Sterile cultivars (or close to it) — is this a viable option for the nursery industry?©

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INTRODUCTION

Some popular landscape plants have proven over time to exhibit invasive tendencies. The realization that these plants are invasive has led to legal bans of known invasive ornamental species in some states. For example, *Berberis thunbergii* and *Euonymus alatus* have been illegal to grow, sell, and transport since 2004 in New Hampshire and since 2009 in Massachusetts. In 2013, New York began a legal phasing out of *Berberis* and *Euonymus*, and Minnesota and Wisconsin have initiated partial bans on the most fecund Japanese barberry cultivars.

Many of the characteristics that make plants invasive, also make them good landscape plants. Invasive plants are typically tough adaptable plants that perform at a high level in managed landscapes. In addition, they often are highly ornamental and some are unpalatable to deer, making them even more useful in regions where deer populations have exploded. Use of native species or non-invasive exotic species as alternatives to invasive species has had some success. However, there are some invasive species for which it is hard to find replacement plants that provide the same set of ornamental characteristics and landscape performance traits that are delivered by the invasive plant. For these hard to replace invasive species, there is considerable interest in the development of sterile forms of these plants. Gagliardi and Brand (2007) found that the green industry strongly supported the development of sterile forms of ornamental plants as a solution to the invasive issue.

DEVELOPING STERILE FORMS OF INVASIVE PLANTS

Species undergoing breeding work

Several university and arboretum plant breeders are focusing considerable effort on development of sterile forms of important landscape plants that are invasive. The list of taxa that breeders are working on includes *Acer platanoides, B. thunbergii, Buddleja davidii, Campsis, Cotoneaster, E. alatus, Hibiscus syriacus, Hypericum, Ligustrum, Malus, Miscanthus, Prunus,* and *Spiraea*. We are just beginning to see some of the bred sterile plants enter the market. An example of sterile plants that have been big sellers recently are some of the newer *Buddleja* hybrids that are either completely sterile, or produce much reduced numbers of seed.

Breeding and evaluating plants for sterility is a long-term process, which can be technically challenging. In addition to the challenges inherent in developing sterile plants, there are many other impediments to the use and acceptance of sterile landscape plants. Some states already have legislative bans of invasive species in place. These bans include all forms of a species, including horticultural cultivars. In states with existing plant bans, new legislation will be required that will allow for exemptions for sterile cultivars before they can be used. Reversing existing legislation is often even more difficult to make happen than establishment of the original legislation. Undoubtedly there is also some loss of market for particular species where plant bans have been in effect. Customers who have gotten the message about the invasiveness of a particular species will need to be re-educated about new sterile forms in order to overcome concerns they now have about invasiveness.

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Public trust issues

In a broader sense, there are probably some significant trust issues the public will have with sterile cultivars of invasive ornamentals. To a great extent this lack of trust stems from the public's poor understanding of plant genetics, plant growth, and plant reproductive biology. Further exacerbating this situation are "pseudo scientists" who use the internet and other venues to spread their conviction about sterile plants, which is often not founded in scientific fact or evidence.

Misinformation about two ornamental plants, *Lythrum virgatum* 'Morden Pink' and *Pyrus calleryana* 'Bradford', has placed a great mistrust of plant breeders and the nursery industry in the public's psyche. Almost without exception, when presenting the topic of sterile invasive plants to the gardening public, or general public, I am questioned about whether I can "guarantee that the plants I breed won't revert or change to fertile plants like Morden Pink loosestrife or Bradford pear did." Information about *L. virgatum* 'Morden Pink' from the Agriculture Canada Morden Research Center was misinterpreted initially and some catalogs listed the genotype as sterile, even though it was known to be female fertile by the scientists working with it. 'Morden Pink' was used in crosses to create 'Morden Gleam' and 'Morden Rose' clearly demonstrating a lack of sterility. Incorrect plant catalog information became "fact" over time and soon everyone believed 'Morden Pink' was sterile. To make the situation worse, isolated garden plants of 'Morden Pink' appeared sterile to growers and gardeners because of the complex tristylous reproductive mechanism used to force outcrossing in this genus (Anderson and Ascher, 1993). In tristylous plants, the combination of flowers styles and stamens on each genotype will be short, medium, and long in length. Only stamens and styles of the same length produce seed set. Therefore, 'Morden Pink' has to outcross with another genotype in order to match its style length with another genotype or species with appropriate stamen length. When other *Lythrum* genotypes or species are present, 'Morden Pink' produces lots of seed.

Pyrus calleryana 'Bradford' is another ornamental plant that has added to the public's negative perception about sterile plants. *P. calleryana* was brought to the USA as a fire blight resistant rootstock and for potential use in breeding to incorporate fire blight resistance into *P. communis* (Whitehouse et al., 1963a). Many sources incorrectly state that 'Bradford' was bred to be sterile. However, it was a seedling selected from seed obtained from China (Whitehouse et al., 1963b). Initially, 'Bradford' was observed to produce little fruit, but this lack of fruit production was due to self-incompatibility, not sterility (Zielinski, 1965). Exposure to new cultivars or genotypes of *P. calleryana* resulted in significant fruit and seed production by 'Bradford' (Culley and Hardiman, 2007). This scenario has resulted in the public believing that "sterile" Bradford reverted to a fertile condition and that sterile plants will all eventually become fertile again. The bottom line is the public no longer trusts plant breeders or the nursery industry when it comes to the topic of plant sterility. This is a significant impediment to the use and acceptance of sterile invasive plants.

Methods to produce sterile or near sterile plants

On the positive side, methods do exist that can be used to create sterile or near-sterile forms of plants. These plants will not spontaneously "revert" to a fertile condition. Significant advances have been made in transgenic technologies that can be used to create sterile plants. However, the use of transgenic methods to produce sterile forms of ornamental invasives is not currently a viable option. The negative public opinion about transgenic plants and the regulatory hurdles that must be cleared are currently too large for pursuit of this strategy to develop sterile invasive landscape plants.

Ploidy manipulation is the most often used method for the creation of invasive plants that produce no seeds or few seeds. In most cases, the goal is to develop triploid plants, which will typically have low fertility due to unpaired chromosomes during meiosis. One way to make triploid plants is to take advantage of triploid endosperm tissue that is produced as a result of double fertilization in the ovule. Three 1N nuclei are produced by a pollen grain that lands on the stigma. One becomes the tube nucleus, which forms the pollen tube through the stylar tissue. The other two nuclei are generative nuclei, which enter the ovule. One generative nucleus fertilizes the 1N egg to form the 2N embryo and the second generative nucleus combines with a pair of 1N polar nuclei to create the 3N endosperm, which develops into an important food source for the growing embryo.

In non-endospermic seeds the endosperm food reserves are transferred relatively quickly to the cotyledons of the developing embryo making it challenging to take advantage of this tissue. In endospermic seeds, the cotyledons are small and the endosperm remains large even in a fully developed seed. For endospermic seeds, the 3N endosperm tissue is accessible and can be used as a source of natural triploid cells. Triploid endosperm cells can be induced to form callus in vitro and eventually to form shoots. Shoots can then be rooted to form triploid plantlets. Endosperm derived triploid plants will have two sets of maternal chromosomes and one set of paternal chromosomes. Triploid *Euonymus alatus* has been produced using this procedure (Thammina et al., 2011).

Triploid endosperm is only useful for a limited number of plants due to inaccessible endosperm or recalcitrance in vitro. So a more common approach to creating triploids is to first create tetraploids from diploid plants. Mitotic inhibitors such as colchicine or oryzalin are used to double the chromosome number in plant cells. Meristematic tissues, such as the plumules of germinating seeds or shoot apical and lateral buds are the targets of mitotic inhibitor treatments. Plants are produced from the tetraploid shoots and grown to flowering size. Crosses are then made between tetraploid and diploid plants to create triploids.

Triploid plants must be thoroughly evaluated to determine their level of fertility. Some will be fully sterile, but others will express low and variable levels of fertility. In genera or families where apomixis is known, triploid plants can utilize asexual embryo formation to produce large numbers of viable seeds. While development of triploids can be relatively straight forward in some species, other species possess a triploid block where it is very difficult to obtain triploid seeds, most often due to failure in endosperm development that ultimately results in embryo failure (Köhler et al., 2010).

Berberis thunbergii has a strong triploid block. Despite extensive efforts to generate triploid barberry in my breeding program by crossing 4N and 2N plants, I have only been able to generate four individuals. All four plants have not produced any seeds despite flowering and producing fruit. Unfortunately, all of these triploids have green foliage and are not as desirable as ornamentals as they would have been with purple foliage. Although triploid barberry has been a difficult achievement, it has been relatively easy to produce large numbers of autotetraploids. While many autotetraploid barberry have been fertile, others have been highly seed infertile and we have dwarf or compact tetraploid genotypes with purple, yellow, or green foliage that will soon be available in the trade.

Another method that has been used to create infertile landscape plants has been wide interspecific or intergeneric hybridization. In *Buddleja*, interspecific hybridization, especially when three or more species are involved in the cross, has produced very reduced seed production or even complete sterility (Werner and Snelling, 2011). Similarly, in *Berberis*, a tri-specific cross involving *B. verruculosa, B. gagnepainii*, and *B. vulgaris* has exhibited low seed set (Brand, unpublished). Two of the species involved have blue/black fruit and one has red fruit, so having genomes from different ends of the *Berberis* spectrum helps reduce fertility.

Regardless of how putative sterile plants have been produced, it is of the utmost importance that they are thoroughly studied and documented to perform as claimed. Without thorough confirmation of the level of sterility, there is the risk of a plant becoming another *L.* 'Morden Pink' or *P. calleryana* and further eroding the public's confidence in sterile plants. First, ploidy should be confirmed using flow cytometry and chromosome counts. If a plant is an intergeneric hybrid, its hybridity should be confirmed through both morphological and genetic analysis.

To accurately document seed production, putative sterile plants must be planted with appropriate fertile controls in a replicated planting. One must provide for genetic outcrossing and outcrossing with various ploidy levels by including multiple genotypes. Plants must be allowed to mature enough to insure that reproductive capacity isn't overlooked simply because plants are too young. Brand et al. (2012) found that barberry cultivars on average increase fruit production over 1000% when comparing 5-year old to 10-year old plants. Several plants that appeared sterile at 5 years of age were producing seed at 10 years of age.

Reduced fertility an acceptable option?

When completely sterile plants cannot be achieved are plants with reduced fertility an acceptable option? How reduced does seed production need to be in order to be acceptable? Knight et al. (2011) make the case that long-lived woody plants will need to have extremely low levels of seed production in order to insure no population growth. Brand et al. (2012) developed predictive information about Japanese barberry seedling establishment in the wild using a combination of seed production, seed germination, and seedling survival data. Barberry genotypes producing about more than 50 seeds per year would likely result in one or more seedlings becoming established in an unmanaged woodland. To be most useful, similar data will need to be established for each invasive species that is being considered for sterile cultivar development if absolute sterility cannot achieved.

The nursery and landscape industry has been supportive of cultivar exemptions for sterile or near sterile genotypes of important invasive landscape plants. The best example of a working cultivar exemption for sterility is one established in Oregon for *Buddleja* (Oregon Department of Agriculture, 2011). Regulation of *Buddleja* is through the Oregon Department of Agriculture (ODA). To be approved for sale, a *Buddleja* genotype must produce 2% or less viable seed or be documented to be an interpecific hybrid. In 2015 there were 18 approved cultivars of *Buddleja* that were legal to use in Oregon. To gain approval for exemption, one can either submit independent research documenting the level of fecundity to the ODA for review, or they can pay to have Oregon State University evaluate the fecundity of a plant. For interspecific hybrids, proof of parentage information must be submitted to ODA for review. The *Buddleja* cultivar exemption program in Oregon seems to be successful so far and can serve as a model for other states to follow with additional plant species (Contreras and McAninch, 2013).

In New York, where Japanese barberry has recently been legally banned, a decision-making tree was developed to support a cultivar exemption program. Barberry cultivars do not necessarily have to be completely sterile to be approved for sale, but must produce low numbers of seed and meet several other criteria that collectively would result in low risk of establishment in unmanaged areas. Minnesota and Wisconsin have taken a slightly different approach with their recent bans of *B. thunbergii* and cultivar acceptability. They used data developed at the University of Connecticut (Brand et al., 2012), which documented seed production levels for 45 cultivars. Minnesota and Wisconsin legislation bans the species (*B. thunbergii*) plus 25 cultivars, which produce high numbers of fruit. Lower fruiting cultivars are still legal, but the language included in the legislation states that when horticulturally acceptable seedless cultivars become available revisions should be made to reduce the seediness considered acceptable for use.

Massachusetts, which has a long-standing ban on all *B. thunbergii* and cultivars, has formed a committee to explore the possibility of sterile cultivar exemptions. New Hampshire is not considering cultivar exemptions to its barberry ban at this time. A concern that is often voiced by those considering support for cultivar exemptions for sterile plants is how can one be sure of the identity of a plant. Often, sterile cultivars may be hard to distinguish from fertile forms of the same plant. Mechanisms need to exist to help prevent the sale of fertile plants either intentionally or accidentally. As genetic testing of plants becomes increasingly routine and affordable, it will become reasonable to require random genetic checks to confirm the identity of sterile plants on the market. In addition, sterile plants will all be patented and licensed to specific growers, making tracking of plant material fairly straightforward. Sterile plants will probably all be sold with individual plant tags that get carried forward with the plant from propagation to final sale, again making plant tracking easier.

Given the number of plant breeders currently focusing effort on the development of sterile forms of invasive landscape plants, there will undoubtedly be numerous new sterile plants arriving in the market in the next decades. It is likely that exemptions in plant bans to allow for the use of sterile cultivars will become widespread and commonplace.

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