Seed Production Levels and Seedling Traits of *Berberis thunbergii* Cultivars©

Jonathan M. Lehrer and Mark H. Brand
Department of Plant Science, University of Connecticut, 1376 Storrs Rd. U-67, Storrs, Connecticut 06269-4067 U.S.A.

***Berberis thunbergii* (Japanese barberry) is both a widespread invasive plant and an important horticultural crop in the form of various ornamental cultivars. Little research has previously been dedicated to evaluating the invasive potential of current commercial cultivars and/or developing new sterile forms that lack potential invasiveness. A research program initiated at the University of Connecticut combines both of these elements.**

Initial results indicate that *B. thunbergii* 'Atropurpurea' and 'Rose Glow' demonstrate gross seed production and levels of seed production per canopy volume similar to or greater than naturalized wild-type Japanese barberry. Values for 'Atropurpurea Nana' (syn. 'Crimson Pygmy') and 'Aurea' are reduced. The foliage color of seedlings derived from 'Atropurpurea', 'Rose Glow', and 'Atropurpurea Nana' varies widely from specimen to specimen, with some plants producing overwhelming percentages of purple seedlings and others yielding large percentages of green seedlings.

INTRODUCTION

Invasive exotic plants threaten native species and ecosystems through abundant fruit and seed production, effective dispersal mechanisms, rapid establishment and growth, and the ability to competitively exclude native vegetation (Mehrhoff, 1998). According to Reichard (2001), greater than 80% of the 235 woody plants considered invasive in natural areas across the United States were introduced primarily for horticultural purposes. These species contribute to environmental damages and losses estimated at greater than $138 billion per year and the increased imperilment of many threatened and endangered species (Pimentel et al., 2001).

Berberis thunbergii was introduced to the United States in 1875 as a garden plant and in subsequent decades began spreading from cultivation to occupy varied habitats in unmanaged areas (Silander, 1999). Currently, the plant is considered naturalized in over 30 states, primarily throughout the central and eastern U.S.A. (U.S.D.A., 2003). Japanese barberry is spread predominately via avian vectors who in fall and winter consume the red fruit produced by shrubs (Silander, 1999). In suitable locations such as closed forests, woodlands, pastures, fence rows, and waste places *B. thunbergii* may form vast dense thickets that threaten native plant communities (Silander, 1999).

Japanese barberry is represented in cultivation primarily by more than 40 cultivars that have been selected for unique ornamental attributes such as purple foliage, yellow foliage, variegated foliage, and dwarf growth habit (Dirr, 1998). While few observers dispute the invasive potential of wild-type green *B. thunbergii*, the invasive ability of horticultural cultivars is unknown. Named cultivars such as *B. thunbergii* 'Atropurpurea' (also known as *B. thunbergii* var. *atropurpurea*), 'Atropurpurea Nana' (syn. 'Crimson Pygmy'), 'Rose Glow', and 'Aurea' remain popular

among green industry producers and consumers for their attractive ornamental traits, ease of growth, hardiness, and adaptability. In Connecticut alone, the combined annual value of the Japanese barberry crop and another woody ornamental plant considered invasive, *Euonymus alatus* (winged euonymus), is $15–$20 million (Heffernan, 2002). With such large economic stakes, it is prudent to study the invasive potential of Japanese barberry cultivars and develop horticultural strategies to reduce this problem before making any decisions regarding the future of *B. thunbergii* as a horticultural crop.

MATERIALS AND METHODS

The ongoing University of Connecticut barberry research program consists of four primary modules to study the invasive potential of current Japanese barberry cultivars and develop new sterile forms:

Cultivar Seed/Seedling Evaluation. From late October to early December fruit are collected from *B. thunbergii* cultivars of verifiable nomenclature located in landscapes. Additional information is recorded such as the size of each mother plant (height, width, and depth), its growing conditions and proximity to other barberry clones. After the material is weighed, counted, and cleaned the seeds are given an appropriate period of cold stratification. Two hundred seeds from each accession are then sown in community flats that are placed in a greenhouse and monitored weekly for germination rate, seedling survival, and seedling color. Fifteen seedlings are randomly selected from each accession and potted up into 1-qt pots. These plants are randomized in flats and grown outdoors for a full growing season at 100% ambient sunlight. Upon leaf abscission in fall/winter, these seedlings are harvested and measures of plant size and weight are recorded.

Cultivar Seedling Evaluation Under Shaded Conditions. Fruit collection and seed processing proceeds as per the cultivar seed/seedling evaluation discussed above. Seeds derived from four cultivars — wild type, 'Atropurpurea', 'Rose Glow', and 'Atropurpurea Nana' — are sown in community flats and placed under shade cloth blocking 30%, 50%, and 70% ambient sunlight. A set of seed lots is also grown in 100% ambient sun as a control. The flats are monitored weekly for seed germination rate, survival, and leaf color before 30 seedlings from each cultivar and shade level are randomly selected and potted into 1-qt pots. These plants are grown outdoors under shade cloth for one season, wintered over, transplanted into 1-gal pots, and grown for a second season before being harvested. Appropriate measures of plant size and weight are recorded.

Establishment of a Research Collection. There inevitably exists great variation among the cultivated Japanese barberry plants from which seeds are collected for these studies. A research collection of Japanese barberry cultivars has therefore been installed at the University of Connecticut in Storrs, Connecticut. The plot consists of three plants each of over 40 cultivars randomly arranged in rows under uniform conditions. This collection will act as a future source for research material and facilitate ornamental comparison of the various cultivars.

Induction of Tetraploidy in Japanese Barberry. A project has been undertaken to breed Japanese barberry cultivars expressing triploid sterility derived from crosses between diploid plants and tetraploid plants induced using the mitotic

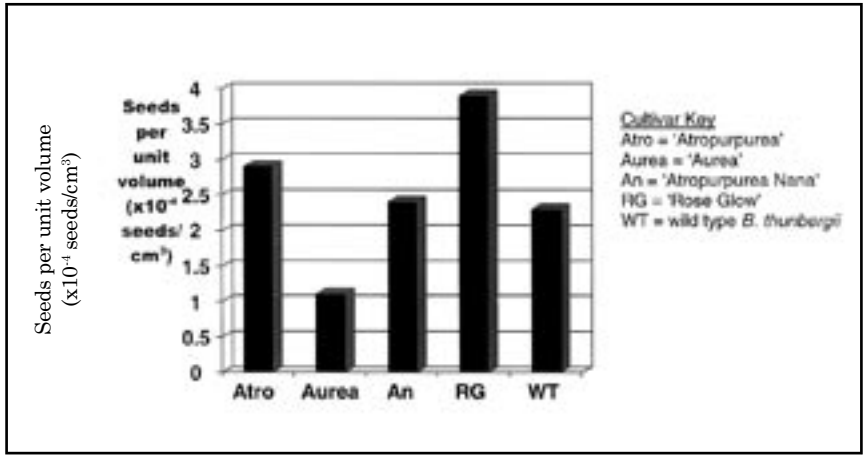

Figure 1. Average seed production per canopy volume of popular Japanese barberry cultivars.

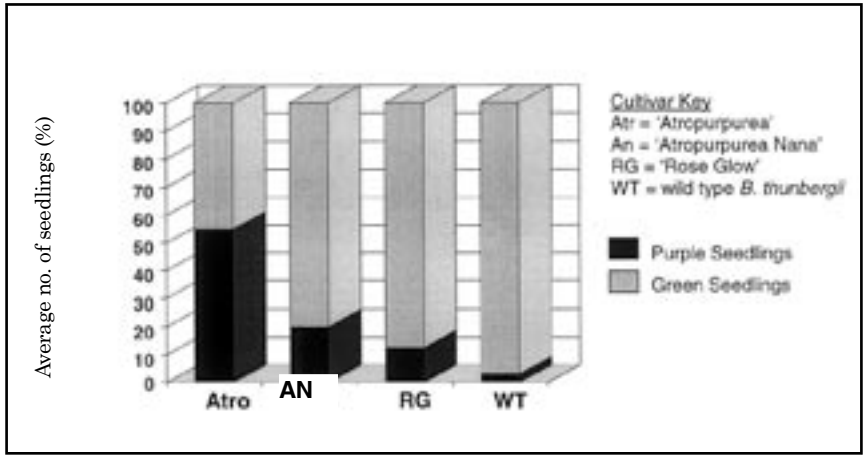

Figure 2. Foliage color of seedlings derived from popular Japanese barberry cultivars.

Table 1. Gross seed production of popular Japanese barberry cultivars.

Cultivar	Avg. seed production	Standard deviation
Atropurpurea	996	382
Aurea	120	67
Atropurpurea Nana	250	487
Rose Glow	1575	1165
Wild Type	1132	390

spindle poison colchicine. *Berberis thunbergii* 'Atropurpurea', 'Atropurpurea Nana', and 'Rose Glow' have been established in tissue culture according to the protocol established by Uno and Preece (1987). Induction of tetraploidy proceeds via two pathways:

In vitro Protocol. Following a protocol adapted from Rose et al. (2001), sterile shoot explants are taken from barberry stock cultures and immersed in tubes containing sterile solutions of colchicine (0 mM, 0.01 mM, 0.05 mM, 0.1 mM, 0.15 mM, and 0.2 mM) for 1, 2, or 3 days. Surviving lines are maintained in culture and analyzed for ploidy level via flow cytometry following extraction of cellular nuclei from plant tissue.

In vivo Protocols. Stratified Japanese barberry seeds are immersed in tubes containing nonsterile aqueous solutions of colchicine (0%, 0.02%, 0.05%, 0.1%, and 0.2%) for 6, 12, or 24 h based on work by McEwen (1990), Leach (1961), and others. Following treatment the seeds are washed, sown in community flats, and placed in a greenhouse. Seedlings that survive are analyzed for ploidy level using flow cytometry following extraction of cellular nuclei from plant tissue.

As a modification of this protocol, one drop of nonsterile aqueous colchicine solutions (0%, 0.02%, 0.05%, 0.1%, or 0.2%) is applied to the growing point of young seedlings using a dropper every morning and evening for either 1, 3, or 5 consecutive days. Cellular nuclei are extracted from the tissue of surviving plants for ploidy analysis via flow cytometry.

RESULTS

Thus far quantitative data has only been collected and processed for the cultivar seed/seedling evaluation. Of the major commercial cultivars studied, 'Rose Glow' produced the highest estimated seed production relative to canopy volume at 3.9×10^{-4} seeds/cm^3 followed by 'Atropurpurea' at 2.9×10^{-4} seeds/cm^3, 'Atropurpurea Nana' at 2.4×10^{-4} seeds/cm^3, and 'Aurea' at 1.1×10^{-4} seeds/cm^3 (Fig. 1). As a control, wild type *B. thunbergii* produced 2.3×10^{-4} seeds/cm^3, though this estimate may be low due to the shaded conditions under which naturalized Japanese barberry normally grows (Fig. 1).

While the average gross seed production of popular commercial cultivars varies widely from plant to plant of the same cultivar, some trends are evident. Large-growing purple-leaf cultivars such as *B. thunbergii* 'Atropurpurea' and 'Rose Glow' on average produce greater than 1000 seeds per plant, a level comparable to or greater than wild-type Japanese barberry (Table 1). The yellow-leaf 'Aurea' and the dwarf purple-leaf 'Atropurpurea Nana' produce significantly fewer seeds on average (Table 1).

The foliage color of seedlings derived from popular Japanese barberry cultivars varies widely as measured by the ratio of green and purple seedlings. While some accessions of 'Atropurpurea', 'Rose Glow', and 'Atropurpurea Nana' produced large percentages of purple-leaf seedlings (80% to 90%) with the remainder green, other specimens produced predominately green seedlings. As a result, on average these cultivars produced no more than 50% purple seedlings (Fig. 2). Wild-type Japanese barberry produces almost 100% green seedlings with only an occasional purple individual (Fig. 2).

DISCUSSION

The initial results of this study indicate that important commercial Japanese barberry cultivars such as 'Atropurpurea' and 'Rose Glow' demonstrate levels of gross seed production and seed production per canopy volume that approximate or exceed the levels expressed by wild-type naturalized *B. thunbergii*. The figures for 'Aurea' and 'Atropurpurea Nana' are somewhat lower. While such findings suggest that these cultivars are not without invasive potential, other factors not measured as part of this study may more accurately predict the invasive ability of these plants.

Seeds derived from 'Atropurpurea', 'Atropurpurea Nana', and 'Rose Glow' produce ratios of purple and green seedlings that differ widely from plant to plant. Empirical observations appear to indicate a relationship between these ratios and the proximity of other Japanese barberry to the mother plant. For example, seed collected from a specimen of 'Atropurpurea' growing immediately adjacent to plants of 'Rose Glow' and 'Atropurpurea Nana' may produce greater than 80% purple seedlings, while a specimen of 'Rose Glow' growing in isolation may produce greater than 90% green seedlings. These observations indicate that cross-pollination plays an important role in barberry seedling traits, an idea echoed by Dirr and Heuser (1987). It is clear that purple-leaf Japanese barberry cultivars can produce large numbers of green seedlings that are indistinguishable from those derived from invasive *B. thunbergii* populations. It is impossible to say at this time, however, what percentage — if any — of the naturalized Japanese barberry found throughout the central and eastern U.S.A. is derived from cultivars in the landscape.

LITERATURE CITED

Dirr, M.A. 1998. Manual of woody landscape plants. Stipes Pub., Champaign, Illinois.

Dirr, M.A. and **C.W. Heuser**. 1987. The reference manual of woody plant propagation. Varsity Press, Athens, Georgia.

Heffernan, R. 2002. Managing a tough issue. Amer. Nurseryman 15 Nov., 34-39.

Leach, D.G. 1961. Rhododendrons of the world. Scribner's, New York.

McEwen, C. 1990. The Japanese iris. University Press of New England, London.

Mehrhoff, L.J. 1998. The biology of plant invasiveness. Conservation Notes New England Wildflower Soc. 2(3):8-10.

Pimentel, D., S. McNair, J. Janecka, J. Wightman, C. Simmonds, C. O'Connell, E. Wong, L. Russel, J. Zern, T. Aquino, and **T. Tsomondo.** (2001). Economic and environmental threats of alien plant, animal, and microbe invasions. Agriculture, Ecosystems and Environment 84:1–20.

Reichard, S.H. 2001. Horticulture as a pathway of invasive plant introductions in the United States. BioScience 51: 103-113.

Rose, J.B., J. Kubba, and **K.R. Tobutt.** 2001. Induction of tetraploids for breeding hardy ornamentals. Acta Hort. 560:109-112.

Silander, J.A. and **D.M. Klepeis.** 1999. The invasion ecology of Japanese barberry (*Berberis thunbergii*) in the New England landscape. Biol. Invasions 1:189-201.

Uno, S. and **J.E. Preece.** 1987. Micro- and cutting propagation of 'Crimson Pygmy' barberry. Hortscience 22:488-491.

U.S.D.A., NRCS. 2003. The Plants database, version 3.5 <http:plants.usda.gov>. National Plant Data Center, Baton Rouge, LA 70874-4490 U.S.A.