Development of Autopolyploid *Syringa reticulata* subsp. *pekinensis* for Breeding©

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INTRODUCTION

Tree lilacs are generally considered to be popular and smart choices for street and utility plantings because of their hardiness, rugged nature, and ability to thrive under adverse urban conditions. In particular cultivars of the Japanese tree lilac (*Syringa reticulata* subsp. *reticulata* (Blume) H. Hara) and Peking lilac (*S. reticulata* subsp. *pekinensis* Rupr.) are often promoted for municipal plantings and for home use (Jull, 2011; Gerhold, 2007). Virtues of these trees include the ability for use in U.S.D.A. hardiness from Zones 3(4) through 7, tolerance to a wide range of biotic and abiotic stresses; as well as, ornamental flowering and in some cases exfoliating bark which adds to aesthetic appeal.

Syringa reticulata may be further divided into two subspecies, with *S. reticulata* subsp. *reticulata* and *S. reticulata* subsp. *amurensis* (Rupr.) P.S. Green & M.C. Chang (Li et al., 2012). *Syringa reticulata* subsp. *pekinensis* is native to northeastern China, *S. reticulata* subsp. *amurensis* native to northeastern China, Korea, and Siberia while *S. reticulata* subsp. *reticulata* is native to northern Japan. The three taxa were independently introduced to cultivation from the mid to late nineteenth century (Dirr, 2009). Under cultivation in North America, garden escapes have been documented in Massachusetts, Michigan, Minnesota, New Hampshire, New York, Pennsylvania, Vermont, Wisconsin, Wyoming, and in Ontario (Canada) (Kartesz, 2013; Schimpf et al., 2009; Jordan et al., 2008; Springer and Parfitt, 2007; Sorrie, 2005). Most of these escapes have been reported as *S. reticulata* and have occurred as few plants or self propagating in localized areas; however, sampling from escaped colonies in Minnesota suggest that the tree lilacs may be shade tolerant during invasion allowing plants to persist until a canopy openings allow for rapid growth and fruit production (Schimpf et al., 2009). Shade tolerance, along with other characteristics such as prolific seed set and tolerance to a wide range of environmental conditions may facilitate the plant's ability to seed out from original plantings to less managed areas. At the Morton Arboretum in Lisle, Illinois, seedlings are frequently observed and removed from cultivated beds (personal observations). To prevent garden escapes and to decrease land management inputs, the use of sterile or low fertility cultivars from some "weedy taxa" have been promoted within some regions (Oregon Department of Agriculture, 2013; Brand et al., 2012; Knight et al., 2011; Alvey, 2009; Gagliardi and Brand, 2007). One technique used to develop sterility is ploidy manipulation.

Ploidy refers to the number of sets of chromosomes within an organism's genome. Humans and most animals are diploid having two sets of chromosomes ($2x$), while polyploid plants (plants with more than two sets of chromosomes) occur frequently in nature. Polyploids because of their enlarged genomes often have "giga" sized cells that are larger than their diploid counterparts. The layering of the enlarged cells can impact the appearance and behavior of the polyploid plant (Kher, 1996). Plants with three sets of chromosomes are referred to as triploids ($3x$). Triploids are generally considered to be sterile because the chromosomes fail to divide evenly during meiosis. Triploids may be created by hybridization of tetraploid plants (plants with four sets of chromosomes, $4x$) and diploid plants. In some cases such as bananas, the hybridization of two species with different ploidy levels was used successfully to created triploids that produce seedless fruit. In *Syringa* there are no reports of naturally occurring polyploids (Fiala, 1988). Artificial induction of polyploidy in *Syringa* with the chemical mutagen colchicine was successfully demonstrated by Fiala (1988) for shrub lilacs and with *S. reticulata*. Oryzalin is another chemical mutagen that is also used to produce polyploids in ornamental taxa. In

comparisons between colchicine and oryzalin, oryzalin produces similar conversions to polyploidy at lower concentrations (Ascough et al., 2008; Lehrer et al., 2008).

Therefore, the goal of the research presented in this paper is to produce autotetraploid *S. reticulata* subsp. *pekinensis* with the chemical oryzalin. Tetraploid plants will then be used as breeding stock to retrieve sterile triploid selections.

MATERIALS AND METHODS

Plant Material

Four hundred open pollinated seed were collected at the Morton Arboretum in the fall of 2012 from each of three accessioned *S. reticulata* subsp. *pekinensis;* including cultivars 'Morton', China Snow™ (2391-26*1), 'Zhang Zhiming', Beijing Gold™ tree lilac (319-2002*1), and an unnamed upright selection (459-96*1). The seed were sown into flats (deep propagation flat; Anderson Die & Manufacturing, Portland, Oregon), one flat was sown for each accession, medium was a peat based potting mix. Seeded flats were watered and placed in a walk in cooler for stratification at 4°C for 90 days. After stratification the flats were moved to a glass house (74°F, with fluctuations ~60°F to 94°F) for germination and watered by hand as needed. Six days after the flats were moved to the glasshouse the first emergence was observed.

Inducing Polyploidy

At approximately 9:00 AM on the 7th day following the first emergence, when a majority of the emerging seedlings were in the cotyledon stage, the seedlings were individually plucked from the trays and evenly divided into treatment and control groups. There was one bulked treatment and one bulked control per group of seedlings. In the control groups the seedlings were soaked in deionized water while the treatment groups were soaked in 175 µm oryzalin solution (prepared from Surflan® AS; United Phoshorus, Trenton, New Jersey) for a duration of 4 h. After the treatment was complete, the seedlings were gently rinsed with DI water to remove excess treatment solution. The seedlings were then replanted in plug trays (PL-128; T.O. Plastics, Clearwater, Minnesota) with the same potting mix as above and returned to the glass house to resume growth.

Ploidy and Data Analysis

Approximately 120 days following the treatment a 10 plant sample of the control seedlings and 62 selected vigorous seedlings from the treatment groups (seedlings which had resumed growth and developed true leaves) were screened to detect ploidy levels at the University of Illinois, Roy J. Carver Biotechnology Center. For flow cytometric analysis the samples were prepared by chopping ~0.5 cm^3 leaf tissue with a razor blade in a nuclei extraction buffer (Cystain® Ultraviolet Precise P Nuclei Extraction Buffer; Partec, Münster, Germany) and stained with 4',6-diamidino-2-pheylindole (Cystain® Ultraviolet Precise P Nuclei Extraction Buffer; Partec, Münster, Germany); however, because of poor fluorescence, the samples were also over-stained with 50 ppm propidium iodide before being analyzed using a BD LSR II® flow cytometer (Becton, Dickinson and Company; San Jose, California). Data interpretation was done through review with FCS Express (DeNovo™ Software; Los Angeles, California). All samples were prepared with an internal standard to prevent peak shifting (*Pisum sativum* L. 'Ctriad'; 2C = 8.76 pg) (Greilhuber et al., 2007).

RESULTS AND DISCUSSION

Many of the treated seedlings failed to develop true leaves following the applications of oryzalin. *Syringa* seedlings treated with colchicine similarly had slow growth and development (Fiala, 1988). Because true leaves were needed for ploidy analysis, only samples of treated seedlings were able to be screened through flow cytometry. All of the control plants appeared to have the same genome size and were therefore used to set gates to identify diploid and tetraploid cells in analysis. Tetraploid plants were recovered from

all three oryzalin treatments (Table 1). The conversion rate to tetraploid was similar across treatments and the combined average conversion to tetraploids was 60.3%. Some of the treated seedlings seemed unaffected by the treatments and remained diploid ($2x$) and others were chimeral with a mix of diploid and tetraploid cells ($2x + 4x$). A few plants appeared to be octoploid ($8x$) or mixaploids consisting of tetraploid and octoploid cells ($4x + 8x$). Statistical analysis was not done because treatments were not replicated within genotypes and only samples of the seedlings were screened. Even without analysis similar trends in conversion were observed across genotypes.

Phenotypic differences between polyploid and diploid plants were also observed in the treated seedlings. The tetraploids and some of the mixaploid plants appeared to have darker green leaves and felt thicker than the control and diploid seedlings (Fig. 1). These differences are attributed to increased cell size which may also alter plant behavior (Beaulieu et al., 2008). Similar changes in morphology were observed by Contreras and Ruter in treated *Cyrptomeria japonica* (L.f.) D. Don (Contreras and Ruter, 2010). Using observations based on the appearance and thickness of leaves one may be able to do a preliminary sort to discard diploid plants which had effectively escaped the treatments, although mixaploids may be distorted and closely resemble homogeneous tetraploid plants. Oryzalin proved to be very successful at inducing polyploidy in this study and the flow cytometer offered a means to quickly and accurately determine ploidy levels of the seedlings.

The next steps of the new plant development program will be to continue growing out these lilac seedlings to maturity. The plants ploidy levels will then be rescreened prior to pollinations to ensure that they have remained homogeneous tetraploids over time. The induced autotetraploid lilacs will then be used as seed parents in a series of backcrosses with pollen from diploid plants. When triploid seedlings are recovered they will be evaluated for fertility and selections will be made for ornamental characteristics.

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Table 1. Estimated ploidy levels of *Syringa reticulata* subsp. *pekinensis* seedlings after treatment with oryzalin solution.

Parent ID	Seedlings examined (no.)	2*x*	2*x* + 4*x*	4*x*	4*x* + 8*x*	8*x*
2391-26*1	30	5	4	17	2	2
459-96*1	26	6	4	15	0	1
319-2002*1	6	2	0	4	0	0
Total	62	13	8	41	2	3

Fig. 1. Visible differences between polyploid and diploid *Syringa reticulata* subsp. *pekinensis*. Diploid from control group left (lighter and thinner leaf) and autotetraploid from treatment group right (darker and thicker leaf).