Bark Anthocyanin Levels Differ with Location in Cultivars of Red Maple

Jeff L. Sibley¹ and John M. Ruter²

Department of Horticulture, University of Georgia, Coastal Plain Experiment Station, Tifton, GA 31793

D. Joseph Eakes²

Department of Horticulture, Auburn University, AL 36849

Additional index words. *Acer rubrum*, *Acer ×freemanii*, container production, climate, ornamental tree production, environmental conditions, plant pigments

Abstract. The objective of this study was to determine differences in the bulk anthocyanin content of bark tissue of container-grown red maple (*Acer rubrum* L. and *Acer ×freemanii* E. Murray) at two Georgia locations with different environmental conditions. Rooted cuttings and tissue-cultured plantlets of eight cultivars were grown in either Blairsville or Tifton, Ga. [U.S. Dept. of Agriculture (USDA) Hardiness Zones 6b and 8a; American Horticultural Society (AHS) Heat Zones 5 and 8, respectively], from June 1995 until Dec. 1996. Bark tissue from twigs of trees grown in Blairsville was visually redder and contained more total anthocyanin than did that of trees grown in Tifton. Levels of total anthocyanins were higher ($P = 0.0007$) at Blairsville ($0.087 \text{ mg} \cdot \text{g}^{-1}$, $N = 48$) than at Tifton ($0.068 \text{ mg} \cdot \text{g}^{-1}$, $N = 47$). At both locations the levels were highest in 'Landsburg' ('Firedance'TM), followed by 'Franksred' ('Red Sunset'TM) and 'October Glory'. This is the first report to quantify anthocyanin differences in bark tissue of container-grown trees. Cooler nights in Blairsville might have contributed to increased coloration by reducing respiratory losses, thus leaving more carbohydrates available for pigment production.

Anthocyanins, water-soluble polyphenolic substances composed of glycosides of anthocyanidins (Jackman et al., 1987), are responsible for the blue, purple, violet, magenta, and red pigmentation of most plants. Notable exceptions are tomatoes (*Lycopersicon esculentum* Mill.) and beets (*Beta vulgaris* L.), which owe their red-orange and red-purple coloration to the pigments lycopene and betanin, respectively. As many as 247 different anthocyanin pigments have been identified (Jackman et al., 1987). Pigmentation is rarely due to a single anthocyanin, but rather to a mixture. As examples, 21 different anthocyanins occur in wine prepared from one variety of grape (*Vitis vinifera* L.) (Zamorani and Pifferi, 1966) and 15 different anthocyanins have been found in lowbush blueberry (*Vaccinium angustifolium* Ait.) (Francis et al., 1966).

National and regional surveys indicate that red maple is one of the most frequently planted landscape trees (Kielbaso, 1990; U.S. Dept. of Commerce, 1988). About 55 distinct cultivars of red maple are available in the nursery industry, but we have found no references quantifying

anthocyanin levels in bark tissue of container-grown trees. However, an earlier report from this study (Sibley et al., 1997) described differences in coloration in bark tissue of 'October Glory' attributed to anthocyanin levels. Deal et al. (1990) reported that the anthocyanin level in dwarf Japanese maple (*Acer palmatum* Thunb.) foliage was greater under the cool night temperatures common at higher elevations. Red- and purple-leaved seedlings of 'Bloodgood' Japanese maple, selected for superior color and growth under northern climatic conditions, exhibited progressive color loss and reduced growth rates when exposed to hotter southern climates, especially those of the southeastern United States. Many maples maintain dark foliage color and grow well in certain geographic areas of the western United States, even though maximum temperatures are comparable to or greater than those in much of the southern United States. However, local climatic factors and high elevations in the western areas cause rapid radiational cooling at night, producing night temperatures typical of more northern climates. Exposure to supraoptimal night temperatures may reduce photosynthetic efficiency, whereas cool night temperatures reduce dark respiration losses, favor a higher leaf sugar content, and increase synthesis and retention of anthocyanins (Deal et al., 1989; Goodwin, 1976).

Materials and Methods

Plant material. In Apr. 1995, eight red maple cultivars (six *Acer rubrum* and two *Acer ×freemanii*) (Table 1) were obtained as rooted cuttings or tissue-cultured plantlets (height = 5–25 cm) from four nurseries (A.

McGill and Son Nurseries, Fairview, Ore.; Bailey Nurseries, St. Paul, Minn.; Grassland Nursery, Muscle Shoals, Ala.; and J. Frank Schmidt and Sons, Troutdale, Ore.). All trees were transplanted on the same day in Auburn, Ala., to 9.1-L containers in a 6 pine bark : 1 sand (by volume) substrate amended with $8.3 \text{ kg} \cdot \text{m}^{-3}$ of 17N–3P–10K slow-release fertilizer (Osmocote 17–7–12; O.M. Scotts Co., Maryville, Ohio), $0.9 \text{ kg} \cdot \text{m}^{-3}$ minor element mix (Micromax; O.M. Scotts Co.), and $3.0 \text{ kg} \cdot \text{m}^{-3}$ dolomitic lime in May 1995. Trees were grown in full sun under standard nursery practices for 1 month in Auburn and then transported to Blairsville, Ga. ($34^{\circ}53' \text{N} \times 83^{\circ}58' \text{W}$, elevation 577 m), and Tifton, Ga. ($31^{\circ}27' \text{N} \times 83^{\circ}31' \text{W}$, elevation 109 m), in the second week of June 1995. Blairsville and Tifton are in American Horticultural Society (AHS) Heat Zones 5 and 8, and U.S. Dept. of Agriculture (USDA) Hardiness Zones 6b and 8a, respectively (AHS, 1997; USDA, 1990). Trees were arranged at each location on beds covered with landscape fabric as a split plot in a randomized complete-block design with locations considered blocks. Cultivars were the main plot with replications considered subplots. There were six replications within each location with three plants of each cultivar placed pot to pot. To provide adequate tissue, samples were collected from each of the three plants per cultivar for each replication and combined into one unit for analysis. All trees were pruned to a central leader the last week of July 1995. The trees were irrigated daily with 1.25 cm of water. Trees were grown for 6 months until dormant (the second week of Dec. 1995). All trees were transplanted to 38-L containers into substrate amended as previously described. Mean monthly air temperatures and rainfall for each location in 1995–96 were tabulated (Table 2). All trees were transported to a single location for harvest at the end of Dec. 1996.

Table 1. Main effects of location and cultivar on anthocyanin levels [$\text{mg} \cdot \text{kg}^{-1}$ fresh weight (FW)] in bark tissue of red maple cultivars following two growing seasons.

		Anthocyanin ($\text{mg} \cdot \text{kg}^{-1}$ FW)
Location		
Tifton		68 b
Blairsville		87 a ^z
Cultivar		
Landsburg (Firedance TM)		124 a ^y
Franksred (Red Sunset TM)		91 b
October Glory		88 b
Fairview Flame		73 bc
Celzam (Celebration TM)		66 bc
Autumn Flame		65 bc
Armstrong		64 bc
Olson (Northfire TM)		56 c
Source	df	Significance
Location (L)	1	0.0007
Cultivar (C)	7	0.0001
L × C	7	0.1907
Replication (R)	5	0.1568
C × R	35	0.4583

^zMean separation by LSD ($n = 48$) at $P = 0.05$.

^yMean separation by Waller–Duncan K ratio t tests ($n = 12$) at $P = 0.05$.

Received for publication on 23 Mar 1998. Accepted for publication 27 July 1998. Mention of a trademark or proprietary product does not imply endorsement of the products named or criticism of other products not named that may also be suitable. Appreciation is expressed to the Lerio Corporation for supplying the containers used in this study. The cost of publishing this paper was defrayed in part by the payment of page charges. Under postal regulations, this paper therefore must be hereby marked advertisement solely to indicate this fact.

¹Graduate Research Assistant.

²Associate Professor.

Table 2. Mean monthly maximum ($T_{\max}$) and minimum ($T_{\min}$) temperatures, monthly rainfall totals, and length of growing season by location, June 1995–Dec. 1996.

Month	Blairsville, Ga.			Tifton, Ga.		
	Temp (°C)		Rainfall (cm)	Temp (°C)		Rainfall (cm)
	Max	Min		Max	Min	
1995						
June	25.7	13.0	18.3	30.1	19.2	9.4
July	30.2	16.7	12.0	33.2	21.7	9.4
August	29.7	18.8	18.5	32.6	22.0	5.6
September	25.2	14.1	6.5	30.2	18.1	1.6
October	21.2	6.6	22.4	27.2	14.1	4.0
November	13.1	0.4	13.4	18.7	5.0	6.3
December	9.2	−2.4	6.9	15.1	2.7	3.8
Mean	22.1	9.6		26.7	14.7	
Total			98.0			40.1
1996						
January	5.5	−5.4	25.9	15.9	2.2	7.9
February	8.7	−4.6	10.5	17.8	3.9	7.3
March	11.7	−1.9	18.0	17.9	6.0	19.4
April	18.9	2.7	12.4	23.6	10.1	9.7
May	25.1	11.1	12.9	30.0	17.4	2.9
June	27.1	14.8	14.6	31.2	19.7	5.2
July	28.4	16.7	9.2	33.3	21.9	2.5
August	28.1	16.3	9.8	31.6	20.6	15.5
September	24.4	12.7	15.4	29.6	18.3	10.1
October	20.4	6.1	2.8	24.7	11.9	7.1
November	12.1	0.6	14.4	19.4	6.7	3.3
December	11.4	−1.0	16.0	17.4	4.8	9.5
Mean	18.5	5.7		24.4	12.0	
Total			161.8			100.4

Extraction and measurement. Procedures outlined by Lees and Francis (1972), Lister et al. (1996), and Torre and Barritt (1977) were modified slightly to determine total bulk anthocyanin content in bark tissue of twigs. Terminal stem segments, 15 cm in length, were collected arbitrarily from each replication, cultivar, and location and stored immediately in sealed plastic bags at -70°C until analysis. About 300 mg fresh weight of the outermost layer (periderm) of bark was mixed with liquid nitrogen and ground in a mortar and pestle. Four mL of methanol : acetic acid (85:15) extracting solvent was used to transfer the macerate solution to a 30-mL glass tube. The tube was then covered with aluminum foil and stored overnight at 4°C . Samples were then filtered through 42.5-mm #1 Whatman filter paper in a medium-fritted Buchner funnel into a vacuum-fitted 125-mL side-arm filtering flask. The filtrate was quantitatively transferred to a 10-mL volumetric flask, and the final volume was brought to 10 mL with extracting solvent. The extract was stored in the dark for 2 h at room temperature, and the pH measured to ensure that it was 1.0 ± 0.1 . Absorbance readings were taken using a scanning spectrophotometer (Beckman DU650 UV/VIS, Irvine, Calif.) at 535 nm for total bulk anthocyanin content (Harborne, 1967), using 1-cm cuvettes and deionized water as a blank. The total anthocyanin content of the samples ($\text{mg}\cdot\text{g}^{-1}$ tissue) was calculated using appropriate weights, volume, and dilution factor and the average E value for anthocyanin as follows:

$$\begin{aligned} \text{Total anthocyanin (mg}\cdot\text{g}^{-1}) &= \text{Absorbance} \times \text{dilution factor} \\ &\quad \times 10/\text{Avg } E_{535}^{1\%} \\ &= \text{Absorbance} \times \text{dilution factor}/98.2. \end{aligned}$$

Statistical analysis. All data were subjected to analysis of variance using SAS version 6.04 (SAS Institute, 1988). Mean separations were by Waller–Duncan K ratio t tests and least significant differences (LSD). Differences were considered significant at $P = 0.05$.

Results and Discussion

At harvest, bark tissue was redder and anthocyanin content was greater ($P = 0.0007$) at Blairsville than at Tifton (Table 1). This is the first report to quantify anthocyanin differences in bark tissue of container-grown trees. Anthocyanins are known to be unstable when subjected to heat (Jackman et al., 1987); therefore, cooler nights in Blairsville might have contributed to this pigment accumulation by reducing respiration losses and leaving more carbohydrates available for pigment production (Penning de Vries, 1975). Greatest anthocyanin levels were found in ‘Landsburg’, followed by ‘Franksred’ (‘Red Sunset’TM) and ‘October Glory’ (Table 1). The introduction of ‘Landsburg’ (‘Firedance’TM) was based on notable red fall foliage color and burgundy to purple stems throughout the year in northern Minnesota. In another study, Sibley et al. (1995) noted outstanding fall color for ‘October Glory’ and ‘Franksred’, but no studies have quantified their bark color relative to other cultivars. Furthermore, the low level of anthocyanin found in twig bark of ‘Olson’ (Table 1) was expected, based on a lack of visual red coloration at both locations.

Growth response across cultivars in Blairsville and Tifton was similar, with the exception of ‘Landsburg’ (Sibley et al., 1998a). Stem diameter increase was the same at Blairsville and Tifton for all cultivars with the

exception of ‘Celzam’ and ‘Landsburg’ (Sibley et al., 1998a) (data not shown). No differences were found between locations in dry weights for tissues of each cultivar collected from the late July 1995 pruning (data not shown). Rainfall events differed little between the two locations during the two growing seasons with 122 rainfall events at Blairsville compared with 134 at Tifton; however, total rainfall was greater in Blairsville than Tifton in both years (Table 2). Tifton, with 247 and 282 frostfree days in 1995 and 1996, respectively, had a much longer growing season each year than did Blairsville with 171 and 164 frostfree days in 1995 and 1996, respectively.

Much can be gained from this study regarding cultivar performance under dissimilar environmental conditions. Three cultivars, ‘Celzam’ (‘Celebration’TM), ‘Landsburg’ (‘Firedance’TM), and ‘Olson’ (‘Northfire’TM) are new introductions and have only recently (Sibley et al., 1999) been included in controlled studies. ‘Olson’ and ‘Celzam’ appear to be well adapted to container production in the growing conditions of the Southeast. However, ‘Landsburg’ did not grow as well at Tifton as at Blairsville, even though red coloration was still greater than in other cultivars. Fuentes-Granados and Widrechner (1997) found that the inheritance of anthocyanin production was controlled by a single dominant gene in *Agastache rugosa* (Fisher & Meyer) Kuntze. Future work with red maples should consider the genetic control of anthocyanin production, in particular the heritability of anthocyanin in cultivars such as ‘Landsburg’.

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