Seedling Populations Produced by Colored-leaf Genotypes of Japanese Barberry (*Berberis thunbergii* DC.) Contain Seedlings with Green Leaf Phenotype[1]

Jonathan M. Lehrer[2], Mark H. Brand[3] and Jessica D. Lubell[2]
Department of Plant Science, Unit 4067
University of Connecticut, Storrs CT 06269-4067

Abstract

The leaf color of seedling populations derived from ornamental genotypes of Japanese barberry (*Berberis thunbergii* DC.) was evaluated to determine whether nursery selections of this important landscape plant could be expected to produce green-leaf progeny or seedlings with leaf color resembling the purple-leaf or yellow-leaf parent. This is a compelling inquiry since nearly all *B. thunbergii* plants found within invasive populations possess green foliage and the potential contribution of seedlings by ornamental purple- and yellow-leaf genotypes is unknown. Seed lots collected from cultivated barberry genotypes located in landscape settings were processed and raised in a greenhouse to observe leaf color phenotype. It was found that all genotypes studied produced at least some green seedlings. The percentage of green progeny produced varied widely by genotype. Green-leaf cultivars yielded close to 100% green seedlings and all purple- and yellow-leaf forms produced at least 20% green offspring. Among purple-leaf genotype accessions located adjacent to potential purple-leaf pollen donors, var. *atropurpurea* produced significantly fewer green seedlings (18.5%) than ‘Crimson Pygmy’ (71%) and ‘Rose Glow’ (45%). ‘Rose Glow’ individuals growing adjacent to other purple Japanese barberry forms produced significantly fewer green seedlings (45%) than ‘Rose Glow’ accessions that were isolated from additional purple Japanese barberry (88%). This study demonstrates that some invasive green-leaf *B. thunbergii* could be derived from popular garden forms since purple- and yellow-leaf genotypes readily produce green-leaf offspring which resemble feral barberry. These findings do not, however, provide any definitive link between cultivated and naturalized Japanese barberry.

Index words: invasive plants, horticultural genotypes, leaf color, woody shrub.

Significance to the Nursery Industry

The invasive plant issue is a difficult dilemma facing the United States nursery industry. Many state governments across the country have already imposed or are currently considering legislation that will curtail the sale of valuable horticultural crops with purported invasive potential. This report details our efforts to determine whether colored-leaf ornamental forms of Japanese barberry produce seedlings of similar phenotype or green-leaf (herein referred to as green) progeny that are phenotypically similar to the individuals that comprise invasive populations. The potential for cultivated purple- and yellow-leaf genotypes (herein referred to as purple and yellow) to produce green ‘reversion’ seedlings that resemble feral plants has been the subject of unresolved debate among ecologists and representatives of the nursery industry for many years.

Our research demonstrated that all green, purple and yellow Japanese barberry genotypes studied produced some green seedlings. Percentages of green progeny varied by genotype and proximity to potential purple pollen donors, with the popular commercial cultivars ‘Crimson Pygmy’ and ‘Rose Glow’ yielding significantly more green seedlings than var. *atropurpurea* under conditions of comparable pollen donor proximity. Although the green seedlings produced by commercial barberry selections bear phenotypic resemblance to feral plants, these results alone establish no definitive link.

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[2]Graduate research assistant.

[3]Professor. To whom reprint requests and all correspondence should be addressed: E-mail address: mark.brand@uconn.edu

Introduction

Japanese barberry (*Berberis thunbergii* DC.) is widely recognized as an invasive shrub that has colonized disturbed terrestrial environments throughout more than 30 states across the eastern and central United States (16, 18). The species was introduced into the U.S. as a garden and landscape plant in the late 19th century near Boston and soon began spreading from cultivation into unmanaged habitats (16). Aided by the songbirds, small rodents and wild turkeys that consume its red berries and disseminate its seeds (16, 3), Japanese barberry readily yields abundant feral seedlings that may form dense thickets (6) in a wide variety of habitats (16). Comprehensive work undertaken by Ehrenfeld et al. (7) and Kourtev et al. (10, 11) demonstrated that Japanese barberry invasions perturb ecosystems to the detriment of native flora by altering various parameters of soil chemistry, microbial communities and earthworm populations. The presence of this exotic shrub may place further pressure on indigenous species by preventing the recruitment of new seedlings within invaded habitats (2).

Japanese barberry remains a popular garden plant (17) and valuable nursery commodity (9) nearly 150 years after its introduction into the United States. In Connecticut alone, the *B. thunbergii* crop is valued at more than $5 million each year (9), a figure dwarfed by the production in many larger states. The outward appearance of most garden barberry, however, has changed drastically since 1875. Over the last few decades, horticulturists in the U.S. and abroad have selected more than 40 cultivars of *B. thunbergii* characterized by marked diversions in leaf color (purple, yellow and variegated) and plant form (dwarf and fastigiate) (4). Consequently, there now exists a stark physical dichotomy between these refined, primarily purple landscape plants and their ancestor, the large-growing, green wild-type Japanese barberry that was a mainstay in gardens decades ago and comprises current invasive populations. This purple versus green discrepancy helps fuel resistance within the nursery industry to current and proposed legislation which seeks to summarily ban the sale of Japanese barberry without consideration of cultivar distinctions (8, 15). Personal communication with representatives of the nursery industry reveals widespread disbelief that the barberry cultivars they grow contribute to feral populations. These observers often emphasize that they have never witnessed any feral purple or yellow *B. thunbergii* seedlings, either on their property adjacent to barberry crops or in wooded areas, that resemble the purple and yellow ornamental genotypes they are growing. Their conclusion is that no link exists between ornamental Japanese barberry genotypes and invasive barberry populations.

Previous research exploring invasive Japanese barberry has focused primarily on the invasion biology of green wild type *B. thunbergii* and its ecological consequences (16, 7, 10, 11). Recent work by Lehrer et al. (13) and Lovinger and Anisko (14) provides the only insight available concerning the invasive potential of ornamental Japanese barberry genotypes. Baseline measurements of invasive potential were established by these studies via evaluation of parameters such as fruit and seed production, seed germination rate and seedling vigor. In this paper, we supplement these initial investigations by analyzing the leaf color of seedlings derived from ornamental Japanese barberry genotypes to determine: 1) whether seedling progeny can be expected to resemble either their parents or green barberry with regard to this phenotypic trait, and 2) whether the location of mother plants relative to likely pollen donors influences offspring color. Determining whether colored-leaf genotypes yield green seedlings that resemble feral barberry has important implications for the invasive plant debate.

Materials and Methods

Ripe berries were collected in October and November 2001, 2002 and 2003 from cultivated genotypes of Japanese barberry of verifiable identity located in botanical gardens, university campuses and home landscapes in Connecticut, Massachusetts and New York. The composite data set included 15 mother plants of var. *atropurpurea*, 10 'Aurea', 3 'Aurea Nana', 1 'Bagatelle', 4 'Bogozam', 29 'Crimson Pygmy', 4 'Erecta', 4 'Gentry', 6 'Golden Ring', 4 'Helmond Pillar', 4 'Inermis', 6 'Kobold', 2 'Lime Glow', 1 'Minor', 1 'Monlers', 21 'Rose Glow', 2 'Sparkle' and 18 wild type *B. thunbergii* from naturalized populations in Mansfield, CT.

The proximity of each mother plant to other nearby barberry genotypes was recorded and the identity of these potential pollen donors was determined to study the influence of likely outcrossing scenarios on progeny leaf color. Two proximity classes were defined. Landscape specimens located immediately adjacent to additional genotypes with the same leaf hue were considered 'adjacent.' These plants were typically growing in groupings or hedge situations where they were planted on ≤3 m (10 ft) centers. Mother plants located >91 m (300 ft) from any additional barberry genotypes with the same leaf color were designated 'isolated.' These accessions were typically single specimen plants and were more uncommon since the primary landscape use of Japanese barberry is in groupings, masses and hedges (4). Seeds were extracted from fruit lots by maceration and then stratified for 90 days in moistened, sterilized sand to facilitate germination according to the protocol described by Dirr and Heuser (5).

Seedling color for all genotypes regardless of proximity. Up to 200 stratified seeds from each accession were sown on May 1 in rows in heavy plastic flats (Kadon Corp., Dayton, OH) using Metro Mix 360 Growing Medium (Scotts Co., Marysville, OH) to determine the potential for diverse Japanese barberry genotypes to produce green progeny regardless of potential pollen donor. Seed lots derived from multiple accessions of the same genotype were randomized within different seed flats. The flats were placed in a greenhouse with set points of 21C (70F) day and 17C (63F) night and natural lighting at the University of Connecticut's Floriculture Greenhouses in Storrs, CT. They were irrigated and hand-weeded as needed. Seedlings were allowed to germinate and grow for 6 weeks after which seedling color was recorded. This study was repeated for 3 years and annual data was combined for all accessions of each genotype. Differential proximity to pollen donors for the study plants did not allow analysis of variance and mean separation to be performed for this study. The percentage of green progeny produced by each genotype accession was recorded and averages for each genotype were calculated.

Seedling color for individual purple genotypes adjacent to other purple plants. Seeds and seedlings were managed as described above, but only accessions of var. *atropurpurea*, 'Crimson Pygmy' and 'Rose Glow' located adjacent to purple potential pollen donors were considered in the data set to determine the potential for green seedling production by individual purple genotypes under conditions of optimized pollination by a purple pollen donor. Data for the 3 study years were combined by individual genotype within a completely randomized design and analysis of variance (ANOVA) and mean separation using Fisher's Least Significant Difference ($P \leq 0.05$) was performed using SAS for Windows Version 8.0 (SAS Institute, Cary, NC) and the mixed (PROC MIXED) procedure.

Seedling color for 'Rose Glow' accessions grouped according to proximity. Seeds and seedlings were managed as previously described, but only data for 'Rose Glow' were considered. Accessions were assigned to the two proximity classes defined earlier to determine the influence of likely outcrossing scenarios on offspring leaf color. 'Rose Glow' was chosen for this analysis because its common landscape application as both a specimen and massing plant allowed for commensurate representation in both proximity classes. Analysis of variance and mean separation was performed as described above for purple genotypes.

Results and Discussion

Seedling color for all genotypes regardless of proximity. All green, purple and yellow cultivated genotypes of Japanese barberry studied produced percentages of green seedlings (Table 1). Remaining progeny generated by purple genotypes were predominately purple and yellow for yellow genotypes (data not shown). All green genotypes studied produced a minimum of 90% green offspring (Table 1). Although percentages of green seedlings produced by individual purple and yellow genotypes varied widely, all forms studied produced at least 23% green progeny (Table 1). Relative com-

Table 1. Percentage of green seedlings derived from Japanese barberry (*Berberis thunbergii*) genotypes regardless of proximity to pollen donors.

Genotype	n[z]	Green-leaf progeny (%)
Green genotypes		
'Erecta'	4	99
'Inermis'	4	100
'Kobold'	6	90
'Lime Glow'	2	96
'Minor'	1	100
'Sparkle'	2	100
Wild type *B. thunbergii*	18	96
Purple genotypes		
var. *atropurpurea*	15	31
'Bagatelle'	1	48
'Crimson Pygmy'	29	74
'Gentry' (Royal Burgundy®)	4	77
'Golden Ring'	6	89
'Helmond Pillar'	4	24
'Rose Glow'	21	70
Yellow genotypes		
'Aurea'	10	61
'Aurea Nana'	3	74
'Bogozam' (Bonanza Gold®)	4	45
'Monlers' (Golden Nugget™)	1	52

[z]Number of individual plant accessions per genotype.

parisons between genotypes cannot be made due to differential proximity to pollen donors for the study plants. While these results do not prove that any feral barberry seedlings are derived from landscape plants, it is clear that all cultivated forms of Japanese barberry studied have the potential to produce green seedlings that bear phenotypic similarity to naturalized plants.

Table 2. Percentage of green-leaf seedlings derived from purple-leaf Japanese barberry (*Berberis thunbergii*) genotype accessions located adjacent to (≤3 m (10 ft)) potential purple-leaf pollen donors.

Genotype	n[z]	Green-leaf progeny (%)
'Crimson Pygmy'	22	71a[y]
'Rose Glow'	9	45b
var. *atropurpurea*	12	19c

[z]Number of individual plant accessions per genotype.

[y]Mean separation within column by Fisher's least significant difference test, $P \leq 0.05$.

Seedling color for individual purple genotypes adjacent to other purple plants. Table 2 shows that 'Crimson Pygmy' produced 71% green offspring and 'Rose Glow' yielded 45% green seedlings despite optimal conditions for pollination by purple peers. Accessions of var. *atropurpurea* located adjacent to additional purple Japanese barberry plants yielded significantly fewer green seedlings than the other genotypes at 19% (Table 2). These results suggest that popular purple Japanese barberry forms such as 'Crimson Pygmy' and 'Rose Glow' can be expected to produce substantial percentages of green seedlings even when conditions are optimal for pollination by another purple genotype. Pollination with distant green pollen facilitated by bees and/or the action of a forced outcrossing system such as that discussed below may explain these findings.

Seedling color for 'Rose Glow' accessions grouped according to proximity. Accessions of the purple genotype 'Rose Glow' located adjacent to additional purple peers produced significantly fewer green progeny (45%) than mother plants isolated from additional barberry genotypes (88%) (Table 3). Statistical difference between the two groups suggests that the proximity of 'Rose Glow' mother plants to potential purple pollen donors influences the leaf color of offspring. Proximity to purple peers is inversely related to production of green offspring. Other purple Japanese barberry cultivars, which like 'Rose Glow' are seedling selections of *B. thunbergii* var. *atropurpurea* (4), are likely to follow the same trend and produce the greatest number of green progeny when they are isolated from purple pollen donors.

The large number of green offspring produced by purple barberry genotypes situated in close proximity to potential purple pollen donors (Table 2) is curious given that expression of purple leaf phenotype in *Berberis* has been reported to be mediated by a single recessive gene that acts early in the biosynthetic pathway to control production of the pigment cyanidin (1). Under such a scenario, purple genotypes should be homozygous for the recessive allele and crossing among such plants should yield high rates of purple progeny. Self-pollination should produce a similar result. Our findings suggest that either Cadic's model (1) for purple pigment inheritance in barberry is incomplete or that a high degree of outcrossing with distant non-purple genotypes occurs for purple barberry plants even when they are growing in dense masses/hedges of purple individuals.

The potential for pollination by distant genotypes in *B. thunbergii* is feasible given that the species, while bearing perfect flowers that are self-fertile (1), has evolved a specialized mechanism to facilitate cross-pollination mediated by bees (12). The stamens of *B. thunbergii* flowers are pressed to the outside of the corolla and bear a trip mechanism

Table 3. Percentage of green-leaf seedlings produced by the purple-leaf Japanese barberry (*Berberis thunbergii*) genotype 'Rose Glow' as influenced by proximity to potential purple-leaf pollen donors.

Proximity scenario	n[z]	Green-leaf progeny (%)
Isolated from purple-leaf potential pollen donors[y]	5	88a[x]
Adjacent to purple-leaf potential pollen donors[w]	9	45b

[z]Number of 'Rose Glow' accessions included in each proximity scenario group.

[y]Mother plants located >91 m (300 ft) from any additional purple genotypes.

[x]Mean separation within column by Fisher's least significant difference test, $P \leq 0.05$.

[w]Mother plants located in masses/hedges ≤3 m (10 ft) from additional purple genotypes.

whereby stimulation by visiting insects causes the anther filaments to snap inward and deposit pollen on the vector (12). Despite the close proximity of purple pollen donors to purple genotypes, production of green offspring may be favored by the ease with which bees may transport green pollen to cultivated plants from feral green barberry plants located relatively far away. Japanese barberry may also possess an undocumented forced outcrossing pollination mechanism that favors crosses with dissimilar genotypes over self-pollination in situations where both options are viable.

It is clear that all the ornamental cultivated genotypes of *B. thunbergii* studied maintain the potential to produce some green offspring. Green cultivars can be expected to produce more green seedlings than colored-leaf cultivars. The percentage of green offspring produced by purple barberry genotypes differs by cultivar and is dependent upon proximity to purple pollen donors. Green seedlings produced by cultivated green, purple and yellow Japanese barberry genotypes are visually indistinguishable from plants found within invasive populations. Despite this phenotypic similarity, care should be exercised when interpreting and applying the results of this study since they alone do not establish a definitive link between cultivated Japanese barberry genotypes and invasions in natural areas.

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