 5:663–668
- Ball MC, Butterworth JA, Roden JS, Christian R, Egerton JJG (1995) Applications of chlorophyll fluorescence to forest ecology. *Aust J Plant Physiol* 22:311–319
- Ball MC, Egerton JJG, Leuning R, Cunningham RB, Dunne P (1997) Microclimate above grass adversely affects spring growth of seedling snow gum (*Eucalyptus pauciflora*). *Plant Cell Environ* 20:155–166
- Ball MC, Canny MJ, Huang CX, Heady RD (2004) Structural changes in acclimated and unacclimated leaves during freezing and thawing. *Funct Plant Biol* 31:29–40
- Benowicz A, Guy RD, El-Kassaby YA (2000) Geographic pattern of genetic variation in photosynthetic capacity and growth in two hardwood species from British Columbia. *Oecologia* 123:168–174
- Bertrand A, Robitaille G, Nadeau P, Castonguay Y (1999) Influence of ozone on cold acclimation in sugar maple seedlings. *Tree Physiol* 19:527–534
- Billington HL, Pelham J (1991) Genetic variation in the date of budburst in Scottish birch populations: implications for climate change. *Funct Ecol* 5:403–409
- Blennow K, Lindkvist L (2000) Models of low temperature and high irradiance and their application to explaining the risk of seedling mortality. *For Ecol Manage* 135:289–301
- Boorse GC, Bosma TL, Meyer AC, Ewers FW, Davis SD (1998a) Comparative methods of estimating freezing temperatures and freezing injury in leaves of chaparral shrubs. *Int J Plant Sci* 159:513–521
- Boorse GC, Ewers FW, Davis SD (1998b) Response of chaparral shrubs to below-freezing temperatures: Acclimation, ecotypes, seedlings vs. adults. *Am J Bot* 85:1224–1230
- Burke M, Gusta L, Quamme H, Weiser C, Li P (1976) Freezing injury in plants. *Annu Rev Plant Physiol* 27:507–528
- Butler WL, Kitajima M (1975) Fluorescence quenching in Photosystem II of chloroplasts. *Biochim Biophys Acta* 376:116–125
- Cavender-Bares J (2005) Impacts of freezing on long-distance transport in woody plants. In: Holbrook NM, Zwieniecki M

- (eds) Vascular transport in plants. Elsevier Inc., Oxford, pp 401–424
- Cavender-Bares J, Bazzaz FA (2004) From leaves to ecosystems: using chlorophyll fluorescence to assess photosynthesis and plant function in ecological studies. In: Papageorgiou GC, Govindjee (eds) Chlorophyll a fluorescence: a signature of photosynthesis. Advances in photosynthesis and respiration, vol 19. Springer, Dordrecht, pp 737–755
- Cavender-Bares J, Holbrook NM (2001) Hydraulic properties and freezing-induced cavitation in sympatric evergreen and deciduous oaks with contrasting habitats. *Plant Cell Environ* 24:1243–1256
- Cavender-Bares J, Apostol S, Moya I, Briantais JM, Bazzaz FA (1999) Chilling-induced photoinhibition in two oak species: are evergreen leaves inherently better protected than deciduous leaves? *Photosynthetica* 36:587–596
- Cavender-Bares J, Potts M, Zacharias E, Bazzaz FA (2000) Consequences of CO₂ and light interactions for leaf phenology, growth, and senescence in *Quercus rubra*. *Glob Chg Biol* 6:877–887
- Cavender-Bares J, Cortes P, Rambal S, Joffre R, Miles B, Rocheteau A (2005) Summer and winter sensitivity of leaves and xylem to minimum freezing temperatures: a comparison of co-occurring Mediterranean oaks that differ in leaf lifespan. *New Phytol* 168:597–612
- Cavender-Bares J, Sack L, Savage J (2007) Atmospheric and soil drought reduce nocturnal conductance in live oaks. *Tree Physiol* 27:611–620
- Claussen J, Keck DD, Hiesey WM (1940) Experimental studies on the nature of species. I. The effect of varied environment on western North American plants. Carnegie Institution of Washington
- Conner WH, Mixon WD, Wood GW (2005) Maritime forest habitat dynamics on Bulls Island, Cape Romain National Wildlife Refuge, SC, following Hurricane Hugo. *For Ecol Manage* 212:127–134
- Corcuera L, Morales F, Abadia A, Gil-Pelegrin E (2004) The effect of low temperatures on the photosynthetic apparatus of *Quercus ilex* subsp. *ballota* at its lower and upper altitudinal limits in the Iberian peninsula and during a single freezing-thawing cycle *Trees-Struct Funct* 19:99–108
- Corcuera L, Morales F, Abadia A, Gil-Pelegrin E (2005) Seasonal changes in photosynthesis and photoprotection in a *Quercus ilex* subsp. *ballota* woodland located in its upper altitudinal extreme in the Iberian Peninsula. *Tree Physiol* 25:599–608
- Davis MB (1981) Quaternary history and the stability of forest communities. In: West DC, Shugart HH, Botkin DB (eds) Forest succession: concepts and application. Springer-Verlag, New York, pp 132–153
- Delcourt PA, Delcourt HR (1987) Long term forest dynamics of the temperate zone: a case study of late-Quaternary forests in eastern North America. Springer-Verlag, New York
- Demmig-Adams B, Adams WW, Logan BA, Verhoeven AS (1995) Xanthophyll cycle-dependent energy dissipation and flexible photosystem II efficiency in plants acclimated to light stress. *Aust J Plant Physiol* 22:249–260
- Ducousso A, Guyon JP, Kremer A (1996) Latitudinal and altitudinal variation of bud burst in western populations of sessile oak (*Quercus petraea* (Matt) Liebl). *Ann Sci Forest* 53:775–782
- Dudley SA (1996) The response to differing selection on plant physiological traits: evidence for local adaptation. *Evolution* 50:103–110
- Etterson JR (2004) Evolutionary potential of *Chamaecrista fasciculata* in relation to climate change I. Clinal patterns of selection along an environmental gradient in the Great Plains. *Evolution* 58:1446–1458
- Feild TS, Brodribb T (2001) Stem water transport and freeze-thaw xylem embolism in conifers and angiosperms in a Tasmanian treeline heath. *Oecologia* 127:314–320
- Garcia-Plazaola JI, Faria T, Abadia J, Chaves MM, Pereira JS (1997) Seasonal changes in xanthophyll composition and photosynthesis of cork oak (*Quercus suber* L.) leaves under mediterranean climate. *J Exp Bot* 48:1667–1674
- Garcia-Plazaola JI, Artetxe U, Dunabeitia MK, Becerril JM (1999) Role of photoprotective systems of holm-oak (*Quercus ilex*) in the adaptation to winter conditions. *J Plant Physiol* 155:625–630
- Gordon DM (1992) Phenotypic plasticity. In: Keller EF, Lloyd EA (eds) Keywords in evolutionary biology. Harvard University Press, Cambridge, Massachusetts
- Guo YP, Zhou HF, Zhang LC (2006) Photosynthetic characteristics and protective mechanisms against photooxidation during high temperature stress in two citrus species. *Sci Hort* 108:260–267
- Guy CL (2003) Freezing tolerance of plants: current understanding and selected emerging concepts. *Can J Bot-Rev Can Bot* 81:1216–1223
- Hacker J, Neuner G (2006) Photosynthetic capacity and PSII efficiency of the evergreen alpine cushion plant *Saxifraga paniculata* during winter at different altitudes. *Arct Antarct Alp Res* 38:198–205
- Hanelt D, Huppertz K, Nultsch W (1992) Photoinhibition of photosynthesis and its recovery in red algae. *Bot Acta* 105:278–284
- Holly C, Laughlin GP, Ball MC (1994) Cold-Induced photoinhibition and design of shelters for establishment of eucalypts in pasture. *Aust J Bot* 42:139–147
- Hortensteiner S (2006) Chlorophyll degradation during senescence. *Annu Rev Plant Biol* 57:55–77
- Horton P, Hague A (1988) Studies on the induction of chlorophyll fluorescence in isolated barley protoplasts. IV resolution of non-photochemical quenching. *Biochim Biophys Acta* 932:107–115
- Hovenden MJ, Warren CR (1998) Photochemistry, energy dissipation and cold-hardening in *Eucalyptus nitens* and *E-pauciflora*. *Aust J Plant Physiol* 25:581–589
- Huner NPA, Oquist G, Hurry VM, Krol M, Falk S, Griffith M (1993) Photosynthesis, photoinhibition and low-temperature acclimation in cold tolerant plants. *Photosynth Res* 37:19–39
- Koroleva OY, Thiele A, Krause GH (1995) Increased xanthophyll cycle activity as an important factor in acclimation of the photosynthetic apparatus to high-light stress at low temperatures. In: Mathis P (ed) Photosynthesis: from light to biosphere. Kluwer, Dordrecht, pp 425–428
- Krause GH (1988) Photoinhibition of photosynthesis—an evaluation of damaging and protective mechanisms. *Physiol Plant* 74:566–574
- Lazar D (2006) The polyphasic chlorophyll *a* fluorescence rise measured under high intensity of exciting light. *Funct Plant Biol* 33:9–30
- Lee DW, O’Keefe J, Holbrook NM, Feild TS (2003) Pigment dynamics and autumn leaf senescence in a New England deciduous forest, eastern USA. *Ecol Res* 18:677–694
- Logan BA, Demmig-Adams B, Adams WW (1998a) Antioxidants and xanthophyll cycle-dependent energy dissipation in *Cucurbita pepo* L. and *Vinca major* L. upon a sudden increase in growth PPFD in the field. *J Exp Bot* 49:1881–1888
- Logan BA, Grace SC, Adams WW, Demmig-Adams B (1998b) Seasonal differences in xanthophyll cycle characteristics and antioxidants in *Mahonia repens* growing in different light environments. *Oecologia* 116:9–17
- Lovelock CE, Osmond CB, Seppelt RD (1995) Photoinhibition in the Antarctic moss *Grimmia antarctici* Card when exposed to cycles of freezing and thawing. *Plant Cell Environ* 18:1395–1402

- McLachlan JS, Clark JS, Manos PS (2005) Molecular indicators of tree migration capacity under rapid climate change. *Ecology* 86:2088–2098
- Méthy M, Trabaud L (1990) Caractérisation de la résistance au froid de deux espèces sclérophylles méditerranéennes par la fluorescence de la chlorophylle. *Ann Sci For* 47:141–148
- Muller CH (1961) The live oaks of the series *Virentes*. *Am Midl Nat* 65:17–39
- Nardini A, Salleo S, Lo Gullo MA, Pitt F (2000) Different responses to drought and freeze stress of *Quercus ilex* L. growing along a latitudinal gradient. *Plant Ecol* 148:139–147
- Nilsson JE, Walfridsson EA (1995) Phenological variation among plus-tree clones of *Pinus-Sylvestris* (L) in Northern Sweden. *Silvae Genet* 44:20–28
- Nilsson J-E (2001) Seasonal changes in phenological traits and cold hardiness of F1-populations from plus-trees of *Pinus sylvestris* and *Pinus contorta* of various geographical origins. *Scand J For Res* 16:7–20
- Nixon KC (1985) A biosystematic study of *Quercus* series *Virentes* (the live oaks) with phylogenetic analysis of *Fagales*, *Fagaceae*, and *Quercus*. Ph.D. Dissertation, University of Texas
- Nixon KC, Muller CH (1997) *Quercus* Linnaeus sect. *Quercus* White oaks. In: FoNAE Committee (ed) *Flora of North America North of Mexico*. Oxford University Press, New York, pp 436–506
- Oliveira G, Peñuelas J (2000) Comparative photochemical and phenomorphological responses to winter stress of an evergreen (*Quercus ilex* L.) and a semi-deciduous (*Cistus albidus* L.) Mediterranean woody species. *Acta Oecol-Int J Ecol* 21:97–107
- Oliveira G, Peñuelas J (2001) Allocation of absorbed light energy into photochemistry and dissipation in a semi-deciduous and an evergreen Mediterranean woody species during winter. *Aust J Plant Physiol* 28:471–480
- Öquist G, Huner N (1991) Effects of cold acclimation on the susceptibility of photosynthesis to photoinhibition in Scots pine and in winter and spring cereals: a fluorescence analysis. *Funct Ecol* 5:91–100
- Pruzinska A, Tanner G, Aubry S, Anders I, Moser S, Muller T, Ongania KH, Krautler B, Youn JY, Liljegren SJ, Hortensteiner S (2005) Chlorophyll breakdown in senescent Arabidopsis leaves. Characterization of chlorophyll catabolites and of chlorophyll catabolic enzymes involved in the degreening reaction. *Plant Physiol* 139:52–63
- Rajashakar C, Lafta A (1996) Cell-wall changes and cell tension in response to cold acclimation and exogenous abscisic acid in leaves and cell cultures. *Plant Physiol* 111:605–612
- Rehfeldt GE (2004) Interspecific and intraspecific variation in *Picea engelmannii* and its congeneric cohorts: biosystematics, genealogy, and climate change. United States Department of Agriculture: General Technical Report RMRS-GTR-134
- Rehfeldt GE, Tchebakova NM, Parfenova YI, Wykoff WR, Kuzmina NA, Milyutin LI (2002) Intraspecific responses to climate in *Pinus sylvestris*. *Glob Change Biol* 8:912–929
- Rosenthal SI, Camm EL (1997) Photosynthetic decline and pigment loss during autumn foliar senescence in western larch (*Larix occidentalis*). *Tree Physiol* 17:767–775
- Savitch LV, Gray GR, Huner NPA (1997) Feedback-limited photosynthesis and regulation of sucrose-starch accumulation during cold acclimation and low-temperature stress in a spring and winter wheat. *Planta* 201:18–26
- Schrader S, Sauter JJ (2002) Seasonal changes of sucrose-phosphate synthase and sucrose synthase activities in poplar wood (*Populus x canadensis* Moench ‘robusta’) and their possible role in carbohydrate metabolism. *J Plant Physiol* 159:833–843
- Schreiber U, Neubauer C, Schliwa U (1993) PAM fluorometer based on medium-frequency pulsed Xe-flash measuring light: a highly sensitive new tool in basic and applied photosynthesis research. *Photosynth Res* 36:65–72
- Schreiber U, Bilger W, Neubauer C (1994) Chlorophyll fluorescence as a noninvasive indicator for rapid assessment of in vivo photosynthesis. In: Schulze ED, Caldwell MM (eds) *Ecophysiology of photosynthesis*. Springer-Verlag, Berlin, pp 49–70
- Somersalo S, Krause GH (1989) Photoinhibition at chilling temperature: Fluorescence characteristics of unhardened and cold-acclimated spinach leaves. *Planta* 177:409–416
- Somersalo S, Krause GH (1990) Photoinhibition at chilling temperatures and effects of freezing stress on cold acclimated spinach leaves in the field: a fluorescence study. *Physiol Plant* 79:617–622
- Spector T, Putz FE (2006) Crown retreat of open-grown Southern live oaks (*Quercus virginiana*) due to canopy encroachment in Florida, USA. *Forest Ecol Manag* 228:168–176
- Strand M, Öquist G (1988) Effects of frost hardening, de-hardening and freezing stress on in vivo chlorophyll fluorescence of seedlings of Scots pine (*Pinus sylvestris* L.). *Plant Cell Environ* 11:231–238
- Sultan SE (1995) Phenotypic plasticity and plant adaptation. *Acta Bot Neer* 44:363–383
- Sultan SE (2001) Phenotypic plasticity for fitness components in Polygonum species of contrasting ecological breadth. *Ecology* 82:328–343
- Tobias DJ, Ikemoto A, Nishimura T (1995) Leaf senescence patterns and photosynthesis in four leaf flushes of two deciduous oak (*Quercus*) species. *Photosynthetica* 31:231–239
- Tretiach M, Bolognini G, Rondi A (1997) Photosynthetic activity of *Quercus ilex* at the extremes of a transect between Mediterranean and submediterranean vegetation (Trieste–NE Italy). *Flora* 192:369–378
- Velleman PF (1997) Data desk (version 6.0). Data Description inc., Ithaca, New York
- Verhoeven AS, Adams WW, Demmig-Adams B (1999a) The xanthophyll cycle and acclimation of *Pinus ponderosa* and *Malva neglecta* to winter stress. *Oecologia* 118:277–287
- Verhoeven AS, Adams WW, Demmig-Adams B, Croce R, Bassi R (1999b) Xanthophyll cycle pigment localization and dynamics during exposure to low temperatures and light stress in *Vinca major*. *Plant Physiol* 120:727–737
- Warren CR, Hovenden MJ, Davidson NJ, Beadle CL (1998) Cold hardening reduces photoinhibition of *Eucalypts nitens* and *E-pauciflora* at frost temperatures. *Oecologia* 113:350–359
- Wisniewski M, Davis G, Schaffer K (1991) Mediation of deep supercooling of peach and dogwood by enzymatic modifications in cell-wall structure. *Planta* 184:254–260
- Yamori W, Noguchi K, Terashima I (2005) Temperature acclimation of photosynthesis in spinach leaves: analyses of photosynthetic components and temperature dependencies of photosynthetic partial reactions. *Plant Cell Environ* 28:536–547
- Yasumura Y, Hikosaka K, Hirose T (2006) Seasonal changes in photosynthesis, nitrogen content and nitrogen partitioning in *Lindera umbellata* leaves grown in high or low irradiance. *Tree Physiol* 26:1315–1323
- Zhu XB, Cox RM, Bourque CPA, Arp PA (2002) Thaw effects on cold-hardiness parameters in yellow birch. *Can J Bot-Rev Can Bot* 80:390–398