Purple-leaved Japanese Barberry (var. *atropurpurea*) Genotypes Become Visually Indistinguishable from Green-leaved Genotypes (*Berberis thunbergii* DC.) at Low Light Levels[1]

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Abstract

Japanese barberry (*Berberis thunbergii* DC.) is a popular nursery plant that has escaped from cultivation to establish invasive populations across the United States. Previous studies demonstrate that green-leaved feral plants adapt successfully to low light levels. The leaf color phenotype of purple-leaved Japanese barberry (*Berberis thunbergii* var. *atropurpurea*) seedlings grown under shaded conditions was studied to determine their appearance under low light conditions typical of wooded areas often invaded by barberry. Seedlings from *B. thunbergii* var. *atropurpurea* were grown in full sunlight and under three shade treatments (36, 50 and 72%). Seedlings were also grown in full sunlight and under two higher shade treatments (85 and 95%) in a second study. The hue angle of purple seedlings increased from a mean of 22.3 in full sun to 86.9 at 72% shade, reflecting a color change from violet-purple to green-umber. Foliage color also became more vivid and lighter as shading increased. All purple genotype seedlings appeared green when grown at 95% shade due to lack of anthocyanin production at this light exposure. Escaped barberry seedlings originating from landscape plants of var. *atropurpurea* may not be recognized as purple-leaved forms when they invade densely shaded woodland environments.

Index words: invasive plants, horticultural varieties, seedlings, shading, anthocyanins.

Significance to the Nursery Industry

The invasive plant issue creates difficulties for the U.S. nursery industry. The sale of valuable horticultural plants with purported invasive potential is threatened in some states by legislative action. This report details our efforts to determine the appearance of seedlings derived from purple-leaf Japanese barberry under various lighting regimes, including levels that approximate forest settings colonized by feral barberry. The culpability of purple-leaved Japanese barberry cultivars, which are an important commercial crop, in the production of feral seedlings is the center of unresolved debate among various constituencies with a stake in the invasive plant debate. Much of this uncertainty may be attributed to the variable leaf color of seedling progeny as influenced by genotype and phenotypic expression mediated by lighting exposure.

Our research revealed that leaf color phenotype is strongly influenced by UV-light exposure. Anthocyanin accumulation by purple seedling genotypes decreased to produce a leaf color shift from purple to green as shade levels increased. At very high shade levels the leaf color of purple seedling genotypes could not be differentiated from green seedlings. Visual leaf color alone, therefore, is an unreliable predictor of barberry seedling origin since purple seedlings may be unrecognized in heavily shaded settings.

Introduction

Japanese barberry (*Berberis thunbergii* DC.) has been grown in the United States as an ornamental landscape shrub since its 1875 horticultural introduction in Boston (13). The species soon escaped from cultivation to establish feral populations in unmanaged habitats throughout New England and adjacent areas (15). Japanese barberry is now naturalized in more than 30 central and eastern states (17) where it threatens native plant communities, impacts soil chemistry, changes microbial communities and earthworm populations and interferes with human activities (4, 6, 7, 15). Greenhouse and field studies of feral, wild-type Japanese barberry have demonstrated that both seedlings and established plants can withstand low light levels (1–3% full sun exposure) (15).

Japanese barberry remains an important ornamental plant with an annual crop value of $5 million in Connecticut (5) and large market share elsewhere throughout the United States (16). The commercial barberry crop consists primarily of *B. t.* var. *atropurpurea* — a variant characterized by purple foliage — from which cultivars have been selected that develop dwarf, compact or fastigiate growth habits (3). These cultivated forms differ greatly from the large-growing, green-leaf plants that comprise naturalized Japanese barberry populations (13).

The invasive risk posed by Japanese barberry cultivars has been explored at the New England Invasive Plant Center (http://www.invasivecenter.uconn.edu), based at the University of Connecticut in Storrs, CT, through analysis of their reproductive potential and offspring traits (8, 9). One finding is that *B. t.* var. *atropurpurea* produces both green and purple seedlings whose relative percentages are influenced by parental proximity to purple pollen donors (9). The investigation described herein extends the analysis by studying the impact of light availability on the phenotypic expression of foliage color by these green and purple offspring. Since feral barberry plants in shaded woodland areas present a green-leaf phenotype, observers may conclude that they have not originated from purple-leaved horticultural genotypes. This apparent contradiction has been the source of confusion and controversy among those debating the future of commercial

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barberry sales. Our findings suggest that the absence of purple barberry foliage within feral populations should not necessarily be interpreted as an absence of purple-leaved genotypes.

Materials and Methods

Seeds were collected from cultivated *B. thunbergii* var. *atropurpurea* plants of known provenance and seedlings were raised in a greenhouse in 2002 and 2003. One-year-old, dormant seedlings were placed in either full sunlight or under three shade treatments (36, 50 and 72%) outdoors at the University of Connecticut in Storrs on March 15 in 2003 and 2004 as buds swelled. A single set of experimental groups for both replications in time was maintained using frames constructed of 2.54 cm (1 in) chlorinated polyvinyl chloride (CPVC) pipe covered with black woven shade cloth (DeWitt Co., Sikeston, MO). Cloth transmittance (64%T, 50%T and 28%T) was calculated relative to full sun on a clear day in mid-June using a LI-191 Line Quantum Sensor connected to a LI-189 Quantum Meter (LI-COR, Inc., Lincoln, NE). The control group exposed to full sunlight was maintained adjacent to the shaded treatments.

Barberry plants were maintained in 1040 ml black plastic square pots (Belden Plastics, Roseville, MN) containing Metro Mix 510 Growing Medium (Scotts, Co., Marysville, OH). Plants were hand-weeded, irrigated regularly and provided a soluble 20N-8.74P-16.6K fertilizer (Peters 20-10-20 Fertilizer, Scotts Co.) at 150 ppm N every two weeks during the study.

Plants were allowed to leaf out under full sunlight and shade treatment conditions and were then evaluated for foliage color. Five leaves were randomly chosen from each plant on June 1 to be color analyzed using a CR-400 Chroma Meter connected to a DP-400 data processor (Konica Minolta Sensing Americas, Inc., Ramsey, NJ). Leaf color (L* a* b*) values were measured at the broad part of the ovate barberry leaves. The colorimeter was calibrated at illuminant C with a white standard. Because of the slightly translucent nature of barberry leaf tissue, a standard white background was placed behind each leaf when a CIELAB measurement was taken (10). In addition to the CIELAB coordinated value (L*), the coordinates of hue angle ($\tan^{-1}(b^* / a^*)$) and chroma ($a^{*2} + b^{*2}$) were calculated from a* and b*.

Data from the first year of study in 2003 revealed expression of purple leaf phenotype at the highest shade level (72%). A separate study was initiated in 2005 to further explore the expression of purple-leaf phenotype in Japanese barberry at shade levels approximating the *in situ* extremes (< 20%T) noted by Silander and Klepeis (15). Dormant, one-year-old *B. thunbergii* var. *atropurpurea* seedlings were placed under either full sunlight or two shade levels (85 and 95%) to yield 100%, 15%T and 5%T. Plants and plant management proceeded as described previously and foliage color measurements were conducted as described for the first study.

To determine the foliage color genotype of experimental plants, all were moved from shaded treatments to full sun exposure following color measurement. After 4 weeks of exposure to full sunlight, anthocyanin development allowed for genotype categorization of plants as either green or purple. This step was necessary due to the poor expression of purple phenotype in higher shade treatments.

The study using 36, 50 and 72% shade was repeated twice in time in 2003 and 2004 and data were combined for statistical analysis resulting in 180 total plants per shade treatment. The study using 85 and 95% shade was conducted once in 2005 with 45 plants per shade treatment. Experimentation used a nested, completely random design. *B. thunbergii* var. *atropurpurea* seedling populations include both green and purple foliage plants. Since plants were dormant when assigned to shade treatments, purple and green genotype identity was initially unknown and both color classes were therefore distributed randomly amongst shade treatments and were also placed randomly within shade treatments. Paired t-tests ($P \leq 0.05$) were used to compare means between purple and green genotype plants and were completed using SAS for Windows Version 8.0 (SAS Institute, Cary, NC).

Results and Discussion

Shading influenced the leaf color of purple genotype seedlings. Mean hue angle increased with increasing shade intensities (e.g. from 22.3 in full sun to 86.9 under 72% shade, Table 1) for purple genotype seedlings, but remained unchanged for green genotype seedlings. Purple seedlings were violet-purple in full sun and were greenish umber (less violet-purple) as shading increased.

L* (lightness) and chroma of purple genotype foliage also increased as light levels decreased due to higher shading (Table 1). Chroma values for purple genotype seedlings grown in 72% shade were twice that of seedlings grown in full sun, whereas values for green genotype seedlings remained unchanged. Increase in L* values for purple genotypes, in response to increased shading, was not as dramatic as changes observed for hue angle and chroma. Findings at 72% shade indicate lighter and more vivid foliage color that are consistent with a hue angle shift from purple to green shades, which have higher chroma and L* characters. Color values for chroma, hue angle and L* for purple and green

Table 1. Comparison of CIELAB leaf color parameters for combined green- and purple-leaved seedling progeny derived from *Berberis thunbergii* var. *atropurpurea* grown under light levels received from 0 to 72% shading.

	Leaf color class		
Shade (%)	**Green (n)**	**Purple (n)**	**Significance[z]**
	Chroma		
0	29.2 (66)	9.5 (114)	*
36	32.8 (82)	12.2 (97)	*
50	31.8 (67)	12.8 (113)	*
72	29.6 (74)	18.2 (102)	*
	Hue angle		
0	128.8	22.3	*
36	124.8	28.9	*
50	129.4	42.3	*
72	128.9	86.9	*
	L[y]*		
0	40.3	28.3	*
36	41.5	28.1	*
50	41.1	30.6	*
72	38.9	35.1	*

[z] Mean separation between green and purple genotypes by Fisher's least significant difference test.

[y] A measure of color lightness with a value range of 0 = black to 100 = white.

* indicates significance at $P \leq 0.05$.

Table 2. Comparison of CIELAB leaf color parameters for combined green- and purple-leaved seedling progeny derived from *Berberis thunbergii* var. *atropurpurea* grown under light levels received from 0 to 95% shading.

	Leaf color class		
Shade (%)	Green (n)	Purple (n)	Significance^z
	Chroma		
0	23.1 (11)	7.8 (32)	*
85	34.8 (13)	23.5 (32)	*
95	36.9 (12)	36.2 (33)	NS
	Hue angle		
0	131.2	38.7	*
85	128.2	109.2	*
95	127.4	127.4	NS
	L^{*y}		
0	35.0	28.0	*
85	40.3	37.8	*
95	43.1	42.6	NS

^{z}Mean separation between green and purple genotypes by Fisher's least significant difference test.

^{y}A measure of color lightness with a value range of 0 = black to 100 = white.

* indicates significance at $P \leq 0.05$; NS indicates not significantly different.

genotypes were more similar at 85% shade, but were still significantly different for this treatment (Table 2). At 95% shade, chroma, hue angle and L* were not significantly different for purple and green genotypes (Table 2). All plants exhibited a green phenotype for this treatment.

Alteration of hue angle in response to shading reflects a relative reduction in foliar anthocyanin presence due to reduced illumination by ultraviolet light. Cadic (1) found that the anthocyanin pigment cyanidin was solely responsible for purple leaf coloration in *B. thunbergii*. While accumulation of this pigment in leaves is influenced by many factors, UV-light exposure is a primary determinant (14) since the presence of purple coloring may protect leaf tissue from damaging sunlight levels (2).

Our findings parallel those of Oren-Shamir and Levi-Nissim (14) who found that plants of *Cotinus coggygria* Scop. 'Royal Purple' (purple-leaf common smokebush) exposed to strong light intensities accumulated high concentrations of foliar anthocyanin and appeared deeper purple. Observations under garden conditions indicate a similar response for other purple-leaf woody plant cultivars including *Physocarpus opulifolius* (L.) Maxim. 'Monlo' Diabolo™ (purple-leaf common ninebark) and *Weigela florida* (Bunge.) A. DC. 'Alexandra' Wine & Roses® (purple-leaf old-fashioned weigela) (data not shown).

Differences in color values between green and purple genotype barberry seedlings decreased as shading levels increased from full sun to 85% shade (Tables 1 and 2). At some level of shade higher than 85%, purple and green barberry genotypes become indistinguishable. Reduced foliar accumulation of cyanidin under low UV-light levels effectively masks purple genotype. Our findings suggest that phenotypic similarity between green and purple Japanese barberry genotypes can be expected within heavily shaded natural environments. This phenomenon may account for widespread reports by field biologists, conservation workers and nursery industry representatives that feral Japanese barberry seedlings with purple foliage do not exist or are rarely seen within invasive populations. While purple genotypes may not be prevalent in invasive barberry populations (11), more reliable visual detection of purple genotypes within feral groups may be possible if plants are examined in early spring since Japanese barberry develops foliage before most forest canopy species in the northeastern United States. This habit facilitates early foliar anthocyanin development which may be observed prior to heavy shading produced by full canopy foliation (12).

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