Chlorophyll, Carotenoid, and Visual Color Rating of Japanese-cedar Grown in the Southeastern United States

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Abstract. **Japanese-cedar has been underused in landscapes of the United States until recent years. There are now over 100 cultivars, many of which are grown in the southeast of the United States. Performance of cultivars has been described from U.S. Department of Agriculture (USDA) Zone 6b to USDA Zone 7b; however, there are no reports on how cultivars perform in USDA Zone 8. The current study was conducted to measure chlorophyll *a*, chlorophyll *b*, total chlorophyll, and carotenoid content and assign visual color ratings to determine if there was a relationship between pigment values and perceived greenness, which generally is regarded as a desirable and potentially heritable trait. Total chlorophyll ($P = 0.0051$), carotenoids ($P = 0.0266$), and the ratio of total chlorophyll to carotenoids ($P = 0.0188$) exhibited a positive relationship with greenness after accounting for season and tree effects. In contrast, the ratio of chlorophyll *a* to chlorophyll *b* did not have an effect on greenness. There was a linear relationship between total chlorophyll and carotenoid regardless of season (summer $R^2 = 0.94$; winter $R^2 = 0.88$) when pooled across 2 years. The observed correlation between chlorophyll and carotenoid content suggests they could be used interchangeably as predictors of greenness. There were large differences in rainfall between the 2 years that may have resulted in additional variation. Furthermore, the climate in which the evaluation was conducted differs greatly from the native distribution of japanese-cedar occurring in China and Japan.**

Japanese-cedar [*Cryptomeria japonica* (L.f.) D. Don] is a variable conifer that grows up to 60 m tall in its native range. Wild-type specimens are conical when young and become cylindrical with age (Eckenwalder, 2009). Japanese-cedar traditionally has been used as screening or specimen plantings; however, there are a large number of cultivars displaying varying forms and growth rates (Rouse et al., 2000; Tripp, 1993) that may be used in diverse landscape situations. There are estimates of over 100 different ornamental cultivars (R. Determann, personal communication; Erhardt, 2005) with ≈45 of these grown in the eastern United States (Rouse et al., 2000). Japanese-cedars are native to the warm-temperate zones of south China and Japan. In Japan, they generally are limited to north-facing slopes that receive 180 to 300 cm of rainfall per year (Tsukada, 1967). Japanese-cedars perform well under a number of environmental and soil conditions including the hot, humid summers and heavy clay soils of the southeast United States (Tripp and Raulston, 1992). As a result of this fact, japanese-cedars have been promoted as an alternative to Leyland cypress [×*Cuprocyparis leylandii* (A.B.Jacks. & Dallim.) Farjon (Farjon et al., 2002)], on which numerous problems now occur including bagworms (*Thyridopteryx ephemeraeformis* Haworth) (Lemke et al., 2005), fungal cankers caused by *Seiridium* Nees ex Link spp. and *Botryosphaeria dothidea* (Moug.) Ces. et De Not., and cercospora needle blight (*Cercosporidium sequoiae* Ellis and Everh.) (Martinez et al., 2009). Japanese-cedars exhibit less susceptibility to bagworm infestations, and cultivars are available that have reduced interior branch death (Tripp and Raulston, 1992); however, the species is not problem-free. Redfire (*Phyllosticta aurea* C.Z. Wang) is a fungal pathogen that can attack stressed japanese-cedars and cause stem death, particularly on older foliage (Cox and Ruter, 2013; Tripp, 2005). Also, as Dirr (2009) notes, there is not a fast-growing, tree-like cultivar that remains green during winter. Winter browning in japanese-cedar is often unsightly and undesirable to consumers, which may have contributed to why it has remained underused in landscapes.

Winter browning in japanese-cedar occurs through the conversion of chloroplasts to chromoplasts during winter (Ida, 1981). This transition takes place only in sun-exposed leaves during periods of low temperature, indicating that photoinhibition likely plays a role (Han and Mukai, 1999; Ida, 1981). Plants have several mechanisms to cope with excess light during periods of low temperature when Calvin cycle activity is limiting, including reduction of chlorophyll, pH-dependent xanthophyll cycle, increased levels of carotenoids, and production of antioxidants or reactive oxygen species (ROS) scavenging enzymes. During winter, japanese-cedar has been shown to demonstrate two principle mechanisms to deal with excess light energy. The amount of chlorophyll decreases during winter (Han and Mukai, 1999; Ida, 1981) in both sun- and shade-exposed leaves (Han et al., 2004). This occurs in both wild-type and non-browning mutants (Han et al., 2003), thus reducing the amount of energy absorbed. The other mechanism is the conversion of chloroplasts in sun-exposed leaves to rhodoxanthin-containing chromoplasts during winter to dissipate excess light energy as heat (Ida, 1981).

In a study on 15-year-old japanese-cedar trees in Shizuoka Prefecture, Japan, Han et al. (2004) reported accumulation of rhodoxanthin in sun-exposed leaves beginning in January, reaching maximum levels in February, decreasing significantly in March, and falling to zero by April. However, timing of discoloring in winter and restoration in spring is highly variable and location-specific (personal observation). Han et al. (2003) demonstrated that wild-type leaves that accumulated rhodoxanthin maintained higher levels of photosynthesis with lower levels of zeaxanthin- and antheraxanthin-dependent thermal dissipation than mutants that remained green all winter. The proposed role of rhodoxanthin is to intercept a portion of incident light to help maintain an appropriate balance among light absorption, thermal dissipation, and photosynthesis (Han et al., 2003). Japanese-cedar also accumulated substantial levels of xanthophyll cycle pigments and lutein during

winter (Han et al., 2003; Han and Mukai, 1999).

The overarching objective of the current study was to identify an early predictor of winter foliage color (resistance to leaf browning) in japanese-cedar as a screening tool for identifying superior selections. Specifically, we assessed if quantitating pigments such as total chlorophyll (C_{a+b}), ratio of chlorophyll a (C_a):chlorophyll b (C_b), total carotenoids (C_{x+c}), and ratio of (C_{a+b}):(C_{x+c}) exhibited a strong relationship with greenness as measured by visual color rating at the University of Georgia Tifton Campus (USDA Zone 8b; USDA-ARS, 2012).

Materials and Methods

Plant material and growing conditions. Single plants of the following 12 taxa of japanese-cedar were randomly planted in 1997: 'Araucariodes', 'Ben Franklin', 'Black Dragon', 'Cristata', 'Gyokruga', 'Rasen', 'Sekkan', 'Tansu', 'Tarheel Blue', var. *sinensis*, 'Yaku', and 'Yoshino'. These taxa were not meant to provide an evaluation of each per se as a result of single replicates; rather, they were used to provide a general sampling of the genotypic and phenotypic diversity observed in landscape forms of japanese-cedar. Plants were maintained in field plots at the University of Georgia Tifton Campus (lat. 31°49' N, long. 83°53' W; USDA Zone 8b). Field soil was a Tifton loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudult), pH 5.2. Plots were fertilized in March every year after planting at a rate of 56 kg·ha⁻¹ nitrogen (N) using Super Rainbow 16N-1.8P-6.6K plus minor elements (Agrium U.S. Inc., Denver, CO). An additional 28.5 kg·ha⁻¹ N was applied in late August each year after planting using the product mentioned previously. Southeast-facing branches were flagged during winter 2007–08 and material used for the duration of the study was collected from the same branches. Leaves were collected 8 Feb. 2008, 17 Aug. 2008, 9 Feb. 2009, and 5 May 2009 and frozen at –80 °C until analysis. Temperature and precipitation data at the Tifton Campus for the duration of the study are included in Table 1. Supplemental irrigation was used only at the time of new plant establishment within plots.

Chlorophyll and carotenoid extraction, analysis, and calculations. Three subsamples of leaf tissue were collected from the 12 individuals and C_{a+b} and C_{x+c} were extracted by grinding 85 mg leaf tissue three times in 3.33 mL 80% aqueous acetone and the extract was transferred to a test tube and brought to a final volume of 10 mL. After the third grind in acetone, the leaf material remaining was transferred to the test tube containing the extract and maintained in the dark at 4 °C for 1 h to ensure complete extraction. Two milliliters of the extract was centrifuged for 30 s at 6800 g_n. The supernatant was then transferred to a cuvette and absorbance was measured at 470 nm, 646 nm, and 663 nm using a GENESYS™ 10 Spectrophotometer (Thermo Electron Corp., Madison, WI). Absorbance for all samples at each wavelength was between 0.2 and 0.8. Determination of C_a, C_b, and C_{x+c} was performed using calculations from Lichtenthaler and Wellburn (1983). C_a content was calculated using the formula: C_a (mg·L⁻¹) = $(12.25 \times A_{663}) - (2.79 \times A_{646})$. C_b content was calculated using the formula: C_b (mg·L⁻¹) = $(22.5 \times A_{646}) - (5.1 \times A_{663})$. C_{a+b} content was determined by summing C_a and C_b values. Total carotenoid content was determined using the formula: C_{x+c} (mg·L⁻¹) = $[1000 \times A_{470} - (1.82 \times C_a) - (85.02 \times C_b)]/198$. Chlorophyll and carotenoid contents were expressed in mg·g⁻¹ of dry weight after being corrected for moisture content (MC) as follows: three unanalyzed leaf subsamples were collected to determine mean MC for each individual (replicate) for each harvest date using the formula {MC = [(initial weight – dry weight)/initial weight] × 100}.

Color rating. Plants were observed within 1 week of the four leaf collection dates. Five evaluators assigned color ratings from 1 (very brown/yellow; off color) to 5 (very green). Ratings of 3.5 to 4 would be considered acceptable for landscape use. All plants were addressed from the southeast side, directly in front of flagged branches. Mean rating for each individual was calculated and used for statistical analysis.

Design and statistical analysis. The experimental design used repeated measurements across winter and summer on each individual with subsamples taken on the individual branches. Relevant covariate information, chlorophyll and carotenoid measurements, was obtained for each subsample, whereas the target response, color rating, was characterized at the individual level.

To assess the relationship between chlorophyll and carotenoid measurements with the observed color rating of the trees, data were analyzed using a mixed model framework (PROC MIXED, SAS Version 9.3; SAS Institute Inc., Cary, NC). Random intercept models (linear models with random components with each variant of tree having a different intercept, but the slopes are assumed the same) were constructed to assess the relationship between the chlorophyll and carotenoid measurements with the observed greenness of the trees. These models were of the form:

$$Green_{ijk} = \mu + tree_i + season_j + \beta^* covariate_{ijk} + E_{ijk}, \quad (1)$$
$$tree_i \sim N(0, \sigma_t), E_{ijk} \sim N(0, \sigma_E).$$

To determine an overall best model, Akaike's Information Criterion (AIC) was used, in which a lower number represents a better fit. Also of interest in this research was to test for differences across the seasons in color ratings and chlorophyll and carotenoid easurements. For each measured pigment, a mixed-effects analysis of variance was constructed

$$Pigment_{ijk} = \mu + tree_i + season_j + E_{ijk},$$
$$tree_i \sim N(0, \sigma_t), E_{ijk} \sim N(0, \sigma_E) \quad (2)$$

Hence, examining season term for each of these five models established whether there were seasonal differences in each pigment. The previous two statistical analyses aggregated the subsamples such that a single observation per tree per period was used.

To assess whether summer greenness measures are predictive of winter greenness, a simple regression model was used, for which summer and winter greenness ratings were aggregated for each individual. Given the experimental constraints in which only a single tree from each taxon could be planted, there is no replication of taxa. Hence, statistical methods cannot be used to determine

Table 1. Temperature and precipitation data at the University of Georgia Tifton Campus[z] for the duration of a study to evaluate pigments and color of *Cryptomeria japonica* from Nov. 2007 through Aug. 2009.

Month	Avg daily maximum temp (°C)	Avg daily minimum temp (°C)	Avg daily temp (°C)	Rainfall (cm)
Nov. 2007	20.8	7.4	14.1	2.3
Dec. 2007	19.4	8.0	13.7	14.4
Jan. 2008	14.0	3.9	8.9	8.7
Feb. 2008	18.9	5.6	12.2	19.9
Mar. 2008	20.9	8.5	14.7	8.1
Apr. 2008	24.1	13.0	18.6	7.7
May 2008	28.8	17.1	22.9	4.7
June 2008	32.5	21.0	26.7	5.8
July 2008	32.3	21.8	27.0	8.4
Aug. 2008	30.7	21.9	26.3	31.3
Sept. 2008	29.8	19.3	24.5	0.4
Oct. 2008	23.7	12.5	18.1	16.3
Nov. 2008	18.5	6.7	12.6	9.9
Dec. 2008	18.8	7.5	13.1	7.9
Jan. 2009	15.7	4.7	10.2	4.2
Feb. 2009	17.1	4.3	10.7	5.5
Mar. 2009	20.9	9.6	15.3	20.9
Apr. 2009	24.0	12.6	18.3	21.0
May 2009	27.6	18.6	23.1	16.6
June 2009	32.8	22.3	27.6	5.0
July 2009	31.7	21.6	26.6	17.6
Aug. 2009	31.5	22.0	26.7	17.8

[z]U.S. Department of Agriculture Zone 8b, lat. 31°49' N, long. 83°53' W.

whether certain cultivars or varieties exhibit higher greenness ratings. Rather, our objective here was to use these named taxa to determine if C_a, C_b, C_{a+b}, or C_{x+c} can be used in a model to predict winter greenness for *C. japonica*.

Results

C_{a+b} ($P = 0.0051$), C_{x+c} ($P = 0.0266$), and the ratio of C_{a+b} to C_{x+c} ($P = 0.0188$) exhibited a positive relationship with greenness after accounting for season and tree effects (Table 2). In contrast, the ratio of C_a to C_b was not related to greenness. Using AIC, both C_{x+c} (AIC = 91.7) and C_{a+b} (AIC = 91.9) have superior model fits than ratio of C_{x+c} to C_{a+b} (AIC = 93.2). Similarly, visual color rating correlated with C_{a+b} ($r = 0.535$), total C_{x+c} ($r = 0.375$), and their ratio ($r = 0.520$) when pooled across years (Table 3). In addition, there was linear relationship between C_{a+b} and C_{x+c} regardless of season, summer ($R^2 = 0.94$) and winter ($R^2 = 0.88$), when pooled across 2 years (Fig. 1). The slope of the relationship (y = C_{x+c}; x = C_{a+b}) remained relatively consistent (4.47 and 5.05) across seasons; however, the intercept, a measure of C_{x+c} levels, decreased for 0.52 to –1.35 from summer to winter, respectively (Fig. 1)

Each of the five attributes, observed greenness and measured chlorophyll and carotenoids, exhibited differences between summer and winter with higher values in the summer (Table 4). Using observed summer greenness pooled across years, winter greenness can be modeled ($P = 0.017$, $R^2 = 0.45$) (Fig. 2).

Discussion

Mean C_{a+b} content was similar to previous values for japanese-cedar (Ida, 1981).

There were differences in mean C_{a+b} between seasons with a higher content in summer than winter when taxa were pooled (Table 4). Previous studies also have observed reduction in C_{a+b} content during winter in japanese-cedar (Han et al., 2003, 2004) and sitka spruce [*Picea sitchensis* (Bong.) Carr.] (Lewandowska and Jarvis, 1977). However, no increase in C_{a+b} or alteration of C_a:C_b ratio (discussed subsequently) was observed during spring recovery of Scots pine (*Pinus sylvestris* L.) from winter stress (Ottander and Öquist, 1991).

In Winter 2007–08, chlorophyll fluorescence was measured as an estimate of photosystem II (PSII) efficiency to assess damage. However, the values collected were extremely inconsistent (data not shown) and measurements were discontinued. Previous studies have successfully measured chlorophyll fluorescence (Han et al., 2003, 2004; Han and Mukai, 1999); however, the data we collected were not useful in evaluation of efficiency or damage to PSII. Therefore, we observed the relationship between C_a:C_b in an attempt to estimate the status of the photosynthetic apparatus. The ratio of C_a:C_b was lower in the winter than the summer (Table 4), which agrees with Han and Mukai (1999) who observed a slight decrease in the C_a:C_b ratio of japanese-cedar during the period in which chlorophyll was being lost (early winter). This also agrees with the findings of Wolf (1956) who observed this phenomenon in 25 tree species, although that study was conducted solely on angiosperms. The reverse was observed in sitka spruce, which showed a slight increase in C_a:C_b ratio during winter (Lewandowska and Jarvis, 1977). A decreased ratio of C_a:C_b indicates that PSII reaction centers are affected more than light-harvesting complexes, because C_b

is found only in the light-harvesting complexes and C_a is part of the PSII reaction center (Hoober, 1998). Furthermore, characterization of the subunits of PSII reaction center has identified the D1 subunit's involvement in photoinhibition (He and Malkin, 1998) and Sandmann and Scheer (1998) reported that pheophytin *a* (demetalated C_a) may be used as a marker for PSII.

Differences were observed between seasons for C_{x+c} (Table 4). Robakowski (2005) observed a reduction in total carotenoid content in norway spruce [*Picea abies* (L.) Karst.] and mugo pine (*Pinus mugo* Turra) during winter but carotenoid content remained stable for silver fir (*Abies alba* Mill.). Differences were attributed to the latter species' ability to maintain high photosynthetic capacity during winter as its mechanism for preventing oxidative stress (Robakowski, 2005). We measured total carotenoid content in japanese-cedar leaves, whereas previous studies separated carotenoids (Han et al., 2003, 2004; Han and Mukai, 1999). However, the value for yellow carotenoids presented by Ida (1981) appears comparable to our values.

A positive, linear relationship was found between C_{a+b} and C_{x+c} in the current study in all seasons (Table 3). When data were pooled over 36 taxa belonging to Gymnospermae, Ida (1981) found a similar relationship. This relationship seems counterintuitive because chlorophyll decreases and carotenoids increase during winter (Han et al., 2003, 2004). Oserkowsky (1932) reported data that appeared to fit a positive linear relationship between yellow pigments (xanthophyll) and chlorophyll of hardy pear (*Pyrus communis* L.). That study cites other research (Sjöberg, 1931; von Euler and Hellström, 1929) that reported a simultaneous increase in green and yellow pigments in etiolated seedlings. The physiological basis for the concomitant increase in chlorophyll and carotenoids has been established as a means to prevent photooxidative damage resulting from ROS (Lambers et al., 1998). Excess energy is present, particularly during winter, because light capture and energy transfer occur more rapidly than the downstream biochemical reactions. However, carotenoids accumulate during winter and prevent damage by dissipating this excess energy as heat (Lambers et al., 1998).

There was a statistically significant relationship between both C_{a+b} and C_{x+c} content with visual rating. The hypothesis at the beginning of the experiment was that C_{a+b} content may be used as a predictor of eventual field performance; the higher C_{a+b} content, the more green the plants would remain in winter. We failed to reject this hypothesis. Previous studies have observed linear (Marquard and Tipton, 1987; Yadava, 1986) and quadratic (Netto et al., 2005) relationships between SPAD readings [SPAD-501 (Marquard and Tipton, 1987; Yadava, 1986); SPAD-502 (Netto et al., 2005)] and total chlorophyll content determined spectrophotometrically. In our study, C_{x+c} also had a predictive relationship with observed greenness. This may be explained by the simultaneous increase in

Table 2. Mean total chlorophyll [$C_{(a+b)}$], ratio of chlorophyll *a* (C_a):chlorophyll *b* (C_b), total carotenoid [$C_{(x+c)}$], and visual color rating (Rating) of 12 replicates comprised of the same number of different taxa of *Cryptomeria japonica* evaluated at the University of Georgia Tifton Campus[z] during winters of 2007–08 and 2008–09 and summers of 2008 and 2009.

Season	C_{a+b}[y]	C_a:C_b[x]	C_{x+c}[w]	C_{a+b}:C_{x+c}	Rating[v]
Winter 2007–08	2.82	2.57	0.85	3.28	2.98
Summer 2008	3.98	2.78	0.83	4.83	3.23
Winter 2008–09	2.30	2.59	0.7	3.24	2.56
Summer 2009	4.8	2.81	0.91	5.28	3.60

[z]U.S. Department of Agriculture Zone 8b, lat. 31°49′ N, long. 83°53′ W.
[y]Mean C_{a+b} in mg·g^{-1} dry weight.
[x]Mean ratio of C_a:C_b.
[w]Mean C_{x+c} in mg·g^{-1} dry weight.
[v]Visual color rating based on color with 1 = yellow or brown and 5 = green.

Table 3. Correlation between total chlorophyll (C_{a+b}), ratio of chlorophyll *a*:chlorophyll *b* (C_a:C_b), total carotenoids (C_{x+c}), ratio of C_{a+b}:C_{x+c}, and visual color rating (Rating) of 12 replicates comprised of the same number of different taxa of *Cryptomeria japonica* evaluated at the University of Georgia Tifton Campus[z] during winters of 2007–08 and 2008–09 and summers of 2008 and 2009.

	C_{a+b}	C_a:C_b	C_{x+c}	C_{a+b}:C_{x+c}	Rating
C_{a+b}	1.00	0.048	0.805***	0.840***	0.535***
C_a:C_b		1.00	−0.259	0.304*	0.165
C_{x+c}			1.00	0.382**	0.375**
C_{a+b}:C_{x+c}				1.00	0.520**
Rating					1.00

[z]U.S. Department of Agriculture Zone 8b, lat. 31°49′ N, long. 83°53′ W.
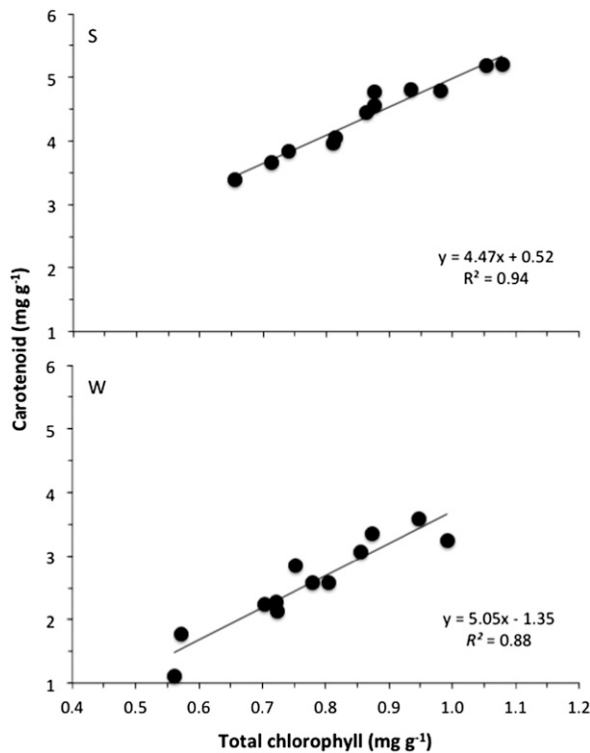*, **, *** indicate significance at $P \leq 0.05$, $P \leq 0.01$, and $P < 0.0001$, respectively.

Fig. 1. Linear regression of total chlorophyll content over total carotenoid content in summer (S) or winter (W), pooled across 2 years, 12 replicates comprised of the same number of different taxa of *Cryptomeria japonica* evaluated at the University of Georgia Tifton Campus (U.S. Department of Agriculture Zone 8b, lat. 31°49′ N, long. 83°53′ W). The values for S and W are means of the 2 years.

Table 4. A mixed effects analysis of variance[z] (ANOVA) table to evaluate seasonal differences, summer minus winter, of total chlorophyll (C_{a+b}), ratio of chlorophyll *a*:chlorophyll *b* (C_a:C_b), total carotenoids (C_{x+c}), ratio of C_{a+b}:C_{x+c}, and visual color rating (Rating) of 12 replicates comprised of the same number of different taxa of *Cryptomeria japonica* evaluated at the University of Georgia Tifton Campus[y] pooled across years 2007–09 (winters) and 2008–09 (summers).

Covariate	Estimate	SE	P
C_{a+b}[x]	1.8260	0.2078	<0.0001
C_a:C_b	0.2144	0.04233	<0.0001
C_{x+c}[w]	0.09274	0.03953	0.0248
$C_{(a+b)}$:$C_{(x+c)}$	1.7999	0.1158	<0.0001
Rating	0.6167	0.1449	<0.0001

[z]$Pigment_{ijk} = \mu + tree_i + season_j + E_{ijk}, tree_i \sim N(0, \sigma_t), E_{ijk} \sim N(0, \sigma_E)$
[y]U.S. Department of Agriculture Zone 8b, lat. 31°49′ N, long. 83°53′ W.
[x]Mean C_{a+b} in mg·g^{-1} dry weight.
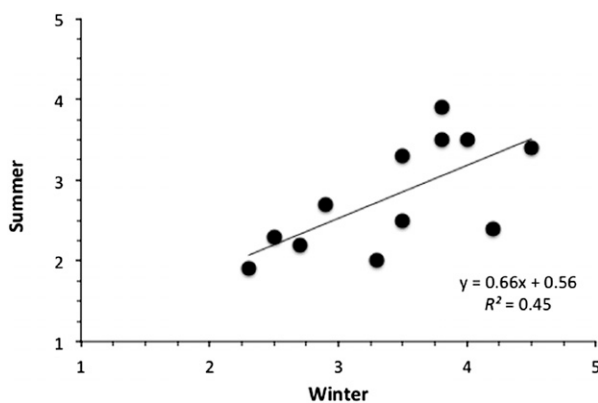[w]Mean C_{x+c} in mg·g^{-1} dry weight.

Fig. 2. Linear relationship of summer vs. winter greenness determined by visual color rating [1 (very brown/yellow; off color) to 5 (very green)] for *Cryptomeria japonica* evaluated at the University of Georgia Tifton Campus (U.S. Department of Agriculture Zone 8b, lat. 31°49′ N, long. 83°53′ W). Data points for 12 individuals are means over both years of the study.

carotenoids and chlorophyll discussed previously, which is supported by the fact that we also observed a positive relationship between the ratio of C_{a+b} to C_{x+c} with greenness. This correlation may be used to predict winter foliage color of japanese-cedar; however, it is unclear if measurement of seedlings is a strong predictor of eventual performance of mature trees.

There was a substantial difference in rainfall between the 2 years of the study. From Nov. 2007 to Sept. 2008, the evaluation plot received 112 cm of precipitation, whereas from Oct. 2008 to Aug. 2009, there was 143 cm of rain. Supplemental irrigation was only used for short periods during new plant establishment; therefore, the 30-cm difference in rainfall between the 2 years likely had a confounding effect on the study. Most of the native range of japanese-cedar receives between 120 cm and 180 cm of rainfall; however, the optimum growing conditions appear to be the north-facing slopes of Japan that receive between 180 and 300 cm of precipitation. Our Tifton, GA, site has a south-facing slope and receives ≈120 cm, the low end reported to sustain japanese-cedar. Furthermore, the field soil [Tifton loamy sand (fine-loamy, siliceous, thermic Plinthic Paleudult)] has low water-holding capacity, further limiting the available water. Other studies also have reported that drought stress was a major limiting factor in the survival of plants from northern Japan (Widrlechner et al., 1998). There was a 17% lower level of C_{a+b} during the first year of the study under reduced precipitation (Table 2). This effect is lower than but consistent with the trend observed by Guerfel et al. (2009) for two olive cultivars that showed an average of 36% reduction in chlorophyll contents under drought stress. It is possible that we would have observed a similar reduction under comparable drought stress, but we did not quantify leaf water potential or other metrics to precisely measure the level of stress. Anecdotal evidence is also provided by growers in the southeastern United States who have reported that increased irrigation on japanese-cedar before, and during, winter maintains greener foliage than when the substrate/soil is allowed to dry in both containerized and field-grown plants (personal communication).

The current research was conducted to evaluate several biochemical properties of japanese-cedar grown in USDA Zone 8 and determine if there was a relationship with winter color. We sought to identify a single biochemical property such as chlorophyll content that would allow early screening of seedlings to predict winter browning of foliage or lack thereof. We determined chlorophyll, carotenoids, and their ratio can be used to explain greenness in mature japanese-cedar taxa. As a result of the importance of drought stress in landscape performance of japanese-cedar, different results may be obtained if consistent supplemental irrigation is used such that water is not limiting. Also, performance of a number of cultivars that were included as replicates in the current

study is altered when grown in cooler climates that are more similar to the native range of the species (personal observation). Japanese-cedar has a native range that includes Akita Prefecture, Japan, which has a mean yearly temperature, mean yearly high temperature, and mean yearly low temperature of 11.1, 15.1, and 7.4 °C compared with 18.6, 24.4, and 12.6 °C for Tifton, GA. The increased temperature coupled with the relatively low rainfall of Tifton, GA, and other USDA Zone 8 sites often results in poor performance of cultivars. Some cultivars that languished in the current study have performed much better in cooler climates (USDA Zone 6 to 7; personal observation), but all selections exhibit increased winter browning in cooler climates. Even in areas as near as Athens, GA (USDA Zone 8a), 10-year-old specimens of 'Yoshino' planted on the University of Georgia campus exhibit a marked increase in winter browning compared with comparable size plants in Tifton, GA (personal observation). The increase in winter browning likely is the result of the lower temperatures being more limiting to enzymatic reactions, causing the plant to rely more heavily on the xanthophyll cycle to dissipate excess energy to prevent oxidative damage.

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