Foliar Heat Tolerance of Three Holly Species (Ilex spp.): Responses of Chlorophyll Fluorescence and Leaf Gas Exchange to Supraoptimal Leaf Temperatures

Thomas G. Ranney
Department of Horticultural Science, Mountain Horticultural Crops Research and Extension Center, North Carolina State University, 2016 Fanning Bridge Road, Fletcher, NC 28732-9216

John M. Ruter
Department of Horticulture, Coastal Plain Experiment Station, University of Georgia, Tifton, GA 31793-0748

Additional Index Words. Ilex aquifolium ‘Monler’ Sparkler, Ilex cornuta ‘Barfordii’, Ilex rugosa, carbon exchange rate, high temperature, photosynthesis, respiration

Abstract. Temperature sensitivity of CO2 assimilation (ACO2), dark respiration, and chlorophyll fluorescence was evaluated among three taxa of hollies including I. aquifolium L., I. cornuta Lindl. & Paxt., and I. rugosa Friedr. Schmidt. Variations in foliar heat tolerance among these species were manifested in temperature responses for ACO2. Temperature optima of ACO2 for I. rugosa, I. cornuta, and I. aquifolium were 22.0, 26.3, and 27.9 °C, respectively (LSDB = 2.9). Temperature responses of respiration were similar among taxa and did not appear to be contributing factors to variations in ACO2 At 40 °C, potential photosynthetic capacity, measured under saturating CO2, was 4.1, 9.4, and 14.8 μmol m−2 s−1 for I. rugosa, I. aquifolium, and I. cornuta, respectively (LSDB = 5.1). Variations in the relative dark-acclimated fluorescence temperature curves were used to assess thresholds for irreversible heat injury. The critical fluorescence temperature threshold (Tcr) was similar (48.0 °C) for all taxa. The fluorescence temperature peaks (Tm) were 52.0, 52.8, and 53.5 °C for I. rugosa, I. cornuta, and I. aquifolium, respectively (LSDB = 0.9). Based on these results, I. rugosa was the most heat-sensitive species, followed by I. aquifolium and I. cornuta. Ilex cornuta also had substantially greater potential photosynthetic capacity than the other species at 40 °C, indicating superior metabolic tolerance to high temperatures.

Hollies are important landscape plants native to a broad range of latitudes, elevations, and associated climates (The New Royal Horticultural Society, 1992). Evaluation of growth and survival of hollies in warm climates suggests considerable differences in heat tolerance among species. For example, I. cornuta typically grows well throughout the warm climate of the southern United States, whereas I. rugosa grows poorly in those areas (Dirr, 1990). Many of the holly taxa that exist in commercial production are of unknown provenance and/or are hybrids between diverse taxa; thus, it is difficult to predict thermotolerance of a given cultivar based on the origin and adaptability of its respective parents.

Inhibition of growth and/or plant decline under supraoptimal temperatures can result from thermal effects on many physiological and developmental processes (Fitter and Hay, 1987). Photosynthesis is one of the most heat-sensitive processes that govern plant growth (Björkman et al., 1980). Consequently, physiological adaptation of photosynthetic processes can be a key factor in adaptability of plants to high temperatures. Temperature responses of CO2 assimilation (ACO2) can provide an integrated measure of a plant’s temperature adaptability. The optimal temperature for ACO2 is usually correlated with the optimal temperature for plant growth under a given set of environmental conditions and can reflect a plant’s native (evolutionary origin) climate (Björkman and Björkman, 1980; Björkman et al., 1980; Friend and Nelson, 1976; Fryer and Ledig, 1972; Larcher, 1995; Pisek et al., 1973). Considerable variation occurs in the thermotolerance of ACO2 among different plant genera (Björkman et al., 1980), species (Hällgren et al., 1982; Koike and Sakagami, 1985; Moon et al., 1987; Ranney and Peet, 1994; Ranney et al., 1995), and, in some cases, ecotypes and provenances (Fryer and Ledig, 1972; Mebrahtu et al., 1991; Slatyer and Ferrar, 1977). When plants are acclimated to high temperatures, the optimal temperature for ACO2 is a measure of the upward limit of this temperature optimum and can be used as an effective index of heat tolerance (Ranney et al., 1995).

The capacity for a plant to tolerate and survive prolonged high temperature extremes can also be an important survival mechanism in certain environments. In the southern United States, foliage temperatures of hollies can exceed 50 °C (Pair and Still, 1982; J.M. Ruter, personal observation). Changes in chlorophyll fluorescence can be used as a sensitive probe for determining threshold temperatures for heat injury to the photosynthetic apparatus (Bilger et al., 1984). Minimal, dark-acclimated fluorescence (F0) is typically stable at increasing temperatures until a critical temperature (Tc) is reached, followed by a sudden rise and peak (Tm) in Fm (Schreiber and Berry, 1977). The values of Tc and Tm provide relative indices of progressive heat damage to the thylakoid membrane and have been associated with an inhibition of energy transfer to the reaction centers and irreversible heat injury (Bilger et al., 1984; McCain et al., 1989; Smillie and Nott, 1979).

A greater understanding of heat tolerance of different taxa, including the specific characteristics and mechanisms that influence heat tolerance, would aid in evaluation, selection, and improvement of hollies for various climates. The objectives of this research were to (1) evaluate foliar heat tolerance of selected species of holly based on temperature responses of ACO2 and critical temperature thresholds for tissue injury based on chlorophyll
fluorescence and 2) study mechanisms contributing to variation in temperature responses of $A_{CO_2}$.

**Materials and Methods**

Three taxa of hollies, representing diverse habitats of origin, were studied, including English holly (*Ilex aquifolium* 'Monler' Sparkler), Chinese holly (*I. cornuta* 'Burfordii'), and prostrate holly (*I. rugosa*). Plants were propagated as stem cuttings from stock plants at the North Carolina State University Arboretum in Fall 1993. In April 1995, plants were transplanted into 19-L containers filled with milled pine bark amended with 2.97 kg·m$^{-3}$ dolomitic limestone and 2.37 kg·m$^{-3}$ fertilizer (5N-0.4P-0K, Micro-Start, Sta-Green Co., Sylacauga, Ala.). Plants were grown in an unshaded glass house with day/night temperatures of 21/18 °C, irrigated as needed, and fertilized weekly with water soluble fertilizer (20N–8.8P–16.6K; Scotts Co., Marysville, Ohio). Fertilizer solution (100 ppm N) was applied in sufficient amounts to ensure leaching. On 10 July 1995, the temperature in the greenhouse was raised to day/night temperatures of 30/25 °C to acclimate plants to warm temperatures. Plants were arranged in a completely randomized design with six replicate plants per taxon.

Gas-exchange measurements were determined on individual, attached shoots enclosed in a temperature controlled cuvette (DDG-9000, Data Design Group, La Jolla, Calif.). Leaves of all branches were from the most recent growth flush and similar in age. Gas-exchange measurements were conducted using an open-flow gas-exchange measurement system as described by Renney and Peet (1994). Leaf temperature was monitored using a thermocouple on the underside of a representative leaf. The CO$_2$ and vapor pressure deficit in the cuvette were maintained at a mean of 355 ppm (sd = 12) and 1.05 kPa (sd = 0.29), respectively, during gas-exchange measurements, regardless of temperatures. Artificial light was supplied with a quartz filament lamp providing an irradiance of 1400 μmol·m$^{-2}$·s$^{-1}$ photosynthetically active radiation (400–700 nm) at the uppermost surface of a representative leaf.

Plants were maintained outside under ambient conditions before being measured. Plants were brought into a laboratory and branches with four to eight leaves were placed inside the cuvette and allowed to acclimate for 15 min at 15 °C before the first measurement. Leaf temperature was then increased 5 °C at 1 °C/min, the leaf was allowed to acclimate for 15 min, and the next measurement was taken. This procedure was repeated up to 40 °C. Equations for calculating $A_{CO_2}$, stomatal conductance (g), and calculated concentration of internal leaf CO$_2$ (C) followed von Caemmerer and Farquhar (1981). Temperature optima of $A_{CO_2}$ for each plant were estimated by calculating the leaf temperature at which the first-order derivative of the temperature response of $A_{CO_2}$ was equal to 0. Potential photosynthetic capacity (Larcher, 1995) at 40 °C was measured under saturating CO$_2$ (mean = 1216 ppm) following a 30-min acclimation period.

Dark respiration rates were measured during daylight hours. The same temperature regime protocol and gas-exchange measurement system was used as described for $A_{CO_2}$ measurements, except that the sample cuvette was enclosed in aluminum foil to exclude light. Dark respiration of shoots was expressed on a leaf area basis. Temperature coefficients ($Q_{10}$) for dark respiration were calculated as [rate at $(t + 10 °C)/$(rate at $t$)], where $t$ = the base temperature used for the calculation. Separate $Q_{10}$ coefficients were calculated for base temperatures of 15, 20, 25, and 30 °C.

Chlorophyll fluorescence was measured on individual leaves using a pulse modulation chlorophyll fluorometer (OS-500; PP Systems, Haverhill, Mass.). $F_{v}$ was measured over a range of 30 to 60 °C. Leaf temperatures were increased from 30 °C in 1 °C min. Measurements were taken each minute using a pulsed measuring light for 10 s with 0.46 μmol·m$^{-2}$·s$^{-1}$ (660 nm) at the sensor head following a 10-s far-red illumination (735 nm) of 0.0133 μmol·m$^{-2}$·s$^{-1}$. $F_{v}$ and $F_{m}$ were calculated for each sample following Bilger et al. (1984).

Measurements were conducted from 0800 to 1700 hr from 10 Aug. through 13 Sept. 1995. For the month before and for the period during measurement, the mean daily and mean daily high temperatures in the greenhouse were 29 and 35 °C, respectively.

The experimental design was a randomized complete block with measurements of gas exchange and chlorophyll fluorescence blocked over time. Each plant was measured in random order within each of six blocks. Different branches were sampled for gas exchange in the light, dark respiration, and chlorophyll fluorescence. The treatment design was a two-way factorial with independent variables of taxa and temperature. Analysis of variance (AOV) was conducted using a general linear model (PROC GLM; SAS Institute, Cary, N.C.). Least significant difference (LSD$_{0.05}$) values were calculated based on a pooled mean square errors from...
the AOV including all taxa and temperatures. Simple linear or polynomial curves were fit to the data when significant trends were identified in separate regression analyses (PROC REG; SAS) for each taxa.

**Results**

Temperature responses of $A_{CO_2}$ varied by species as indicated by a significant species by temperature interaction ($P < 0.05$). Regression analysis revealed a significant cubic responses for all three taxa (Fig. 1). The temperature optima for maximum $A_{CO_2}$ for *I. rugosa*, *I. cornuta*, and *I. aquifolium* were 22.0, 26.3, and 27.9 °C, respectively ($LSD_{0.05} = 2.9$). Mean calculated $A_{CO_2}$ maxima (calculated from individual plant regression equations at the temperature optimum) for *I. rugosa*, *I. cornuta*, and *I. aquifolium* were 7.5, 12.1, and 11.1 mmol-m$^{-2}$-s$^{-1}$, respectively ($LSD_{0.05} = 2.2$). Although $A_{CO_2}$ was inhibited at 40 °C for all taxa, *I. aquifolium* maintained higher $A_{CO_2}$ than the other two taxa. Potential photosynthetic capacity (under saturating $CO_2$) at 40 °C was significantly higher than $A_{CO_2}$ at ambient $CO_2$ concentrations for *I. cornuta* and *I. aquifolium*, but not for *I. rugosa* (Fig. 1, solid symbols). Potential photosynthetic capacity at 40 °C was highest for *I. cornuta*, followed in descending order by *I. aquifolium*, and *I. rugosa*.

There was a significant interaction between temperature and taxa for both $g_{s}$ and $C_{i}$. Temperature responses of $g_{s}$ were quadratic for *I. aquifolium* and cubic for *I. cornuta* and *I. rugosa* (Fig. 2A). Temperature optima for $g_{s}$ were generally similar to those for $A_{CO_2}$ and decreased at supraoptimal temperatures. At 40 °C, *I. aquifolium* maintained higher $g_{s}$ than the other two taxa. Temperature responses of $C_{i}$ were cubic for *I. aquifolium* and *I. cornuta* but showed no significant trend for *I. rugosa*. At 40 °C, $C_{i}$ for *I. cornuta* was significantly lower than for the other two taxa (Fig. 2B).

Dark respiration increased with increasing temperatures in a similar manner for all taxa and there were no interactions (Fig. 3). Mean values of $Q_{10}$ for dark respiration varied from 1.9 to 2.3 over the temperature range and were similar among taxa (data not presented).

Base level fluorescence had a mean $T_{m}$ of 48.0 °C and was similar for all taxa (Fig. 4). The $T_{m}$ varied by species and was 52.0, 52.8, and 53.5 °C for *I. rugosa*, *I. cornuta*, and *I. aquifolium*, respectively ($LSD_{0.05} = 0.9$).

**Discussion**

Variations in $A_{CO_2}$ among taxa indicated distinct differences in physiological tolerance to high temperatures. The greater temperature optima of $A_{CO_2}$ for *I. aquifolium* and *I. cornuta*, above that of *I. rugosa*, indicated that the former two species had greater foliar heat tolerance, even when grown and acclimated to similar temperatures. Although the exact origin of these taxa is not known, variations in heat tolerance were consistent with the native climates of these three species. *I. rugosa* is a boreal (subalpine) species native to cool mountains of northern Japan (Ohwi, 1984; The New Royal Horticultural Society, 1992). *I. aquifolium* has a broad range extending from temperate southern and western Europe to subtropical/Mediterranean northern Africa and western Asia (The New Royal Horticultural Society, 1992). *I. cornuta* is native to temperate Korea and the subtropical lower Yangtze provinces in China (Hu, 1949; Hume, 1953).

*I. rugosa* was characterized by having a low temperature optimum for $A_{CO_2}$, a low $A_{CO_2}$ maximum, and low $g_{s}$ relative to the other taxa. At temperatures below 35 °C, low $A_{CO_2}$ rates were most likely related to low $g_{s}$. At these lower temperatures, $g_{s}$ and $C_{i}$ of *I. rugosa* were generally below 0.06 mmol-m$^{-2}$-s$^{-1}$ and 160 ppm $CO_2$, respectively—levels that are photosynthetically limiting for most C$_3$ plants (Sharkey, 1985). As temperatures were increased to 40 °C; however, $A_{CO_2}$ decreased and $C_{i}$ increased for *I. rugosa* indicating that high temperatures were directly limiting $A_{CO_2}$ independent of g (Farquhar and Sharkey, 1982). Increasing $CO_2$ to saturating levels at 40 °C had no significant effect on $A_{CO_2}$ of *I. rugosa*, further indicating that g was not limiting $A_{CO_2}$ at high temperatures. *I. rugosa* is typically found as an understory plant in boreal, coniferous forests (Ohwi, 1984). In this environment, the combination of low light and desiccation stress (particularly in winter) may have resulted in the natural selection of plants having conservative water use (regulated via limited g) and a low $A_{CO_2}$ capacity. Low maximal $A_{CO_2}$ and g are typical of evergreen plants native to cool, alpine areas (Ramey et al., 1995; Tieszen and Wieland, 1975). Such an adaptation, however, could indirectly reduce tolerance to higher temperatures by reducing the photosynthetic efficiency coefficient (i.e., gross photosynthesis/respiration) and result in depletion of carbohydrate at higher temperatures (Crawford and Palin, 1981; Deal et al., 1990; Lambers, 1985; Larcher, 1995; Mooney, et al., 1964).

*I. aquifolium* maintained the highest $A_{CO_2}$ at 40 °C (under ambient $CO_2$) and one of the highest temperature optima for $A_{CO_2}$. This greater $A_{CO_2}$, particularly at 40 °C, appeared to result from...
greater \( g_t \), which in turn provided for greater \( C_t \) and \( A_{CO_2} \). However, when saturating \( CO_2 \) was provided at 40 °C, the potential photosynthetic capacity of \( I. aquifolium \) was only intermediate between \( I. rugosa \) and \( I. cornuta \).

**Ilex cornuta** had one of the highest temperature optima for \( A_{CO_2} \), but \( g_t, C_t, \) and \( A_{CO_2} \) decreased rapidly at temperatures above 30 °C. When \( CO_2 \) was increased to saturating concentrations at 40 °C, \( A_{CO_2} \) for \( I. cornuta \) increased from 3 to 17 \( \mu \text{mol-m}^{-2} \text{s}^{-1} \). The response indicated that these plants maintained a considerable capacity for \( A_{CO_2} \), but that stomata were primarily responsible for limiting \( A_{CO_2} \) at high temperatures and ambient \( CO_2 \) levels, despite a low vapor-pressure deficit. This decrease in \( g_t \) at high temperatures may be an adaptation for desiccation avoidance. In the Zhejiang province of China, *Ilex cornuta* grows on warm, exposed rocky hillside and water deficit stress can be limiting (Lancaster, 1989).

**Fig. 3.** Temperature response of dark respiration for three species of holly (*Ilex*). The solid line represents predicted temperature response based on regression analysis. Symbols represent means \( (n = 6) \). Vertical bars are LSD at \( P < 0.05 \) based on the pooled mean square error from an analysis of variance including all taxa and temperatures.

*Ilex cornuta* and *I. aquifolium* maintained significantly higher \( A_{CO_2} \), maxima than *I. rugosa*. Increased photosynthetic capacity within the optimal temperature range may also enhance heat tolerance by improving carbon balance and compensating for the higher respiratory demand experienced during warmer diurnal and seasonal periods. Photosynthetic capacity at the temperature optimum is typically greater for plants from warmer climates (Mooney and Gulmon, 1979).

Temperature responses of dark respiration were similar and did not appear to be a contributing factor to variations in \( A_{CO_2} \). Although photosynthesis was not measured in this study, the temperature dependence of photosynthesis has been found to be an important factor in differential temperature sensitivity of photosynthesis among taxa (Mebrahtu et al., 1991; Samuelson and Teskey, 1991).

Variations in foliar heat tolerance were not apparent based on differences in the critical temperature thresholds for tissue injury as indicated by changes in base chlorophyll fluorescence-temperature curves \( (T_c) \). However, \( T_m \), a more extreme indicator of thylakoid perturbation, varied significantly among species. These \( T_m \) were similar to the temperature range \( (52–54 °C) \) that Ruter (1993) assessed as the critical midpoint heat killing temperature for *I. xmeserveae* S.Y. Hu 'Blue Prince' and *I. rugosa* X cornuta Lindl. & Paxt. ‘Mesdab’ (China Boy), based on electrolyte leakage techniques.

Results from this study indicate that *I. rugosa* was the most heat-sensitive species as indicated by a low-temperature optimum for \( A_{CO_2} \) and a low \( A_{CO_2} \) maximum. *Ilex aquifolium* and *I. cornuta* had greater heat tolerance as indicated by their higher temperature optima for \( A_{CO_2} \) and higher \( A_{CO_2} \) maxima. However, *I. cornuta* had the greatest potential photosynthetic capacity at 40 °C and saturating \( CO_2 \), indicating superior metabolic tolerance to high temperatures.

**Literture Cited**


**Fig. 4.** Temperature response of relative dark-acclimated fluorescence \( (F_o) \) of three species of holly (*Ilex*). The critical fluorescence-temperature threshold \( (T_m) \) was similar for all taxa at 48.0 °C. The fluorescence temperature peak \( (T_p) \) varied by taxa and was 52.0, 52.8, and 53.5 for *I. rugosa*, *I. cornuta*, and *I. aquifolium*, respectively \( (LSD_{0.05} = 0.9) \). Symbols represent means \( (n = 5) \). Vertical bars are LSD at \( P < 0.05 \) based on the pooled mean square error from an analysis of variance including all taxa and temperatures.


