Hereditary variation is a vital component in the development of new and improved cultivars of landscape plants. Sources of hereditary variation include naturally occurring variation, recombination due to controlled crosses, artificial mutagenesis, and genetic modification via biotechnology. Here I explore all methods with the exception of genetic modification via biotechnology.

In *Acer* I evaluated naturally occurring cytometric variation. Cytometric variation refers to variation in genome size and ploidy. Genome size and ploidy data can be used in the development of targeted breeding projects including interploidy hybridization. I used flow cytometry and traditional root squashes to measure genome size and determine ploidy. Chemical mutagenesis through the application of ethyl methane sulfonate was utilized to induce hereditary phenotypic variation in *Ornithogalum candidans* (cape hyacinth). Phenotypic variation was evaluated by collecting field data of mutant seedlings. Interspecific hybridization through controlled crosses was
evaluated as a method to increase phenotypic variation in Sections Dasanthera, Saccanthera, and Penstemon of Penstemon.

I found that there is indeed natural ploidy and genome size variation in Acer. Genome size and ploidy data collected in this survey will add to the growing body of knowledge relative to angiosperms. Additionally, the identification of natural polyploids will be of use in developing breeding programs focused on sterile cultivar development. Ethyl methanesulfonate is an effective means of inducing phenotypic variation in Ornithogalum candidans and was shown to lead to a reduction in both plant height and fertility. Interspecific hybridization of sections of interest in Penstemon has some challenges. Challenges include identifying the appropriate environment for controlled crosses and ascertaining the fertility and crossability of target species and garden cultivars used in breeding germplasm.
Inducing and Evaluating Phenotypic and Cytometric Variation in Landscape Plants: Observations from Acer, Ornithogalum, and Penstemon

by
Kim Shearer Lattier

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Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

____________________________________________
Kim Shearer Lattier, Author
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CONTRIBUTION OF AUTHORS

Dr. Ryan Contreras assisted with design, editing, and funding.
DEDICATION

To

Kim Haeng Sun Shearer

1956 - 2008

The bravest woman I ever did know.
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Inducing and Evaluating Phenotypic and Cytometric Variation in Landscape Plants: Observations from *Acer*, *Ornithogalum*, and *Penstemon*

Chapter 1: General Introduction

Phenotypic variation in plants can be attributed to two factors: genetics and environment. While it is understood that environment can play a significant role in phenotypic expression, genetic or hereditary variation is the primary focus of a plant breeding program. Ideally, cultivars developed in a breeding program are evaluated in multiple environments to ascertain the stability of phenotypic expression of traits. In this thesis, I will focus on exploring hereditary variation that can be manipulated and exploited by plant breeders.

**Hereditary Variation in Plant Breeding**

Hereditary variation is essential in plant breeding. In developing a breeding program, one of the first steps a breeder must take is acquiring or creating a genetically variable population of plants for their germplasm collection. The greater the variation in a group of plants, the greater the potential of developing improved cultivars adapted to various growing conditions or exhibiting novel traits. Sources for hereditary variation include natural variation due to point mutations or recombination, recombination from controlled crosses, induced mutations, or artificial introduction of extraneous DNA using biotechnology (Sleper and Poehlman, 2006).
Inducing Phenotypic Variation

There are a number of tools at a breeder’s disposal for inducing phenotypic variation. A classic example of inducing phenotypic variation is wide hybridization through controlled crosses. Wide hybridization can include crosses between two different species of the same genus or crosses between two taxa with different ploidy—the number of sets of chromosomes—and on rare occasions, crosses between two species from different genera (Ranney and Eaker, 2003). Traits that have been targeted using wide hybridization for landscape plants cover a wide range from aesthetic appeal to heat and drought tolerance, from disease resistance to physiological responses to environmental cues (Kuligowska et al. 2016). In Chapter 4, I will discuss the potential of interspecific hybridization of Penstemon species for the introgression of moisture tolerance.

Other methods of inducing phenotypic variation include chemical and physical mutagenesis. Physical mutagenesis refers to the use of radiation treatments, such as application of gamma rays, X-rays, neutrons, beta radiation, and ultraviolet radiation to induce random molecular changes in plant tissues (Fehr, 1991). Irradiation treatments have been successfully used for development of novel foliage and flower color, improved habit, and dwarfing (Maluszynski et al., 1992). The widespread use of irradiation-mediated mutation breeding is evident in the electronic Mutant Variety Database (MVD) hosted by the Food and Agriculture Organization of the United Nations (FAO) and the International Atomic Energy Agency (IAEA) through the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture. Through this database, plant
breeders can register new cultivars developed using irradiation techniques. Examples of recently registered ornamental releases include *Chrysanthemum ‘Kesar’, Schefflera ‘Sparkles’,* and *Dracaena sanderiana* ‘Sunbeam’ (MVD, 2016).

Chemical mutagenesis is the application of chemicals including, but not limited to, ethyl methane sulfonate (EMS), diethyl sulfate (DES), ethyleneimine (EI), ethyl nitroso urethane (ENV), ethyl nitroso urea (ENH), and methyl nitroso urea (MNH) (Fehr, 1991). In Chapter 3, I will focus on the application of EMS in ornamental plant development and the effects of inducing point mutations in *Ornithogalum candidans*.

**Evaluating Cytometric Variation**

Cytometric variation refers to differences in measurements of characteristics of the cell. Historically, measurements of the cell and the ensuing documentation of those measurements were time-consuming, tedious endeavors. Once upon a time—before the advent of desktop computers, software with user-friendly graphical user interfaces (GUI), and digital cameras—people had to sit at microscopes for days, months, maybe even years, in order to measure and record the sizes, shapes, and numbers of cells and their various components. However, today we have the benefit of decades of technological advances that enable us to not only view cells through a microscope and photograph them, but we also have the flow cytometer that can measure the content of a cell organelle.

Today’s flow cytometer is a bench top machine that utilizes sheath flow technology developed by the United States military during World War II (Shapiro, 2004), laser and ultraviolet light technology, photo-detector technology, and computers.
(Robinson and Grégori, 2007). Before our bench top models, the first commercial introduction of the flow cytometer was in 1974 with the introduction of the Hemalog D system (Mansberg et al. 1974). Initially, this technology was used to count and analyze cells from blood samples and pap smears (Shapiro, 2004). These samples of cells were suspended in liquid and had no cell walls. Before science could make the leap to analyzing the nuclei of plant cells, there needed to be innovative work in developing protocol for extracting nuclei from solid plant tissue and suspending the nuclei in liquid without lysis of the nuclear membrane.

While Mansberg et al. (1974) were putting together their publication announcing the first commercial flow cytometer, the first study reporting protocol developed for the analysis of plant cells in a flow cytometer was published. In a 1973 publication, F.O. Heller published an analysis of the nuclear DNA content of the faba bean (*Vicia faba*). In this study, Heller outlined a multi-step process for extracting cell nuclei from root tips. He took the biochemical approach of using various enzymes to break down cell walls and digest protein debris. While this was groundbreaking work, there was a major disadvantage in the protocol. The enzymatic step would have to be optimized each time a researcher wanted to investigate DNA content of a different plant species (Doležel et al., 2007).

Occam’s razor—the simplest solution, or hypothesis, is most likely the best. This was the case with the advent of efficient plant cell analysis in a flow cytometer. Probably the most significant break-through in efficient extraction of nuclei from plant cells was the use of a razor blade. In 1983, Galbraith et al. published the first protocol
using a razor blade, a petri dish, and a buffer solution. Finally, a method that anyone could implement no matter the level of experience. As a result, there are now thousands of publications on the analysis of plant cells using flow cytometry.

In Chapter 2, I will explore differences in *Acer* genome size and ploidy. While the mass of genetic material within nuclei can be measured using a flow cytometer, the ploidy of a plant can be inferred using the same information. When the genome size of a particular species is calibrated to chromosome number using traditional cytology work at a microscope, we can determine the ploidy of other samples that may vary by using the monoploid (one set of chromosomes: 1Cx) value of the calibrated species.

Variation in genome size can be used by breeders to confirm interspecific hybridization (Keller et al., 1996; Shearer and Ranney, 2013) in cases where the parent species have significantly different genome sizes; and it can be used to screen germplasm for ploidy variation (Rounsaville et al. 2010; Parris et al. 2010; Lattier et al., 2014; Gillooly et al, 2015). Ploidy variation can be a very useful tool for a breeder in mitigating potential invasive qualities of landscape plants. As demonstrated by Oates et al. (2014), where a cross between an autotetraploid trumpet-vine and its diploid progenitor resulted in highly reduced fertility.

While there are techniques for doubling the number of chromosomes in plant cells, this can take a significant amount of time and effort. Screening germplasm and discovering polyploids can sometimes save years in the development of sterile or infertile woody plant cultivars. Consider the Norway maple (*Acer platanoides*), this particular species of maple has been banned from the state of Massachusetts, is listed
as a noxious weed in the state of Connecticut, and is listed in weed reports for the
Northeastern region of the United States and the state of Wisconsin (USDA, 2016b).
One solution would be to eradicate and eliminate the species from the landscape and
market; however, according to the 2014 USDA Census of Horticultural Specialties
(2016), this one species of tree generated $13.6 million in total sales (includes wholesale
and retail) for the nation; and almost $3 million of Norway maples were sold in the state
of Oregon in 2014 (USDA, 2016).

The alternative solution is to design a breeding program for the development of
sterile, or highly infertile, Norway maples using interploid crosses. This is currently
underway at Oregon State University in the Ornamental Breeding program directed by
Dr. Ryan Contreras. In 2010, tetraploid cytotypes were developed using oryzalin and are
currently growing in an isolated block with diploid cytotypes at the OSU Lewis-Brown
Farm (Corvallis, OR); and they are still juvenile. The juvenility period for maples can last
5 – 20 years (Oterdoom, 1994). A much more expeditious means of attaining this
breeding goal would be to identify mature polyploid material available on the market or
in a public garden. As has been demonstrated with other taxa, there can be wild and
cultivated polyploid material already available for intraspecific or interspecific interploid
hybridization (Bottini et al., 2000; Jones et al., 2007).

**Value of Landscape Plants: Why put effort into landscape plants?**

Landscape plants are those that are planted within our developed landscapes,
commonly referred to as ornamental plants; and the category ornamental plants can
also include house plants. In this thesis, I will focus on the landscape plants. As
reported by the George Morris Centre (Brethour et al., 2007) there is a growing body of literature that demonstrates the many and varied benefits of ornamental plants environmentally, socially, and economically.

With the imminent threat of climate change, there is growing concern over the effects of extreme temperatures on plant material in urban areas. Urban heat islands (UHI) are described by the Environmental Protection Agency (EPA) as “areas that are hotter than nearby rural areas.” On average, UHIs can be 1 – 3 °C warmer than surrounding areas. The impacts of this difference in temperature are wide-ranging: increased energy usage, increased household costs, increased pollutants, negative health effects, and reduced water quality. One way to mitigate these effects is to integrate more green spaces into urban complexes. Green spaces where landscape plants can be used include street trees, parks, vertical gardens, patio gardens, and rooftop gardens (EPA, 2016). The continued development of landscape plants will meet the demand for material adapted to these types of niches.

The scientific consensus is that global warming is largely due to anthropogenic effects (Cook et al., 2013). This is attributed to the increased production of carbon dioxide that is accumulating in our atmosphere exacerbated by a historical decrease in forested areas (Hartmann et al., 2013). Studies have shown that perennial woody landscape plants can provide a carbon sink by sequestering carbon in urban environments with high levels of pollution (Brack 2002; Nowak and Crane, 2002). The impact of reports such as these is evident in current events such as the recent attempt to break Pakistan’s world record for number of trees planted in 24 hours (847,275
trees). In the Uttar Pradesh state of India, more than 800,000 community members united to plant 50 million trees provided by the local government in response to reports at the 2015 Paris Climate Change Summit (Banerjee, 2016).

In the horticulture industry, landscape plants are considered nursery stock. Nursery stock as defined by the USDA (2016) includes finished “deciduous shade and flowering trees, coniferous and broadleaf evergreens, shrubs, bushes, ground covers, fruit and nut trees, grapevines, small fruit plants, and vines.” The 2014 Census of Horticultural Specialties reported total sales of nursery stock in the nation near $4.3 billion. In a report on 2014 figures, the Oregon Department of Agriculture (2015) ranks the greenhouse and nursery industry at number two in Oregon’s top 20 commodities with a value of $830 million, behind cattle and calves by less than $1 million.

The value of landscape plants is not limited to environmental and economic benefits. Socially we, too, benefit from the increase of green spaces in our public arenas. Growing interest in the positive effects of green spaces on overall health and well-being and social interaction is evident in the many emerging city planning and social studies (Matsuoka and Kaplan, 2008; Hunter et al., 2014; Lee et al., 2015). Ornamental landscape plants are environmentally, economically, and socially relevant crops to focus on in a breeding program.

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Inducing and Evaluating Phenotypic and Cytometric Variation in Landscape Plants: Observations from *Acer, Ornithogalum*, and *Penstemon*

Chapter Two:
Genome size, Ploidy and Base Composition of Wild and Cultivated *Acer* L.

*Acer* is a well-known, popular and prevalent genus recognized by experts and novices alike by its leaf morphology and fruit. In addition to notable fall color display, there are a number of features that garner ornamental interest including bark, form, and sometimes even flowers. Interest in maples as a street tree, specimen tree, or shade tree is reflected in the 2014 USDA Census of Horticultural Specialties. This census reported the overall sales of maples, including retail and wholesale, as accounting for $173.4 million nationwide; while in the state of Oregon, total reported sales were more than $63 million—more than half of the sales of deciduous shade trees statewide.

With approximately 129 – 200 (Suh et al., 2000; Zhang et al., 2010; Li, 2011) species worldwide, *Acer* is a diverse genus that pervades developed landscapes as well as the world’s forests. The diversity of the genus is expressed morphologically in terms of leaf shapes and sizes, vegetative buds, inflorescence type, bark, habit, and habitat. Thus there has been considerable taxonomic research in attempting to determine the classification of species within the genus and how these species relate to one another on the evolutionary time scale (Ackerly et al., 1998; Suh et al., 2000; Pfosser et al., 2002; Tian et al., 2002; Li et al., 2006; Grimm et al., 2007; Renner et al., 2008; Zhang et al., 2010; Li, 2011). There has also been considerable debate relative to these taxonomic relationships and phylogenetic order of the genus. However, relatively little has been
reported in regards to genome size and ploidy level within the genus. Three original papers (Olszewska and Osiecka, 1984; Loureiro et al., 2007; Siljak-Yakovlev et al., 2010) report genome sizes for maple species with just 11 species represented. Of these three papers, not a single one reported research focused on maples.

Genome size data has been shown to reflect taxonomic relationships in Cornaceae (Shearer and Ranney, 2013) while also being reflective of genome evolution (Johnston et al., 2005; Yotoko et al., 2011). Genome size data can be used to determine ploidy in a genus when properly calibrated using chromosome counts as demonstrated in Ericaceae, Cornaceae, Magnoliaceae, Berberidaceae, and Lamiaceae (Jones et al., 2007; Parris et al., 2010; Rounsaville and Ranney, 2010; Contreras and Ruter, 2011; Shearer and Ranney, 2013). Genome size and ploidy data are useful tools in a breeding program as they can provide greater insight into a genus and thus aid in developing breeding objectives.

The base chromosome number of Acer is $x = 13$. Cytological reports for maples include a range of ploidy levels (Darlington et al., 1956; van Gelderen et al., 1994). Natural polyploidy has been reported in Section Rubra including hexaploid ($2n = 6x = 78$) A. pycnanthum, hexaploid and octoploid ($2n = 8x = 104$) A. rubrum, and tetraploid ($2n = 4x = 52$) A. saccharinum (Foster, 1933; Santamour, 1971). Most other maples investigated have been reported as diploid ($2n = 2x = 26$) and are presented in Table 2.1.

Polyploidy, or whole genome duplication, can be manipulated in a breeding program such that wide hybrid crosses become attainable (Sanford, 1983), or sterile ornamental cultivars can be developed (Olsen et al., 2006; Trueblood et al., 2010).
Polyploidy can also provide a barrier to successful hybridization in some cases (Sanford, 1983), and thus knowledge of ploidy in a group of taxa can be greatly beneficial in a developing breeding program.

Measuring genome size in plants can be quickly and effectively accomplished using flow cytometry. This has been demonstrated by a number of genome size surveys of angiosperms (Jones et al., 2007; Rounsaville and Ranney, 2010; Parris et al., 2010; Shearer and Ranney, 2013; Lattier et al., 2014). While these genome size and ploidy surveys are useful to ornamental plant breeders, they also answer the call put forth by Galbraith et al., (2011). In their 2011 review, the authors proposed a coordinated global census of genome size, referred to as C-value, of angiosperms. According to the authors, genome size and ploidy data can aid in developing priorities for whole genome sequencing.

There are a number of fluorochromes, or stains, that can be used for flow cytometry. They are broken down into three categories: nucleic acid dyes, protein dyes and functional probes. For genome sizing, the most commonly used stains are DAPI (4',6-diamidino-2-phenylindole) and PI (propidium iodide). These two fluorochromes have contrasting binding characters. DAPI binds to A-T rich regions of the nuclear genome; while PI is an intercalating agent that binds indiscriminately to all genetic material including RNA. These contrasting binding characters can be used to infer the base composition of a genome (Meister and Barow, 2007; Parris et al., 2010; Rothleutner, 2012). Base composition can be expressed as either GC%, for proportion
of genome composed of guanine and cytosine, or as AT%, for proportion of genome composed of adenine and thymine.

The objectives of the current study are to determine relative genome sizes and ploidy levels of a diverse and wide-ranging selection of taxa within *Acer* providing a foundation to facilitate future breeding efforts, determine the base composition of a subset of maples in this study, and to add to the growing body of knowledge of genome sizes in angiosperms.

**Materials and Methods**

*Plant material*

Relative genome size was determined for 192 accessions representing 88 species and 18 taxonomic sections (Table 2.2). Plant material from Heritage Seedlings, Hoyt Arboretum, J. Frank Schmidt and Whitman Farm was collected by the author. Other plant material was collected and shipped to OSU by staff at the following institutions: Arnold Arboretum (Harvard University, Boston, MA), Cornell Plantations (Cornell University, Ithaca, NY), Morris Arboretum (University of Pennsylvania, Philadelphia, PA), The Morton Arboretum (Lisle, IL), Quarry Hill Botanical Garden (Glen Ellen, CA), and the United States National Arboretum (Washington, D.C.). Cuttings from each accession were collected and placed in plastic bags. Cuttings sent from other institutions were shipped overnight with ice packs. All material was kept in a walk-in cooler maintained at ~4 °C.

Collection information associated with material was provided by institutions directly or through public databases; in some cases, collection information such as
Chinese province information was inferred through the publicly available literature reporting and summarizing plant collection expeditions (Aiello and Dosmann, 2016; Arnold Arboretum, 2016). Taxonomic relationships were adapted from van Gelderen et al. (1994), eFlora (2016), and Li (2016).

**Flow cytometry**

The relative 2C genome sizes were determined using flow cytometry. *Pisum sativum* ‘Ctirad’ (2C = 8.76 pg) was used as an internal standard based on its common use as a reference standard (Greilhuber et al., 2007; Bai et al., 2012). Three samples were analyzed for each accession, unless otherwise noted in Table 2.2. For each sample, approximately 1 – 2 cm² or 20 mg of fresh expanding leaf and vegetative bud tissue was finely co-chopped with *P. sativum* ‘Ctirad’ in a polystyrene petri dish with 400 µL of nuclei extraction buffer (Cystain® Ultraviolet Precise P Nuclei Extraction Buffer; Sysmex, Görlitz, Germany) using a sharp razor blade. The nuclei suspension was then filtered into sample tubes through 30-µm gauze filters (Partec Celltrics®) and stained with 1.6 mL DAPI staining buffer (Cystain ultraviolet Precise P Staining Buffer; Partec). For base composition analysis, a subset of the samples was measured following the same methods for chopping and filtering using 500 µL nuclei extraction buffer and 1.5 mL of PI solution. The PI solution contained propidium iodide, RNase, and staining buffer; and was prepared according to manufacturer instructions (Cystain® PI Absolute P). After staining, samples were incubated on ice in dark for at least 20 minutes for RNase digestion of RNA. The relative genome size was determined using a flow cytometer with
fluorescence excitation (Partec Cyflow ® Ploidy Analyser). Relative genome size (2C) DNA contents was calculated as:

$$2C = DNA \text{ content of standard} \times \frac{\text{mean fluorescence value of sample}}{\text{mean fluorescence value of the standard}}.$$ 

The relationship between ploidy level and genome sizes was initially determined using documented data (Foster, 1933; Takizawa, 1952; Bayliss and Gould, 1974; Meurman, 2010). Mean 1Cx genome size was calculated as (mean 2C genome size ÷ inferred ploidy level) (Table 2.3). The CV% of relative fluorescence measured was always less than 10 and usually less than 7.

The base pair composition of 51 accessions representing 34 species and 18 taxonomic sections was evaluated. Base pair composition was estimated according to the equation: % AT = % AT for internal standard × [(mean fluorescence standard, DAPI ÷ mean fluorescence sample, DAPI) / (mean fluorescence standard, PI ÷ mean fluorescence sample, PI)]^{1/binding length} (Godelle et al., 1993), where % AT of internal standard = 61.5 and binding length of DAPI = 3.5 bp (Meister and Barow, 2007) (Table 2.4).

**Cytology**

Cytology was completed on 12 species representing nine taxonomic sections (Figures 2.1 and 2.2) representing diploids and one octoploid. For cytological analysis cuttings were rooted or plants were grown from seed. Actively growing root tips were collected before 11 am on mornings following two sunny days. Roots tips were suspended in a pre-fixative solution of 2mM 8-hydroxyquinoline + 0.24 mM cycloheximide in glass vials and incubated in dark on ice for approximately 3 h. After pre-fixative treatment, root
tips were rinsed 3–4 times in distilled H₂O and placed in Carnoy’s solution (one part glacial acetic acid: 3 parts chloroform: 6 parts 100% ethanol) and incubated in dark at room temperature over-night. The following morning, root tips were rinsed four times using 70% ethanol and then stored in refrigerated vials of 70% ethanol until needed.

In preparation for enzyme digestion, tissue was excised from root apical meristems using a scalpel. A 0.5% enzyme solution including cellulase, pectolyase, and cytohelicase dissolved in 50 mM citrate buffer was used to break down cell walls. Root tips were placed in an Eppendorf tube containing enzyme solution, the tube was floated in a water filled beaker, and incubated for approximately 3 hours at ~32 °C. Following incubation, excised tissue was removed from tube using a glass pipette and placed on a microscope slide. Excess liquid was wicked away using single-ply low lint tissue. A drop of modified carbol fuchsin (Kao, 1975) was placed on the excised tissue, and then a coverslip was placed on top. After approximately three minutes, the material was gently squashed. Slides were scanned and micrographs were captured using a light microscope at ×630 and ×1000 (Axio imager.A1: Zeiss, Thornwood, NY; AxioCam MRm, Zeiss). A minimum of 3–5 cells were counted for each accession.

Statistical Analyses

Data analysis for this paper was generated with SAS software, Version 9.4 of the SAS system for Windows Version 6.1.7601. Copyright© 2002 – 2012 SAS Institute Inc. (Cary, NC, USA.) For monoploid (1Cx) genome size means comparison, PROC MIXED (SAS 9.4) was used with the macro pdmix800 for generating lettered groups (Saxton, 2000).
Additional data analysis was completed using RStudio v3.3.1. A paired t-test was used to determine significant difference between the means of relative genome sizes measured using the two different fluorochromes (RStudio v3.3.1); Tukey’s HSD ($\alpha = 0.05$) was used to determine minimum significant difference. Tukey’s HSD at $\alpha = 0.05$ (SAS 9.4) was applied to mean 1Cx values of each section using PROC GLM.

**Results and Discussion**

The 2C relative genome size ranged from 1.39 pg in *A. negundo v. interius* to 6.10 pg in *A. rubrum v. trilobum* reported in Table 2.2. The mean monoploid genome size (1Cx) of taxonomic sections, species, and grex reported in Table 2.3 ranged from 0.43 pg in *A. carpinifolium* (Section *Indivisa*) to 1.66 pg in *A. caudatifolium* (Section *Macrantha*).

Cytology confirmed accessions of *A. campestre* observed in this study are diploids with a mean relative 2C genome size of 1.50 pg (PI) (Table 2.4) compared with the reported 2C value of 1.38 pg (Table 2.1) (Darlington et al., 1956); however, I did not observe any tetraploid *A. campestre* ($2n = 4x = 52$) (Kew RBG, 2016). Another species reported to be tetraploid in the literature is *A. heldreichii* (Section *Acer*). While I did not confirm this directly through cytology, the 2C genome size indicates that this is likely ($2C = 3.51$ pg), although the 2C value measured in this study does not align with the reported value from previous studies ($2C = 5.16$ pg) (Kew RBG, 2016). The monoploid genome size of Section *Acer* was calibrated using root squashes of *A. saccharum*. The tetraploid *A. pseudoplatanus* (Section *Acer*) was also confirmed through genome sizing. Reported 1C value for *A. pseudoplatanus* (Kew RBG, 2016) was 1.35 pg. Based on analysis of *A.
pseudoplatanus (USNA 2836) using PI, this is supported by the current study (1Cx = 0.71 pg, 1C = 1.40 pg).

Cytology confirmed A. rubrum OSU14-0193 is an octoploid (2n = 8x = 104). This chromosome count provided the calibration necessary to determine the ploidy levels of other accessions of Section Rubra. Genome size calibrated with cytology led to the discovery and confirmation of a natural ploidy series in A. rubrum with hexaploids (2n = 6x = 72) and octoploids observed in this study. There was no evidence of tetraploid A. rubrum. The tetraploid A. saccharinum was confirmed through cytological analysis (2n = 4x = 52). While the monoploid genome size of species within Section Rubra appear to be consistent (1Cx = 0.66 pg) among many of the accessions, there still needs to be more work done with A. pycnanthum. This is an uncommon species endemic to the island of Honshu, Japan that has been reported as a hexaploid (2n = 6x = 78); although, if the monoploid of Section Rubra is consistent, then the measured genome size would indicate it is an octoploid (van Gelderen et al., 1994).

The reported tetraploid A. carpinifolium (Section Indivisa) has not been confirmed. This is due to difficulty in sufficiently breaking down the cells walls, and it is the only species available within Section Indivisa. While the cell walls of all other species in the cytological study were effectively broken down with enzyme digestion, A. carpinifolium proved to be recalcitrant. It may be necessary to attempt another method of cell wall digestion such as a combination of enzyme digestion followed by hydrochloric acid hydrolysis. Hydrochloric acid hydrolysis has proven to be an effective
method with woody plants in previous studies (Parris et al., 2010; Lattier et al., 2013; Shearer and Ranney, 2013).

Often genome size data among diverse taxa of a given family are compared using the monoploid genome size due to ploidy variation. There were some significant differences between sections detected when considering monoploid genome size. The greatest mean monoploid genome size ($1C_x = 1.32$ pg) (Table 2.3) was of Section *Macrantha*, significantly greater than all but three sections: *Oblonga*, *Lithocarpa*, and *Parviflora*. Section *Indivisa* had the smallest monoploid genome size based upon reported ploidy and cytometric analysis ($1C_x = 0.43$ pg). Monoploid genome size was wide-ranging, and if intersectional hybridization is possible, monoploid genome size could be used as a tool for assessing hybridization. Monoploid genome size within a section does not appear to be significantly different, with the exception of Section *Pentaphylla* and potentially Section *Rubra*, if *A. pycnanthum* is proven to be a hexaploid.

Based on average monoploid genome sizes calibrated by intrasectional cytological analysis, three potential natural triploids ($2n = 3x = 39$) have been identified. These are the species *A. elegantulum* and *A. sinsense* of Section *Palmata*; and a hybrid accession from The Morton Arboretum (Lisle, IL) identified as a cross between *A. griseum* and *A. triflorum* (Section *Trifoliata*). Confirmation of triploidy through cytological assessment would be ideal. While *A. elegantulum* is the accepted name of the species, another accession in this study carries the synonym *A. olivaceum*. The relative genome sizes of these two plants is significantly different, $2C = 3.01$ pg and $2C = 2.08$ pg, respectively. If the two can be lumped taxonomically, then this difference in
genome size would further support the putative triploidy of *A. elegantulum*. If *A. elegantulum* is indeed a diploid, then this genome size data could support the taxonomic split of the two.

The triploid hybrid at The Morton Arboretum has not yet flowered (personal correspondence, Kris Bachtell). It is of particular interest due to the ex situ conservation efforts of the American Public Gardens Association (APGA) North American Plant Collections Consortium. The paperbark maple, *A. griseum*, is quite common in the ornamental landscape as an attractive small landscape tree that provides multiple seasons of interest; however, in its native habitat it has become rare and was listed as endangered in 2004 (Sun et al., 2014). More recently, there have been significant efforts to evaluate genetic diversity through APGA member institutions including The Morton Arboretum and Hoyt Arboretum (Portland, OR). Preliminary reports indicate that there is little genetic diversity in cultivated clones which can be tracked to just three genetic sources (personal correspondence, Kris Bachtell). Additional evaluation of natural genetic diversity continues with collections from small pockets of populations still found in China. Future breeding efforts will focus on developing diversity through hybridization and potentially mutation treatments. The natural putative triploid hybrid observed in this study may present a dead end, or an opportunity.

In an effort to develop sterile cultivars, the landscape plant breeding program at OSU has developed autotetraploids of several maple species. I confirmed a triploid accession of *A. buergerianum* OSU12-008-004 from this collection that was not developed through interploid crosses. This particular accession can be used to begin
evaluating fertility of triploid cytotypes in *A. buergerianum*. The confirmed tetraploids of *A. platanoides*, *A. buergerianum* and *A. tataricum* ssp. *ginnala* developed in the program will be valuable germplasm in the continued development of sterile cultivars of maples.

In the fluorochrome comparison, there was a significant difference in mean relative genome size measurements at $\alpha = 0.05$ [$t(53)=14.24$, $P < 0.001$] with samples measured using DAPI being consistently larger. This pattern was noted in a study conducted by Doležel et al. (1992) that compared the fluorochromes PI, DAPI, and mithramycin. They concluded that there may be a number of factors resulting in this difference. These had been reported by prior studies and include differences in base composition between the internal standard and the measured sample, differences in binding properties of the fluorochromes, and differences in sequences between the internal standard and the measured sample. Thus there is the potential for overestimation of genome size when using base-specific fluorochromes such as DAPI; however, it is still a useful, effective, efficient, and inexpensive means to the end of estimating a relative genome size. Additionally, it can provide a tool for estimating base composition when used in conjunction with an intercalating dye such as PI.

In a recent genomic characterization study, base composition for sugar maple (*A. saccharum*) was determined using whole genome sequencing. They determined that the GC% for the sugar maple sample was 38.1 (Staton et al., 2015). While *A. saccharum* was not evaluated using PI, four accessions of Section *Acer* were and the average AT% of these four species was 58.8 (Table 2.4), or GC% was 41.2. While monoploid genome size
was wide-ranging with significant differences present, there is little apparent variation in AT\% among the taxa evaluated in this study ranging from just 56.71 – 61.39.

In 1943, Duffield evaluated stomatal measurements as a potential tool for identification of ploidy variation in natural *A. rubrum* populations. He concluded that stomatal comparisons were not a useful tool for identifying natural ploidy differences, but suggested that results may vary for artificial autopolyploids. I evaluated stomatal measurements as a tool for identifying ploidy variation in the *A. buergerianum* ploidy series developed by Contreras. I found no visible differences between the 2x, 3x, and 4x; however, there were visible differences between the 2x and 4x *A. platanoides*. The usefulness of stomatal measurements in identifying ploidy variation in maples may be species specific.

**Conclusion**

This study provides valuable information for maple breeding programs, contributes to the growing database of angiosperm genome size, and provides additional data for flow cytometry methods and material. While overall the coverage of the genus in this study is broad, there are some taxonomic sections in which there is a considerable depth of coverage including Sections *Acer*, *Macrantha*, *Palmata*, and *Platanoidea*. This depth of coverage reflects the current availability of material in arboreta, botanical gardens, and nurseries. Future work can focus on expanding our knowledge of the genus in the areas that have less coverage. Additionally, there is potential for continued cytological studies to provide clarification regarding ploidy and genome size. While the use of DAPI was
not accurate in the measurement of genome size, it certainly was precise and was the
most inexpensive means to acquire a significant amount of data.

References


<http://sites01.lsu.edu/faculty/exstlab/wp-content/uploads/sites/87/2016/04/pdmix800.txt>


Table 2.1. Previously reported chromosome numbers and genome sizes (pg) for maple species evaluated in this study.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Synonyms</th>
<th>Previous findings</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. argutum Maxim.</td>
<td></td>
<td>2n = 2x = 26</td>
<td>Darlington et al., 1956</td>
</tr>
<tr>
<td>A. campestre L.</td>
<td></td>
<td>2n = 2x = 26, 2C = 1.38</td>
<td>Darlington et al., 1956</td>
</tr>
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<td></td>
<td></td>
<td>2n = 4x = 52, 2C = 2.70</td>
<td>Kew RBG, 2016</td>
</tr>
<tr>
<td>A. carpinifolium Siebold &amp;</td>
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<td>2n = 4x = 52, 2C = 1.52</td>
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<td>Zucc.</td>
<td></td>
<td></td>
<td>RBG, 2016</td>
</tr>
<tr>
<td>A. cirsinatum Pursh</td>
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<td>2n = 2x = 26</td>
<td>Darlington et al., 1956</td>
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<td>A. crataegifolium Siebold &amp;</td>
<td></td>
<td>2n = 2x = 26</td>
<td>Darlington et al., 1956</td>
</tr>
<tr>
<td>Zucc.</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A. diabolicum Blume ex K.</td>
<td></td>
<td>2n = 2x = 26</td>
<td>Darlington et al., 1956</td>
</tr>
<tr>
<td>Koch</td>
<td></td>
<td></td>
<td></td>
</tr>
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<td>A. griseum (Franch.) Pax</td>
<td></td>
<td>2n = 2x = 26</td>
<td>Darlington et al., 1956</td>
</tr>
<tr>
<td>A. heldreichii Orph. ex</td>
<td></td>
<td>2n = 4x = 52, 2C = 5.16</td>
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<td>Boiss.</td>
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<td>A. japonicum Thunb.</td>
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<td>Darlington et al., 1956</td>
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<td>A. miyabei Maxim.</td>
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<td>Darlington et al., 1956</td>
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<td></td>
<td></td>
<td></td>
<td>2016</td>
</tr>
<tr>
<td>A. nikoense (Miq.) Maxim</td>
<td></td>
<td>2n = 2x = 26</td>
<td>Darlington et al., 1956</td>
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<td>A. opalus ssp. obtusatum</td>
<td>A. obtusatum</td>
<td>2n = 2x = 26, 2C = 1.56</td>
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<td>(Waldst. &amp; Kit. ex Willd.)</td>
<td>Waldst. &amp; Kit. ex Willd.</td>
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<td>Gams</td>
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<td>A. palmatum Thunb.</td>
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<td>Darlington et al., 1956</td>
</tr>
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<td>A. pictum Thung. ex Murray</td>
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<td>Darlington et al., 1956</td>
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<td>2n = 3x = 39</td>
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<td>(Pax) Kom.</td>
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<td>A. rubrum L.</td>
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<td>2n = 6x = 78</td>
<td>Darlington, 1956; Santamour, 1965</td>
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<td>A. rufinerve Siebold &amp; Zucc.</td>
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<td>2n = 8x = 104</td>
<td>Santamour, 1965</td>
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<tr>
<td>A. saccharum Marshall</td>
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<td>Darlington, 1956; Santamour, 1971; van Gelderen et al., 1994</td>
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<tr>
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<td>Darlington, 1956; Santamour, 1971; van Gelderen et al., 1994</td>
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<td>A. tataricum L.</td>
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<td>A. tataricum ssp. ginnala L.</td>
<td>A. ginnala Maxim.</td>
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<td>Darlington et al., 1956; Santamour, 1971</td>
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<tr>
<td>A. tschonoskii Maxim.</td>
<td></td>
<td>2n = 2x = 26</td>
<td>Darlington, 1956; Santamour, 1971</td>
</tr>
</tbody>
</table>


Table 2.2. Mean relative genome sizes, putative ploidy, and provenance and/or native range of Acer species, cultivars and hybrids using DAPI.

ARN = Arnold Arboretum (location), COR = Cornell Plantations (location), HOYT = Hoyt Arboretum (Portland, OR) JFS = J. Frank Schmidt Arboretum (location), MRS = Morris Arboretum (location), MRT = Morton Arboretum (location), OSU = Oregon State University Ornamental Plant Breeding Collection, OSU-campus = OSU campus landscape plants, OSU-FL = field planted material located at the Lewis-Brown Farm (Corvallis, OR), QHBG = Quarry Hill Botanical Garden (location), USNA = United States National Arboretum (Washington D.C.), WHT = Whitman Farms (Salem, OR); *Provenance: W = collected in wild, Z = from cultivated plant of known wild origin, G = cultivated plant of garden origin, U = unknown. If *, then area listed is known to be where seed or propagule was collected from. Native distribution indicates general distribution of the species; †indicates ploidy was determined with cytology; ‡Only two values used to calculate average. ‡Putative ploidy was inferred using the 1Cx value of other species of the same Section, however additional cytology work should be conducted to confirm the accuracy of this assessment. ‡‡Putative ploidy reported in the literature, should be confirmed with additional cytology work.
Table 2.2. Mean relative genome sizes, putative ploidy, and provenance and/or native range of Acer species, cultivars and hybrids using DAPI.

<table>
<thead>
<tr>
<th>Taxa</th>
<th>Source/ Accession #</th>
<th>Mean relative 2C GS (pg) [mean ± SEM]</th>
<th>Putative ploidy level (x)</th>
<th>Provenance/Native distribution</th>
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<td><strong>Section Acer</strong></td>
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<td><em>A. caesium</em> Wall. ex. Brandis</td>
<td>QHBG1995-051</td>
<td>2.06 ± 0.02</td>
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<td>W; Tibet*</td>
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<td><em>A. caesium</em></td>
<td>MRS1994-005</td>
<td>2.06 ± 0.04</td>
<td>2</td>
<td>W; W. Himalayas*</td>
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<td><em>A. grandidentatum</em> Nutt.</td>
<td>MRT276-742</td>
<td>1.67 ± 0.01</td>
<td>2</td>
<td>W; Guadalupe Mts., Eddy Co., NM</td>
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<td><em>A. heldreichii v. macropterum</em> Pax</td>
<td>ARN200-85A</td>
<td>3.47 ± 0.04</td>
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<td><em>A. heldreichii ssp. troutvetteri</em> (Medw.) A.E. Murray</td>
<td>MRS2004-172</td>
<td>3.55 ± 0.06</td>
<td>4</td>
<td>W; Caucasus, N. Turkey*</td>
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<td><em>A. hyrcanum</em> Fisch. &amp; C.A. Mey.</td>
<td>MRT67-2001*1</td>
<td>1.88 ± 0.03</td>
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<td>W; W. Asia*</td>
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<td><em>A. opalus</em> Mill.</td>
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<td><em>A. pseudoplatanus</em> L.</td>
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<td><em>A. saccharum</em> Marshall</td>
<td>OSU14-0147</td>
<td>1.84 ± 0.09</td>
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<td>1.70 ± 0.02</td>
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Table 2.2 (Continued)

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<th>Putative ploidy level (x)</th>
<th>Provenance/Native distribution</th>
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<td>Z</td>
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<td><em>A. saccharum</em> ssp. <em>skutchii</em></td>
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<td>Z; Mexico - Guatemala</td>
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<td><em>A. × coriaceum</em> Bosc. ex Tausch</td>
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<td><em>(A. monspessulanum x A. opalus)</em></td>
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</table>

**Section Arguta**

<p>| <em>A. acuminatum</em> Wall. ex D. Don           | QHBG1993-076           | 1.97 ± 0.003                          | 2                         | W; Himachal Pradesh, India*    |
| <em>A. acuminatum</em>                           | QHBG1993-039           | 1.93 ± 0.04                           | 2                         | W; Himachal Pradesh, India*    |
| <em>A. acuminatum</em>                           | QHBG1993-139           | 1.93 ± 0.02                           | 2                         | W; Himachal Pradesh, India*    |
| <em>A. acuminatum</em>                           | MRS1994-009            | 1.92 ± 0.04                           | 2                         | W; Himalayas                   |
| <em>A. argutum</em> Maxim.                       | ARN640-77B             | 1.91 ± 0.06                           | 2                         | W                              |
| <em>A. argutum</em>                              | OSU14-0194             | 1.78 ± 0.01                           | 2*                        | G                              |
| <em>A. barbinerve</em> Maxim.                    | MRT258-2002<em>1          | 1.96 ± 0.02                           | 2                         | W; Shaanxi Province, China</em>    |
| <em>A. barbinerve</em>                           | USNA68777              | 1.77 ± 0.00                           | 2                         | W; Jilin Province, China*      |
| <em>A. stachyophyllum</em> ssp. <em>betulifolium</em>   | MRT854-2005<em>2          | 1.94 ± 0.01                           | 2                         | W; Gansu Province, China</em>      |</p>
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<tr>
<th>Taxa</th>
<th>Source\ Accession #</th>
<th>Mean relative 2C GS (pg) [mean ± SEM]</th>
<th>Putative ploidy level (x)</th>
<th>Provenance/Native distribution†</th>
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<td><em>A. forrestii</em> Diels</td>
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<td><strong>Section Trifoliata</strong></td>
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<td><em>A. griseum</em> (Franch.) Pax</td>
<td>HOYT1998-023</td>
<td>1.93 ± 0.06</td>
<td>2</td>
<td>*G</td>
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<td><em>A. maximowiczianum</em> Miq.</td>
<td>OSU14-0148</td>
<td>2.30 ± 0.22</td>
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<td>Heritage Seedlings, Salem, OR</td>
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<td><em>A. nikoense</em> (Miq.) Maxim</td>
<td>HOYT1969-3958</td>
<td>1.90 ± 0.06</td>
<td>2</td>
<td>*U</td>
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<td><em>A. triflorum</em> Kom.</td>
<td>OSU14-0143</td>
<td>2.05 ± 0.01</td>
<td>2*</td>
<td>*U</td>
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<td><em>A. triflorum</em></td>
<td>USNA58016</td>
<td>1.80 ± 0.03</td>
<td>2</td>
<td><em>W; Korea</em></td>
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<tr>
<td><em>A. griseum</em> × <em>A. nikoense</em> [Rochester Group]</td>
<td>MRT243-94*1</td>
<td>1.96 ± 0.02</td>
<td>2</td>
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<tr>
<td><em>A. griseum</em> × <em>A. triflorum</em></td>
<td>MRT70-2011*1</td>
<td>2.85 ± 0.04</td>
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<td>*G</td>
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<td><em>A. maximowiczianum</em> × <em>A. griseum</em></td>
<td>ARN641-91A</td>
<td>2.14 ± 0.01</td>
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Table 2.3. Average monoploid genome size (pg) for taxonomic sections, species, and grex of *Acer*.

<table>
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<tr>
<th>Taxonomic section</th>
<th>1C* (pg)</th>
<th>Species/grex</th>
<th>1C* (pg)</th>
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<tbody>
<tr>
<td>Acer</td>
<td>0.93 ± 0.02 DE³</td>
<td><em>A. caesium</em></td>
<td>1.03 ± 0.02</td>
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<tr>
<td></td>
<td></td>
<td><em>A. heldreichii</em></td>
<td>0.88 ± 0.01</td>
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<tr>
<td></td>
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<td><em>A. grandidentatum</em></td>
<td>0.84 ± 0.00</td>
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<tr>
<td></td>
<td></td>
<td><em>A. hyrcanum</em></td>
<td>0.97 ± 0.03</td>
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<tr>
<td></td>
<td></td>
<td><em>A. monspessulanum</em></td>
<td>1.01 ± 0.01</td>
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<tr>
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<td><em>A. opalus</em></td>
<td>1.02 ± 0.03</td>
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<td><em>A. pseudoplatanus</em></td>
<td>0.86 ± 0.03</td>
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<tr>
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<td><em>A. saccharum</em></td>
<td>0.89 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. sempervirens</em></td>
<td>0.95 ± 0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>A. velutinum</em></td>
<td>0.89 ± 0.05</td>
</tr>
<tr>
<td></td>
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<td><em>A. xcoriaceum</em></td>
<td>0.93 ± 0.02</td>
</tr>
<tr>
<td>Arguta</td>
<td>0.95 ± 0.01 CDE</td>
<td><em>A. acuminatum</em></td>
<td>0.97 ± 0.01</td>
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<tr>
<td></td>
<td></td>
<td><em>A. argutum</em></td>
<td>0.92 ± 0.03</td>
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<tr>
<td></td>
<td></td>
<td><em>A. barbinerve</em></td>
<td>0.93 ± 0.05</td>
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<tr>
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<td></td>
<td><em>A. stachyophyllum</em></td>
<td>0.97 ± 0.01</td>
</tr>
<tr>
<td>Ginnala</td>
<td>0.80 ± 0.01 EFH</td>
<td><em>A. tataricum</em></td>
<td>0.83 ± 0.01</td>
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<tr>
<td></td>
<td></td>
<td><em>A. tataricum ssp. aidzuense</em></td>
<td>0.82 ± 0.01</td>
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<td><em>A. tataricum ssp. ginnala</em></td>
<td>0.79 ± 0.01</td>
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<td>Glabra*</td>
<td>0.79 ± 0.00 EFGHI</td>
<td><em>A. glabrum</em></td>
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<tr>
<td>Indivisa</td>
<td>0.43 ± 0.02 I</td>
<td><em>A. carpinifolium</em></td>
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<tr>
<td>Lithocarpa</td>
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<td><em>A. sterculiceum</em></td>
<td>1.14 ± 0.01</td>
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<tr>
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<td><em>A. yangbiense</em></td>
<td>0.95 ± 0.02</td>
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<td>Macrantha</td>
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<td><em>A. crataegifolium</em></td>
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<td><em>A. davidii</em></td>
<td>1.28 ± 0.01</td>
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<td><em>A. forrestii</em></td>
<td>1.39 ± 0.04</td>
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<td><em>A. laxiflorum</em></td>
<td>0.81 ± 0.01</td>
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<td><em>A. morrisonense</em></td>
<td>1.46 ± 0.02</td>
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<td><em>A. pectinatum</em></td>
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<td><em>A. pensylvanicum</em></td>
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<td><em>A. rubescens</em></td>
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<td><em>A. rufinerve</em></td>
<td>1.40 ± 0.06</td>
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<td><em>A. tegmentosum</em></td>
<td>1.26 ± 0.04</td>
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<td><em>A. tschonoskii</em></td>
<td>1.08 ± 0.02</td>
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<td></td>
<td><em>A. davidii x tegmentosum</em></td>
<td>1.38 ± 0.03</td>
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<td>Macrophylla</td>
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<td><em>A. macrophyllum</em></td>
<td>0.83 ± 0.01</td>
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Table 2.3. (Continued)

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<th>Species/grex</th>
<th>1Cx (pg)</th>
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<td>A. henryy</td>
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<td>A. negundo</td>
<td>0.72 ± 0.02</td>
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<tr>
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<td>A. oblongum*</td>
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<td>A. amoenum*</td>
<td>1.00 ± 0.01</td>
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<td>A. erianthum*</td>
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<td>A. fabri</td>
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<td>A. japonicum*</td>
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<td>A. olivaceum*</td>
<td>1.04 ± 0.01</td>
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<td>A. pauciflorum</td>
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<td>A. pseudosieboldianum</td>
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<td>A. pubinerve</td>
<td>1.05 ± 0.02</td>
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<td>A. pubipalmatum</td>
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<td>A. wuyuanense</td>
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<td>A. yui*</td>
<td>0.78 ± 0.01</td>
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<td>1.11 ± 0.03</td>
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<td>A. campestre</td>
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<td>A. miyabei</td>
<td>1.00 ± 0.04</td>
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<td>A. okamotoanum</td>
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<td>A. pictum</td>
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Table 2.3. (Continued)

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<th>Taxonomic Section</th>
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<th>Species/grex</th>
<th>1Cx (pg)</th>
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<td>A. shenkanense</td>
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<td>A. truncatum</td>
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<td>0.95 ± 0.01</td>
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<tr>
<td>A. platanoides x A. truncatum</td>
<td>0.91 ± 0.01</td>
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<td><strong>Pubescentia</strong></td>
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<td>A. spicatum*</td>
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<td><strong>Spicata</strong></td>
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<tr>
<td>A. maximowiczianum</td>
<td>1.11 ± 0.02</td>
<td>A. griseum x A. nikoense*</td>
<td>0.98 ± 0.01</td>
</tr>
<tr>
<td>A. nikoense*</td>
<td>0.95 ± 0.03</td>
<td>A. griseum x A. triflorum*</td>
<td>0.95 ± 0.01</td>
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<tr>
<td>A. triflorum*</td>
<td>0.90 ± 0.06</td>
<td>A. maximowiczianum x A. griseum*</td>
<td>1.07 ± 0.01</td>
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<td>A. maxima</td>
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*Three samples of one accession was used to calculate the average 1Cx value.

Sectional means separated using Tukey’s multiple means comparison test (α = 0.05). Means followed by the same letter are not significantly different.
Table 2.4 Base pair composition determined by comparison of two fluorochromes DAPI and PI.

<table>
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<th>Source/Acc.</th>
<th>Relative 2C genome size (pg)</th>
<th>%AT&lt;sup&gt;y&lt;/sup&gt;</th>
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<td><em>A. grandidentatum</em></td>
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<td>1.63 ± 0.01</td>
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<td>3.29 ± 0.08</td>
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<td>1.50 ± 0.01</td>
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<td>1.62 ± 0.02</td>
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2 ARN = Arnold Arboretum (location), COR = Cornell Plantations (location), HOYT = Hoyt Arboretum (Portland, OR) JFS = J. Frank Schmidt Arboretum (location), MRS = Morris Arboretum (location), MRT = Morton Arboretum (location), OSU = Oregon State University Ornamental Plant Breeding Collection, OSU-campus = OSU campus landscape plants, OSU-FL = field planted material located at the Lewis-Brown Farm (Corvallis, OR), QHBG = Quarry Hill Botanical Garden (location), USNA = United States National Arboretum (Washington D.C.), WHT = Whitman Farms (Salem, OR)

Tukey’s HSD = 0.20 pg comparing DAPI to PI; ns, nonsignificant; *, significant

% AT = % AT for internal standard × [(mean fluorescence standard, DAPI ÷ mean fluorescence sample, DAPI) / (mean fluorescence standard, PI ÷ mean fluorescence sample, PI)]^{1/binding length} (Godelle et al., 1993), where % AT of internal standard = 61.5 and binding length of DAPI = 3.5 bp (Meister and Barow, 2007).
Figure 2.1 Photomicrographs of root apical meristem cells

(A) *A. albopurpurascens* QHBG2003-088 (2\(n = 2x = 26\)),  
(B) *A. amplum* QHBG2009-235 (2\(n = 2x = 26\)),  
(C) *A. argutum* OSU14-0194 (2\(n = 2x = 26\)),  
(D) *A. buergerianum* OSU14-0158 (2\(n = 2x = 26\)),  
(E) *A. campestre* OSU14-0196 (2\(n = 2x = 26\)),  
(F) *A. davidii* OSU14-0162 (2\(n = 2x = 26\))
Figure 2.1. Photomicrographs of condensed and modified carbol fuchsin stained root apical meristem cells.
Figure 2.2. Photomicrographs of root apical meristems.

(A) *A. macrophyllum* OSU14-0059-01 (2n = 2x = 26), (B) *A. maximowiczianum* OSU14-0150 (2n = 2x = 26),
(C) *A. rubrum* OSU14-0193 (2n = 8x = 104), (D) *A. saccharum* OSU14-0147 (2n = 2x = 26), (E) *A. tataricum* OSU14-0202 (2n = 2x = 26),
(F) *A. triflorum* OSU14-0143 (2n = 2x = 26)
Figure 2.2. Photomicrographs of root apical meristem cells.
Inducing and Evaluating Phenotypic and Cytometric Variation in Landscape Plants: Observations from Acer, Ornithogalum, and Penstemon

Chapter Three:
Evaluating effects of ethyl methane sulfonate (EMS) treatment on Ornithogalum candicans (Baker) J.C. Manning & Goldblatt (Asparagaceae)

Ornithogalum candicans (Baker) J.C. Manning & Goldblatt (synonym Galtonia candicans Decne.) is an herbaceous perennial native to elevations of 1350 – 2150 m in the Drakensberg region of the Great Escarpment in South Africa (Hilliard and Burtt, 1988). It is naturally self-pollinating and can be clonally propagated by bulb division or twin-scaling. Fragrant white flowers are borne on racemes that emerge from a crown of strap-like foliage from August to September in the Northern Hemisphere (Armitage, 2008). Inflorescences of O. candicans can grow up to four feet; however, it has been noted that the species suffers from lodging due to the length of the peduncle and lack of sufficient support (personal correspondence, Ryan Contreras). Lodging is when the inflorescence of the plant falls over at ground level. Ornithogalum candicans exhibits hardiness for gardens of North America (Armitage, 2008), and there has been little cultivar development. The only cultivar that has been described is the double-flowered O. ‘Moonbeam’, discovered in a New Zealand garden in 1982 (Hammett, 1993).

Mutagenesis is a common method used by plant breeders to induce genetic variation in plants, and can be particularly useful for naturally self-pollinating plants (Waugh et al., 2006). Ethyl methane sulfonate (EMS) treatment is sometimes a preferred mutagenesis method due to ease of access, its chemical mode of action, and cost when compared to methods such as exposure to radiation. EMS treatments result
in point mutations in plants by attaching an ethyl group to the oxygen atom of guanine. The direct result of this mutation is the transition from a G/C pairing to an A/T pairing in the genome (Waugh et al., 2006). Prior studies have demonstrated varying effects on germination and survival rates relative to varying concentrations of EMS treatment, as well as changes in seed dormancy (Greer and Rinehart, 2009; Talebi et al., 2012).

Weigle and Butler (1983) reported hereditary dwarfing effects after EMS treatment of Impatiens platypeta Lindl. seeds; and in Capsicum annuum L., Jabeen and Mirza (2004) demonstrated EMS was effective in inducing dwarf mutations and sterility.

In 2011, seeds of *O. candicans* were treated with EMS at Oregon State University with the objective of producing an improved cultivar for gardens. The criteria for improved cultivars include reduced scape height at first flower. This would provide a potential containerized summer flowering perennial for retail sales. Additionally, this phenotype may reduce lodging. The objectives of this study are to evaluate the effects of varying concentrations of EMS on mutagenesis of *Ornithogalum candicans* and make selections for improved garden forms.

**Materials and Methods**

**Mutagenesis**

In 2011, seed from landscape plants of *O. candicans* at Oregon State University North Willamette Research and Extension Center (Aurora, OR) were collected. An experiment was conducted following a completely randomized design with a factorial arrangement of treatments. Treatments consisted of two factors, a pre-soak factor (pre-soak vs. no presoak) and an EMS concentration factor (0% control, 0.2%, 0.4%,...
0.6%, 0.8%, 1.0%). The 2 x 6 factorial arrangement of treatments produced 12 total treatments. Each treatment was replicated three times on experimental units consisting of 300 seeds each (total of 36 experimental units). In the pre-soak treatment, seeds were soaked in water for 24 hours prior to EMS treatment. EMS treatments were applied as a 24 hour soak. For all soaks, seeds were incubated in a 0.5 L flask placed on a shaker. Following treatments, seed lots were sown in separate 0.2 L containers for germination.

**Germination and field planting of M<sub>1</sub> and M<sub>2</sub> generation plants**

In February of 2012, germinated seedlings were pricked and transplanted to bark media in 0.37 L square polypropylene pots (Kord, Canada); and grown in a poly-house at the OSU Lewis-Brown Farm. In October of the same year, plants were placed in a dark 4 °C cooler for a 90-day vernalization treatment. Following the vernalization treatment, the plants were moved into a greenhouse and grown until May 2013. In May 2013, plants were field planted at the Lewis-Brown farm. Treatments were kept together in rows (not randomized) within the field and the reps for each treatment were blocked. Germination and survival data was collected.

In the fall of 2014, open-pollinated M<sub>2</sub> seed and open-pollinated control seed were collected from field plants and counted. Data was recorded as an average number of seed per capsule. Initially, the objective for developing an M<sub>2</sub> population was to select females with scape height at first flower in the lowest 5%; however, many of the plants with the lowest plant heights were also sterile. Females were selected based upon final plant height and seed availability. Selected females are presented in Table
3.1 with scape height at first flower recorded in 2014. Seeds were germinated in a glasshouse in winter of 2015 in a preliminary germination study with a 16 hour photoperiod. No vernalization was applied to these seedlings. The seedlings were pricked and transplanted to #38 Star Nursery tree tube deep trays in March 2014 and grown in a glasshouse until transplanted in May 2014. Medium was 1 perlite : 2 soilless media (Metro-Mix® 350, Sungro Horticulture Canada Ltd., Agawam, MA). Rows were trenched and bulbs with multiple sets of true leaves were planted at a depth of approximately 20 cm.

A seed germination study was initiated in February 2016. Open-pollinated seeds were collected from nine randomly selected M1 plants in the field. Seeds were sown in soilless media (Metro-Mix® 350) in 0.2 L containers at a depth of ~1 cm and ~2.5 cm apart. Containers were placed in a completely randomized design in a glasshouse of the OSU West Greenhouses, Corvallis, OR with an average temperature of 22 °C. Media was kept moist with regular watering. Due to almost no seed development from 0.4% EMS treatment plants, only control and 0.2% EMS treated plants were evaluated in the germination study. For each experimental unit, 10 seeds were sown. Germination data was collected in May 2016. A one-way ANOVA was performed and a t-test produced confidence intervals.

Field evaluation

Over the summers of 2014 and 2015, M1 generation of field plants were evaluated for morphological differences relative to EMS treatment. Qualitative and quantitative data were collected as response variables for three EMS treatments (0%,...
0.2%, and 0.4%). Quantitative data analyzed included scape height (Figure 3.1) at first flower and average seed set per capsule. Qualitative data analyzed was for lodging (positive or negative). An ANOVA was performed for each of these response variables, and means separation was performed using Fisher’s LSD (PROC GLM, SAS 9.4). PROC LOGISTIC (SAS, 9.4) was used to analyze lodging data.

**Micropropagation**

A completely randomized factorial micropropagation experiment to initiate protocol development was started in September 2015. Explant material used included leaf (L), ovary (O), pedicel (P), ovary + pedicel (OP), and rachis (R) tissue from randomly selected M₁ and control plants. Explant material was collected in the morning before 10 am. Material was sterilized by soaking and swirling in 0.5% sodium hypochlorite solution for ~15 minutes and rinsed 4 times in sterile DI H₂O. Factors in experiment included explant type and media. Data was unbalanced due to availability of material. Four media were prepared with varying concentrations of NAA and BAP and ½ strength MS basal salts and vitamins (PhytoTechnology Laboratories®, Shawnee Misson, KS). Complete media formulation can be found in Table 3.2. Media (15 mL) were aliquoted into 150 mm glass tubes. Explant material was cut on sterile paper plates using sterile scalpels. Sterile forceps were used to place explant tissue on medium surface. Leaf tissue was cut into squares. Ovaries were sliced in cross sections and laid flat on medium. Pedicel tissue was wounded, and stuck in medium. Ovary + pedicel explants were wounded and placed upright in medium with pedicel in medium. Rachis tissue was sliced in cross
sections and placed on medium wounded side down. Tubes were capped and sealed with parafilm, and placed in incubator maintained at room temperature (~25 °C) in dark. Nine weeks after initiation, tubes were transferred to growth chamber maintained at room temperature with a 16-hour photoperiod (50 µmol m\(^{-2}\) s\(^{-1}\)). Light was measured with Apogee Model QMSW Quantum Meter (Logan, UT) with calibration switch using the electric light setting. Data for shoot count, root count, and root hair development was collected 20 weeks after initiation, and each tube of microshoots was subcultured into Magenta™ (Lockport, IL) boxes and 150 mm glass tubes containing Treatment 1 media described in Table 3.2 for continued shoot multiplication. A small sample of excess material with root development was removed from culture and transplanted to soilless media in a humidity chamber under low light. Four weeks after transplanting and transfer to humidity chamber, these plants were moved to the greenhouse and slowly acclimated to greenhouse conditions. Data were analyzed as a two-factor experiment with shoot number and root number as response variables and media and explant type as class variables.

**Fertility assessment**

Flowers were collected from randomly selected M\(_1\) plants (25 control, 26 0.2%, 19 0.4%) in the field for a pollen-staining test. Pollen was wet mounted using a drop of 1% acetocarmine. For each rep and treatment in the field, the pollen of 5 – 12 flowers was analyzed using one slide per flower. For each slide, 100 pollen grains were counted and scored as viable or nonviable. Pollen was scored as viable if stain was taken up into the
pollen grain. If no stain was visible within the pollen grain, then it was scored as non-viable (Figure 3.2).

In November 2015, plants were selected from across treatments based on form for lifting and transplanting into containers. Bulbs were lifted, washed, and dried on brown paper at room temperature (Figure 3.3). After drying, bulbs were stored in brown paper bags in a walk in cooler maintained at ~4 °C. Bulbs were removed from the cooler and planted in 3 L containers in a soilless bark media (Rexius, Eugene, OR) at a depth of ~20 cm in March 2016. Containers were kept in a poly house at the Lewis-Brown Farm until leaf emergence in April 2016.

In July 2016, a female fertility study was initiated. The crossing design for this study is detailed in Table 3.3. While this data has not been collected due to late flowering of some container planted accessions, fruit are developing and seed data will be collected in the coming year. The objective of this study was to clarify the cause of infertility in mutant Ornithogalum. Three female parents were randomly selected from the 0.2% EMS and 0.4% EMS M1 containerized plants. While 0.2% EMS females do set seed, pollen staining studies showed reduced viability in pollen. I would like to determine if these females are being self-pollinated, or if they are out-crossing to control plants nearby. For each female parent, four scapes were randomly selected. All but seven flowers were removed from each scape. Two emasculated scapes were used for controlled crosses using a control (0% EMS) pollen parent and bagged. One scape was bagged for self-pollination, and the fourth scape was left unbagged for open-pollination. Data collected will include fruit set and average seed per capsule.
Results and Discussion

M1 Field Plants

Germination data collected post-EMS treatment demonstrated a significant decrease in germination percent with an increase in EMS concentration in the M1 population (Figure 3.4), as well as lethal effects of increased EMS concentration. However, it should be noted, that germination results in a preliminary germination study of open-pollinated seed from mutant and control plants were notably greater. For example, the initial average germination of control seeds in 2011 was 37%, while the average germination of control seeds in a preliminary germination study in 2015 was 89%. This is may be due to density of seed sown. There were 300 seed sown per experimental unit in 2011, and just 30 seeds sown per experimental unit in 2015. The containers used to germination seedlings in initial EMS experiment and in germination experiment were the same size (0.2 L). Germination percent observed through a second germination experiment conducted in 2016 was consistent with the results of the 2015 experiment.

Density of seed sown has been demonstrated as inhibitory to seed germination, once some seedlings are already established, due to resource competition (Inouye, 1980). In observing germination of O. candicans, I did note that there is a difference in germination timing, with some seeds germinating weeks before others in the same container. A potential cause of inhibition of germination could be allelopathic interactions between the seedlings demonstrating accelerated germination and seeds not yet germinated; or there could be signaling occurring between seeds prior to
germination (Picman and Picman, 1984; Tielbörger, 2009; Houseman, 2015). There was a report addressing the effects of reduced red and infrared radiation exposure; however, sowing depth of that study was relatively shallow, 2 - 6 mm compared to the sowing depth of this study at ~2 cm; and seeds of that study (Lactuca) were much smaller and have a less developed seed coat than that of O. candicans (Woolley and Stoler, 1978). Additionally, the media in which seeds were sown for the initial EMS experiment was bark medium that had been stored outdoors without any protection or sterilization. There is the potential that the medium was contaminated by pathogens. In another germination project in the same year, Contreras (personal correspondence) noted that Pythium was identified as a contaminant in the bark medium causing damping off.

Analysis of scape height recorded in 2014 and 2015 showed significant differences between treatments. Scape height at first flower decreased with increasing EMS concentration (Figure 3.5). Scape height at first flower was evaluated due to the selection criterion for this project—reduced scape height for containerized flowering perennial. Based on field observations, there are a number of forms that are reduced in height as well as producing many flowering scapes throughout the summer.

Initially, reduced fertility was first observed as reduced fruit set during data collection of summer 2014. Due to initial observations, seed germination, pollen staining, and female fertility studies were executed in 2015 – 2016. Fertility of M₁ plants was significantly reduced with increasing EMS concentration. This was evident in seed set and seed germination data collected 2014 – 2016 (Table 3.4).
The mode of sterility was investigated through pollen viability screening in summer 2015. The uptake of 1% acetocarmine by pollen is significantly reduced with increasing EMS concentration suggesting there was a decrease in viability. Control pollen viability was 83%, 0.2% EMS pollen viability was 21%, and the 0.4% EMS pollen viability was 3%. While this is a potential source of self-infertility, there is still the potential of cross-pollination through pollinator visitations. Many pollinators were observed in the field, including flies, bees, and hummingbirds. This could explain the seed found on a 0.4% EMS plant when no viable pollen was observed in samples taken from these plants.

Female fertility will continue to be evaluated in the coming year with data collection from the controlled crosses conducted in July 2016. While pollen appears to have reduced fertility in staining experiments, pollination is occurring in the field. The 0.2% EMS seed parents are receiving pollen from either self-pollination or cross-pollination. The 0.4% EMS seed parents are not being self-pollinated, and rarely cross-pollinated. Potentially this is due to the lack of pollen available to attract most pollinators. Pollen germination experiments may provide additional information regarding 0.2% EMS pollen viability. While pollen may be respiring it is possible that pollen tube growth is stunted.

Lodging data was recorded and analyzed, and there was no significant difference detected in the M₁ data based on a scoring system of 0 for no lodging and 1 for lodging. Proportions of lodging and non-lodging plants are presented in Table 3.5. Field observations indicate that lodging may be affected by cultural practices rather than
genotype. While there was lodging observed in the M<sub>1</sub> field, there were no such observations of controls in the M<sub>2</sub> field. The difference in planting depth most likely contributed to this. Bulblets planted in the M<sub>1</sub> field were planted at a depth of approximately 5 cm compared to planting depth of M<sub>2</sub> plants at 20 cm. We planted the second generation deeper due to noted observations on the exposure of bulb crowns in the shallowly planted field. Other cultural practices to consider are growing conditions of seedlings. Seedlings of the M<sub>1</sub> generation were grown in relatively shallow containers; while M<sub>2</sub> seedlings were transplanted to tubes to provide space for root growth. Figure 3.6 shows the length of roots at the seedling stage prior to transplanting. At field planting, M<sub>2</sub> were considerably larger than the M<sub>1</sub> bulblets (personal correspondence, Jason Lattier).

**Micropropagation**

When media treatment, mutation treatment, and genotype were analyzed as factors, there were no significant effects or interactions. There was no evidence of interaction effects between the factors explant type and media treatment. Explant type proved to have a significant effect on shoot and root number. Two-factor ANOVAs for shoot and root response to the factors of explant type and media treatment were done (Tables 3.6 and 3.7). The explant type with the most shoot development was rachis tissue. The explant types with best root development were rachis and pedicel tissue (Table 3.8). Ovaries did not produce any shoots, and produced very few roots. These results are in partial agreement with those of a study conducted by Drewes and van Staden (1993) in which the best results were produced from pedicel explants.
Rachis tissue and pedicel tissue contain vascular cambium, a source of meristematic cells (Esau, 1953). Meristematic cells provide undifferentiated cells from which organs such as roots and shoots can develop with exposure to the appropriate levels of plant growth regulators (Li et al., 2009). It has been demonstrated in previous studies of *Eucalyptus benthamii* Maiden and Cambage and *Passiflora cincinnata* Mast. that roots and shoots can arise from vascular cambium in vitro (Lombardi et al., 2007; Brondani et al., 2012). While Drewes and van Staden (1993) reported successful hardening off, microplants removed out of culture in this study displayed considerable lag effects.

**M₂ Field Plants**

Following planting, leaves senesced and plants went dormant. Leaf re-emergence began in late July and continued until frost. Flowering began in late August 2015, and continued until frost. The first full growing season of data collection is currently underway and incomplete. Any hereditary mutations cannot be evaluated until data collection is completed. Further selections of M₂ field plants should continue in fall 2016, and optimized propagation protocol should be developed for efficient and effective means of cloning selections. Recommended methods include twin-scaling, division, and micropropagation. Plants produced from this technique should be trialed in a garden or field setting to ensure stability of desired traits.

**Conclusion**

Seed treatment with EMS was an effective method of decreasing height in *Ornithogalum candicans*, and thus inducing phenotypic variation in this group of plants.
Reduced fertility was an unexpected, yet welcome, side effect of EMS treatments.

Observations in the field indicate that *O. candidans* has the potential for weediness. As invasive and weediness potential are a general cause for concern when developing landscape plants, EMS mutagenesis could prove to be a useful tool in the future that may be applied to other landscape crops.

**References**


Table 3.1 Seed parents selected from M$_1$ population of *Ornithogalum candicans* for reduced scape height at first flower.

<table>
<thead>
<tr>
<th>Seed parent from M$_1$ population</th>
<th>EMS treatment (%)</th>
<th>Scape height at first flower (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>30.005</td>
<td>0.0</td>
<td>95.0</td>
</tr>
<tr>
<td>30.037</td>
<td>0.0</td>
<td>71.0</td>
</tr>
<tr>
<td>30.060</td>
<td>0.2</td>
<td>96.0</td>
</tr>
<tr>
<td>30.063</td>
<td>0.2</td>
<td>51.5</td>
</tr>
<tr>
<td>30.072</td>
<td>0.2</td>
<td>99.0</td>
</tr>
<tr>
<td>30.101</td>
<td>0.2</td>
<td>84.0</td>
</tr>
<tr>
<td>30.128</td>
<td>0.2</td>
<td>82.0</td>
</tr>
<tr>
<td>31.087</td>
<td>0.0</td>
<td>96.0</td>
</tr>
<tr>
<td>31.090</td>
<td>0.0</td>
<td>96.4</td>
</tr>
<tr>
<td>32.009</td>
<td>0.2</td>
<td>98.0</td>
</tr>
<tr>
<td>32.022</td>
<td>0.2</td>
<td>55.5</td>
</tr>
<tr>
<td>32.029</td>
<td>0.2</td>
<td>92.0</td>
</tr>
<tr>
<td>32.036</td>
<td>0.2</td>
<td>93.5</td>
</tr>
<tr>
<td>32.046</td>
<td>0.2</td>
<td>96.0</td>
</tr>
<tr>
<td>32.048</td>
<td>0.2</td>
<td>80.0</td>
</tr>
<tr>
<td>32.052</td>
<td>0.2</td>
<td>62.0</td>
</tr>
<tr>
<td>32.053</td>
<td>0.2</td>
<td>107.0</td>
</tr>
<tr>
<td>32.054</td>
<td>0.2</td>
<td>51.0</td>
</tr>
<tr>
<td>32.057</td>
<td>0.2</td>
<td>92.2</td>
</tr>
<tr>
<td>32.058</td>
<td>0.2</td>
<td>89.0</td>
</tr>
<tr>
<td>32.073</td>
<td>0.2</td>
<td>71.0</td>
</tr>
<tr>
<td>32.091</td>
<td>0.2</td>
<td>113.5</td>
</tr>
<tr>
<td>33.023</td>
<td>0.0</td>
<td>84.0</td>
</tr>
<tr>
<td>33.077</td>
<td>0.2</td>
<td>74.5</td>
</tr>
<tr>
<td>33.084</td>
<td>0.2</td>
<td>63.0</td>
</tr>
<tr>
<td>33.087</td>
<td>0.2</td>
<td>68.5</td>
</tr>
<tr>
<td>33.091</td>
<td>0.2</td>
<td>100.0</td>
</tr>
<tr>
<td>33.100</td>
<td>0.2</td>
<td>68.0</td>
</tr>
<tr>
<td>33.107</td>
<td>0.2</td>
<td>59.0</td>
</tr>
<tr>
<td>33.110</td>
<td>0.2</td>
<td>67.0</td>
</tr>
<tr>
<td>33.112</td>
<td>0.2</td>
<td>58.0</td>
</tr>
<tr>
<td>33.116</td>
<td>0.2</td>
<td>68.0</td>
</tr>
<tr>
<td>33.120</td>
<td>0.2</td>
<td>122.0</td>
</tr>
<tr>
<td>33.127</td>
<td>0.2</td>
<td>102.0</td>
</tr>
<tr>
<td>34.012</td>
<td>0.2</td>
<td>77.0</td>
</tr>
</tbody>
</table>
Table 3.2. Tissue culture medium treatments for in vitro micropropagation of *Ornithogalum candicans*.

<table>
<thead>
<tr>
<th>Treatment 1</th>
<th>Treatment 2</th>
<th>Treatment 3</th>
<th>Treatment 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>5.37 μM NAA</td>
<td>5.37 μM NAA</td>
<td>5.37 μM NAA</td>
<td>8.06 μM NAA</td>
</tr>
<tr>
<td>1.33 μM BAP</td>
<td>2.22 μM BAP</td>
<td>4.44 μM BAP</td>
<td>2.22 μM BAP</td>
</tr>
</tbody>
</table>

All treatments contained 2.17 g · L⁻¹ half-strength MS basal salts and vitamins, 30 g · L⁻¹ sucrose, and 1.88 g · mL⁻¹ agar. pH of 5.8 was adjusted prior to addition of agar.
Table 3.3. Controlled crosses conducted in July for female fertility evaluation.

<table>
<thead>
<tr>
<th>Date crossed</th>
<th>Female EMS trt.</th>
<th>Male EMS trt.</th>
<th>Number flowers crossed</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.21.16</td>
<td>33.79</td>
<td>0.2</td>
<td>30.21</td>
</tr>
<tr>
<td>7.21.16</td>
<td>33.79</td>
<td>0.2</td>
<td>31.106</td>
</tr>
<tr>
<td>7.21.16</td>
<td>33.79</td>
<td>0.2</td>
<td>OP</td>
</tr>
<tr>
<td>7.21.16</td>
<td>33.79</td>
<td>0.2</td>
<td>self</td>
</tr>
<tr>
<td>7.21.16</td>
<td>33.93</td>
<td>0.2</td>
<td>30.21</td>
</tr>
<tr>
<td>7.21.16</td>
<td>33.93</td>
<td>0.2</td>
<td>30.36</td>
</tr>
<tr>
<td>7.21.16</td>
<td>33.93</td>
<td>0.2</td>
<td>OP</td>
</tr>
<tr>
<td>7.21.16</td>
<td>33.93</td>
<td>0.2</td>
<td>Self</td>
</tr>
<tr>
<td>7.21.16</td>
<td>30.93</td>
<td>0.2</td>
<td>33.53</td>
</tr>
<tr>
<td>7.21.16</td>
<td>30.93</td>
<td>0.2</td>
<td>31.106</td>
</tr>
<tr>
<td>7.21.16</td>
<td>30.93</td>
<td>0.2</td>
<td>OP</td>
</tr>
<tr>
<td>7.21.16</td>
<td>30.93</td>
<td>0.2</td>
<td>Self</td>
</tr>
<tr>
<td>7.21.16</td>
<td>31.13</td>
<td>0.4</td>
<td>30.36</td>
</tr>
<tr>
<td>7.21.16</td>
<td>31.13</td>
<td>0.4</td>
<td>33.53</td>
</tr>
<tr>
<td>7.21.16</td>
<td>31.13</td>
<td>0.4</td>
<td>OP</td>
</tr>
<tr>
<td>7.21.16</td>
<td>31.13</td>
<td>0.4</td>
<td>Self</td>
</tr>
<tr>
<td>7.21.16</td>
<td>32.107</td>
<td>0.4</td>
<td>30.21</td>
</tr>
<tr>
<td>7.21.16</td>
<td>32.107</td>
<td>0.4</td>
<td>31.106</td>
</tr>
<tr>
<td>7.21.16</td>
<td>32.107</td>
<td>0.4</td>
<td>OP</td>
</tr>
<tr>
<td>7.21.16</td>
<td>32.107</td>
<td>0.4</td>
<td>Self</td>
</tr>
<tr>
<td>7.21.16</td>
<td>34.66</td>
<td>0.4</td>
<td>30.36</td>
</tr>
<tr>
<td>7.21.16</td>
<td>34.66</td>
<td>0.4</td>
<td>30.21</td>
</tr>
<tr>
<td>7.21.16</td>
<td>34.66</td>
<td>0.4</td>
<td>OP</td>
</tr>
<tr>
<td>7.21.16</td>
<td>34.66</td>
<td>0.4</td>
<td>self</td>
</tr>
</tbody>
</table>
Table 3.4. Decreasing seed set, seed germination, and pollen viability with increasing %EMS Treatment in an M$_1$ population of *Ornithogalum candicans* field planted in Corvallis, OR. LSMeans ± SEM is reported for average seed per/capsule and percent pollen viability (PROC GLM, SAS 9.4).

<table>
<thead>
<tr>
<th>Year</th>
<th>Average number of seed/capsule</th>
<th>% seed germination</th>
<th>% pollen viability</th>
</tr>
</thead>
<tbody>
<tr>
<td>2014</td>
<td>18.16 ± 0.96</td>
<td>89.33 ± 2.67</td>
<td>82.90 ± 8.06</td>
</tr>
<tr>
<td></td>
<td>2.89 ± 1.33</td>
<td>56.54 ± 3.81</td>
<td>21.10 ± 7.90</td>
</tr>
<tr>
<td></td>
<td>0.10 ± 5.33</td>
<td>0.00 ± 0.00</td>
<td>2.76 ± 9.24</td>
</tr>
<tr>
<td>2015</td>
<td>20.79 ± 0.87</td>
<td>88.89 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.17 ± 0.88</td>
<td>67.74 ± 0.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00 ± 3.57</td>
<td>.</td>
<td></td>
</tr>
</tbody>
</table>
Table 3.5. Lodging of *Ornithogalum candicans* reported as proportions for each rep and treatment in the M₁ population.

<table>
<thead>
<tr>
<th>Rep</th>
<th>EMS treatment (%)</th>
<th>% lodging per rep</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
<td>56.5</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>40.0</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>33.3</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>55.8</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>40.6</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>36.4</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>50.0</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>41.4</td>
</tr>
<tr>
<td></td>
<td>0.4</td>
<td>0</td>
</tr>
</tbody>
</table>

Average % lodging per treatment

<table>
<thead>
<tr>
<th>± SEM</th>
<th>% lodging per rep</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>54.1 ± 2.06</td>
</tr>
<tr>
<td>0.2</td>
<td>40.7 ± 0.41</td>
</tr>
<tr>
<td>0.4</td>
<td>23.23 ± 11.7</td>
</tr>
</tbody>
</table>
Table 3.6. Two-way factorial ANOVA for shoot number in response to media and explant type of *Ornithogalum candicans* using Type III SS (PROC GLM, SAS 9.4).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>62</td>
<td>6281.08</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>3</td>
<td>102.52</td>
<td>34.17</td>
<td>0.34</td>
<td>0.80</td>
</tr>
<tr>
<td>Explant type</td>
<td>3</td>
<td>959.00</td>
<td>319.67</td>
<td>3.18</td>
<td>0.03</td>
</tr>
<tr>
<td>Media * Exp</td>
<td>8</td>
<td>428.20</td>
<td>53.52</td>
<td>0.53</td>
<td>0.83</td>
</tr>
<tr>
<td>Error</td>
<td>48</td>
<td>4819.71</td>
<td>100.41</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.7. Two-way factorial ANOVA for root number in response to media and explant type of *Ornithogalum candicans* using Type III SS (PROC GLM, SAS 9.4).

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>61</td>
<td>94.97</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Media</td>
<td>3</td>
<td>6.40</td>
<td>2.13</td>
<td>2.11</td>
<td>0.11</td>
</tr>
<tr>
<td>Explant type</td>
<td>3</td>
<td>30.74</td>
<td>10.25</td>
<td>10.16</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Media * Exp</td>
<td>8</td>
<td>7.74</td>
<td>0.97</td>
<td>0.96</td>
<td>0.48</td>
</tr>
<tr>
<td>Error</td>
<td>47</td>
<td>47.40</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 3.8. Average (LSMeans) shoots and roots produced per explant type of micropropagated *Ornithogalum candicans* (PRO GLM, SAS 9.4).

<table>
<thead>
<tr>
<th>Explant type</th>
<th>Number of shoots LSMeans ± SEM</th>
<th>Number of roots LSMeans ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ovary</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Ovary + pedicel</td>
<td>5.99 ± 2.56</td>
<td>1.53 ± 0.26*</td>
</tr>
<tr>
<td>Pedicel</td>
<td>5.60 ± 2.69</td>
<td>2.56 ± 0.32</td>
</tr>
<tr>
<td>Rachis</td>
<td>12.05 ± 2.63*</td>
<td>2.65 ± 0.26</td>
</tr>
</tbody>
</table>

*Significantly different from other treatments for same effect.*
Figure 3.1. Schematic of *Ornithogalum candicans* inflorescence.
Figure 3.2. Photomicrographs (at ×400) of Ornithogalum candidans pollen viability scoring of M₁ plants. (A) non-viable 0.2, (B) viable 0.2, (C) non-viable 0.4, and (D) potentially viable 0.4 pollen.
Figure 3.3. *Ornithogalum candicans* bulbs laid out on brown paper bags prior to rinse. Bulbs are rinsed in water, then laid out on paper to dry for approximately 1 week before bag is sealed and placed in walk-in cooler.
Figure 3.4. *Ornithogalum candicans* germination % as a response to increasing EMS concentration (%) after soaking treatment. Germination decreases with increasing EMS concentration with 0% germination at 1% EMS.
Figure 3.5. Decreasing scape height of the M₁ population of *Ornithogalum candicans* with increasing %EMS concentration in years 2014 – 2015. LSMeans used in place of means due to unbalanced data (PROC GLM, SAS 9.4) with standard error bars.
Figure 3.6. Roots of *Ornithogalum candicans* seedlings just prior to transplanting.
Inducing and Evaluating Phenotypic and Cytometric Variation in Landscape Plants: Observations from *Acer, Ornithogalum, and Penstemon*

Chapter Four: Developing a breeding program for *Penstemon* (Plantaginaceae) with improved garden performance through interspecific hybridization: Compatibility, incompatibility and potential

*Penstemon* is a diverse genus composed of more than 270 species of herbaceous perennials with a distribution in North America from as Alaska to Guatemala in Central America (Wolfe et al., 2006). Their pollinators include bees, wasps, moths, bee flies, and hummingbirds. The genus is most notable for its common use as an ornamental in garden settings due to the display of showy flowers from early spring into the summer. Flowers are coveted for color and size and variable markings within the throat. Additionally, there are a number of forms represented in the genus due to the wide range of habitats including alpine, woodland and prairie.

Historically, backyard breeding of *Penstemon* has been common among gardeners. There are a number of well-known groups of hybrids that have been developed in the past (Way and James, 2003). While breeding has tended to focus on flower color, size and longevity, and sometimes foliage color, there has been little known breeding focused on developing improved garden cultivars for the Pacific Northwest region. *Penstemon* are touted for drought resistance, however moist, cold winters tend to be detrimental to most cultivars and species.

Natural interspecific hybridization has been reported for Subgenus *Penstemon* (Wolfe et al., 1998); and while there has been considerable breeding with species in the
Subgenus *Penstemon*, there has been little published breeding research in the subgenera *Dasanthera* and *Saccanthera*. *Dasanthera* and *Saccanthera* are two of the listed subgenera with plants native to the Pacific Northwest (Lindgren and Wilde, 2003). Of these two subgenera, the focus of this project will be developing garden-worthy hybrids of *Saccanthera* taxa *P. venustus* Douglas ex Lindl. and *P. serrulatus* Menzies ex Sm. and *Dasanthera* taxa *P. newberryi* A. Gray and *P. davidsonii* Greene. *Penstemon serrulatus* is acclimated to moist woodlands and has a dense growth habit. The ability to tolerate moist soils would be a useful trait in developing hybrids. *Penstemon venustus* has larger, flowers than *P. serrulatus*, however it is acclimated to dry, rocky cliffs and is more suited for a scree garden (Lindgren and Wilde, 2003). Additionally, the form of *P. venustus* has been noted as floppy.

*Penstemon davidsonii* is a purple flowered, mat-forming species tolerant of acidic soils. This tolerance of acidic soil is a desirable trait to incorporate into *Penstemon* hybrids. *Penstemon newberryi* is a more moisture tolerant alpine species that can be found in open woodlands. The flower color of *P. newberryi* ranges from pink to reddish-violet (Lindgren and Wilde, 2003).

In a study conducted by Wolfe et al. (2006), molecular evidence suggested subgenus *Saccanthera* and subgenus *Penstemon* were more closely related than previously assumed based on morphology. Thus Wolfe et al. suggested a revision of *Penstemon* that would lump *Saccanthera* into *Penstemon*. In 2007, Lindgren and Schaaf published a summary of interspecific crosses of *Penstemon* taxa. These results demonstrated that crosses between species of the two subgenera were possible.
While most *Penstemon* are prized for their floral show, there have been two unique red-leaved cultivars released by breeder Dale Lindgren. These are *P. digitalis* ‘Husker Red’ and *P. ‘Dark Towers’ of Subgenus Penstemon*. ‘Husker Red’ is a red-leaved form of *P. digitalis* with white flowers that is not tolerant of consistently moist soils (MOBOT, 2016). The red foliage trait was introgressed from a wild-collected specimen that displayed some red pigment. The species *P. digitalis* is native to the northern Mississippi River basin and tends to naturalize in open woodlands, meadows and fields (Lindgren and Wilde, 2003). ‘Dark Towers’ is progeny of ‘Husker Red’ and the interspecific hybrid *P. ‘Prairie Splendor’* (*P. cobaea* Nutt. x *P. triflorus* A. Heller) with red foliage, purple flowers and improved form (Lindgren and Shaaf, 2009) that also is not tolerant of consistently moist soils (MOBOT, 2016). These two cultivars were included in this project to introgress the additional ornamental interest of foliage color, as well as improving moisture tolerance. Demand for landscape plants with novel foliage color is evident in the catalogs of large nurseries such as Monrovia (multiple locations across the nation including Dayton, OR) and Terra Nova Nurseries (Canby, OR). In Monrovia’s 2017 Featured Plants list more than 25% of the plants listed feature novel foliage color including variegated, chartreuse, red and purple (Monrovia, 2016); while Terra Nova nurseries is internationally renowned for their introduction of the foliage plant *Heuchera* in a wide-array of colors.

A genome size survey of *Penstemon* (Broderick et al., 2011) provided evidence of polyploids in the genus. Two notable polyploids are the aforementioned *P. ‘Dark
Towers’ and *P. digitalis* ‘Husker Red’ (*2n = 12x = 96*). The base chromosome count for *Penstemon* is *x = 8*. *Penstemon venustus* was also found to be polyploid (*2n = 8x = 64*).

In the past, University of Nebraska breeder Glen Viehmeyer developed a cultivar (*P. ‘Flathead Lake’*) that has been frequently used as a bridge parent for interspecific hybridization. This particular cultivar was the product of the hybridization between *P. barbatus* (Cav.) Nutt. and a species of the Subgenus *Habroanthus* (Lindgren and Wilde, 2003; Way and James, 2003). The resulting progeny can still be found in the trade as cultivars with names that include the term ‘Prairie’. In this project, *P. ‘Prairie Dusk’* and the *Habroanthus* cultivar, *P. barbatus* ‘Elfin Pink’ were included.

An area of *Penstemon* breeding that appears to be little explored is the use of taxa native to the Southeast United States. This region is known for a climate that is extreme in heat and humidity. While this may mean the germplasm is winter tender, the moisture tolerance would be a valuable trait for introgressing into hybrids for the Pacific Northwest garden. A species of particular interest is *Penstemon tenuis* Small. *Penstemon tenuis* (Subgenus *Penstemon*) is acclimated to wet woodland soils and flowers profusely (Lindgren and Wilde, 2003). These two traits would make *P. tenuis* a desirable parent in a *Penstemon* breeding program. Unfortunately, there are not many cultivars of the species available in the trade; however, the plants are available at native plant nurseries in the South.

The objectives of this study were to evaluate crossability of *Penstemon* including Pacific Northwest natives, a Southeast native, and available garden cultivars in order to develop a breeding program for improved form and moisture tolerance.
Materials and methods

Parent material

Cultivars common in the trade and found in the Willamette Valley or surrounding area were acquired. Species that were included: *P. venustus*, *P. serrulatus*, *P. davidsonii*, *P. newberryi*, and *P. tenuis*. Other species and hybrid represented in the cultivar collection included *P. digitalis*, *P. heterophyllus* Lindl., *P. ×mexicali* and *P. barbatus*. A complete list of germplasm with taxonomic classifications, habitat, and distribution can be found in Table 4.1 (adapted from Lindgren and Wilde, 2012; BM, 2016; LBJWC, 2016). These garden cultivars provide the valued traits of flower color, form and foliage color. These cultivars provide a standard of comparison for what is available in the current nursery market.

Controlled crosses, selfing, and germination

Crosses were conducted in a partial diallel design with simultaneous selfing (Fehr, 1991) (Table 4.2). Flowers were emasculated prior to anthesis. Anthers were desiccated in a petri dish within a magenta box containing anhydrous calcium sulfate for pollen collection. Pollen was refrigerated at 4 °C to prolong shelf-life. Female parents were pollinated through bud pollination after emasculation. This ensured that there was no self-pollination and was thought to improve chances of overcoming compatibility barriers. Plants were grown in 2 part soilless media (Metro-Mix® 350, Sungro Horticulture Canada Ltd.) : 1 part perlite; and controlled crosses were conducted in a glasshouse of Oregon State University West Greenhouses in the spring and summer of 2014. Average temperature in the glasshouse for the month of June was 23 °C with an
average high of 30 °C; and the average temperature the month of July was 25 °C with an average high of 32 °C. During fruit development, plants remained in the glasshouse. Selfing was facilitated by bagging an entire inflorescence on a single plant. Most taxa in this study had approximately 15 – 30 flowers per inflorescence (personal observation), with the exception of *P. davidsonii* and *P. newberryi* which produced one to two flowers per flowering stem. There were not enough flowers for selfing of *P. davidsonii* or *P. newberryi*.

Fruit were collected and counted after browning and prior to dehiscence. This was recorded as fruit set. Seeds were removed and mass (mg) of seed was recorded as they were very small, in some cases almost like dust. Upon completion of seed cleaning and data collection, seeds were stratified at 4 °C in petri dishes lined with moist lab grade filter paper until radicle emergence. Seedlings and remaining seeds were then transplanted to soilless media (Metro-Mix® 350, Sungro Horticulture Canada Ltd.) in 0.37 L square polypropylene pots (Kord, Canada).

**Results and Discussion**

While overall there was considerable fruit set, seed development was limited (Table 4.2). Most seed that began to form appeared to be underdeveloped and aborted. Only one potential hybrid seedling germinated. This was the progeny of *P. ‘Dark Towers’ x P. tenuis*. This seedling appears to be much more vigorous than those produced by selfing on the same female. All other seedlings were the product of the selfing of *P. ‘Dark Towers’, P. tenuis*, and *P. venustus* (Table 4.3).
It is clear, based upon the material that was collected at garden centers and observations of nursery catalogs, that there are still some uncertainties in the market regarding the proper labeling of garden cultivars. A plant labeled as ‘Ruby’ was purchased at a Portland garden center; however, it is reported (Way and James, 2003; Lindgren, 2006) that this is a cultivar of *P. newberryi*. The plant that was purchased is most definitely not *P. newberryi*, and so it has been listed in this thesis as *P. ‘Schöenholzeri’* based on descriptions in these reports. The Royal Horticulture Society (2016) lists this cultivar with the synonyms of ‘Ruby’ and ‘Firebird’. ‘Firebird’ was also purchased at a garden center, and appears to be the same in appearance as the one labeled ‘Ruby’. It has been suggested on one site focused on garden *Penstemon* (PR, 2016) that the reason for this naming confusion is for ease of communication with consumers. For the sake of clarity and to abide by the rules set out by the International Code of Nomenclature for Cultivated Plants (Brickell et al., 2009), this particular cultivar should be listed solely as *P. ‘Schöenholzeri’*. If for the sake of marketing and communication, a nursery would like to give this plant another name, then this should be done as a trademark name and not as the single-quoted cultivar term (e.g. Ruby™ and Firebird™ or *Penstemon* RUBY (‘Schöenholzeri’) and *P. FIREBIRD* (‘Schöenholzeri’)).

In a study exploring interspecific compatibility among *Penstemon* of the Columbia River Gorge (Colrigo), Mackaness (1959) reported successful crosses with *P. serrulatus* as female parent and *P. cardwellii* as male parent. I conducted 122 crosses in this direction and seed parents produced only underdeveloped seed and I recovered no hybrid seedlings. In the Mackaness study, crosses were conducted outdoors in Colrigo
gardens. A potential explanation for the underdeveloped and aborted seed could be the conditions of the growing environment. Since the plants remained in the glasshouse for the duration of fruit development, it is possible that heat stress was a factor in seed abortion. These crosses were conducted in June, and fruit were collected in August. It has been demonstrated that reduced seed set can be the result of heat stress affecting the female and male gametophytes (Young et al., 2004). This heat stress response could also be the factor that prohibited seed set in other female parents of this study that set fruit.

Floral development of *P. davidsonii* was minimal. This species is native to elevations between 1707 – 3658 m where it flowers profusely and there is low humidity. One possible reason for the reduction in flowering is the warm, humid glasshouse conditions where the plants were cultivated. Controlled crosses of this taxa should be attempted outdoors in a pollination cage rather than in glasshouse. In previous studies investigating pollination biology and fertility of high elevation *Penstemon*, controlled crosses were conducted either in situ or in a common garden setting outdoors resulting in sufficient flowering (Mackaness, 1959; Chari and Wilson 2001; Datwyler, 2001; Lara and Ornelas, 2008).

Lindgren (2009) reported in the introduction of ‘Dark Towers’ that there had not been any fertile seed produced in either Canby, OR or North Platte, NE; however, I recovered selfed seed and one hybrid seed that germinated. The vigor of the selfed seedlings appears to be highly reduced (personal observation); however, the potential hybrid seedling is vigorous by comparison.
As many cultivars are reported to be garden hybrids (Way and James, 2003; Lindgren, 2006), this could be the source of sterility in several plants in this study. In interspecific hybrid cultivars, sterility is a common barrier to continued use of material in breeding programs. This can be due to lack of chromosome pairing during Prophase I of meiosis. If there are two different sets of chromosomes in a hybrid cultivar that do not pair, then at anaphase I there is an incomplete set of chromosomes at each pole. This will result in incomplete sets of chromosomes once meiosis is complete. One way to overcome this block to hybridization is ploidy manipulation. Parents of interest can be doubled, thus providing homologous chromosomes that can pair up in meiosis I and potentially overcoming hybrid sterility (Fehr, 1991; Olsen et al., 2006; Contreras et al., 2007).

*Penstemon tenuis* was at least self-fertile in greenhouse conditions, suggesting that heat stress and humidity were not factors in seed abortion relative to the female parent. Additionally, there was considerable fruit set and underdeveloped seed suggesting a potential barrier to interspecific hybridization may have been at the zygotic level during cell division. In the case of crosses by *P. venustus* (2n = 8x = 64) and *P. ‘Dark Towers’* (2n = 12x = 96), a potential culprit in preventing seed development is endosperm balance number (EBN). The EBN hypothesis postulates that in the endosperm, there must be a genetic balance of 2:1 maternal to paternal genetic material in order for the endosperm to develop successfully (Carputo et al., 1999). Both of these male parents are polyploids, and *P. tenuis* is a diploid (2n = 2x = 16).
In cases where there was no fruit set, gametophytic incompatibility could be due to S-locus incompatibility preventing germination of pollen or decelerated tube growth (Sleper and Poehlman, 2006) which is known to be the cause of self-incompatibility in the Plantaginaceae species Antirrhinum (Lai et al., 2002), and in Corylus avellana L. it has been demonstrated to be the source of intraspecific incompatibility (Thompson, 1979; Mehlenbacher and Thompson, 1988). Means of overcoming this incompatibility include applying mixtures of pollen or using growth regulators known for speeding up pollen tube growth. Another means of gametophytic incompatibility could be physical incompatibility due to insufficient length of pollen tube relative to the style. The pistils and pollen tubes have not been measured. This is something that can be confirmed with microscopy work and overcome by shortening the pistil (Fehr, 1991).

Another key to overcoming interspecific incompatibility is the identification of bridge species. This has been done at least once before in Penstemon. A chance hybridization between P. barbatus (Subgenus Penstemon) and a species of Subgenus Habroanthus produced the cultivar P. ‘Flathead Lake’ in 1948 (Lindgren and Wilde, 2003; Way and James, 2003). While not prized for its beauty, this particular cultivar is reported to accept pollen indiscriminately (Way and James, 2003; Lindgren, 2006), and it has been the parent of numerous cultivar releases since then including the wide hybrid P. ‘Prairie Dusk’ included in this study. When crossed with the only pure Habroanthus cultivar included in this study, P. barbatus ‘Elfin Pink’, fruit set and seed set occurred, but seeds aborted in crosses in both directions. This may be due to heat stress or zygotic incompatibility.
Since I did conduct reciprocal crosses and acquired a somewhat diverse collection of germplasm, one method of overcoming interspecific incompatibility at the zygotic level would be to apply a mixture of pollen to the pistil of a female parent. This would be a female that is self-compatible. In this case, I would recommend using *P. tenuis*, *P. venustus*, or *P. ‘Dark Towers’* as the female parent and mixing pollen of the female with pollen of the desired male parent. In this way, one could overcome zygotic incompatibility by fertilizing enough ovules to prevent abortion as described by Fehr (1991).

**Conclusion**

*Penstemon* are an incredibly diverse group of plants with a wide-range of adaptability based on distribution and habitat type. While crosses in this study were not successful, fruit set and seed data did yield some interesting results in considering modes of incompatibility. Moving forward, I would recommend evaluating barriers to hybridization and pollen viability through pollen germination and pollen tube growth experiments. Chromosome doubling could provide unique plant material for use in future breeding projects. Crosses onto polyploids *P. venustus* and *P. ‘Dark Towers’* could potentially produce viable seed if pollen mixtures are used. If this were to be done, then flow cytometry could be used to confirm hybridity of seedlings. Additionally, I would suggest that conducting crosses in a common garden environment or a pollination cage could yield greater success in floral development for *P. davidsonii* and potentially greater seed set in other parent material. Previous studies demonstrate that *P. davidsonii* is floriferous in outdoor conditions.
While *Penstemon* are not known for tolerance to the cold, wet Willamette Valley winters, for a *Penstemon* breeding program it would be wise to consider building the proper beds for a common garden (i.e. scree or trough gardens).

**References**


Table 4.1. Taxonomic classification, habitat and distribution of *Penstemon* germplasm used in crossing experiments as adapted from Lindgren and Wilde (2003), Way and James (2003), Burke Museum (2016), and Calflora (2016).

<table>
<thead>
<tr>
<th>Species and cultivars</th>
<th>Subgenus</th>
<th>Habitat</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. barbatus</em> ‘Elfin Pink’</td>
<td><em>Habroanthus</em></td>
<td>Dry montane; 1356 – 2652 m elev.</td>
<td>S. CO and UT, w. TX, NM, AZ, and n. Mexico</td>
</tr>
<tr>
<td><em>P. cardwellii</em></td>
<td><em>Dasanthera</em></td>
<td>Open or wooded summits or slopes; 396 – 1920 m elev.</td>
<td>WA, OR, n. CA</td>
</tr>
<tr>
<td><em>P. davidsonii</em></td>
<td><em>Dasanthera</em></td>
<td>Rocky alpine, sub-alpine woods and fields; 1707 – 3658 m elev.</td>
<td>N. CA, OR, WA, n. NV, BC</td>
</tr>
<tr>
<td><em>P. digitalis</em> ‘Husker Red’</td>
<td><em>Penstemon</em></td>
<td>Open woodlands, meadows and fields</td>
<td>Northern Mississippi River basin; NE US, ON, QC</td>
</tr>
<tr>
<td><em>P. heterophyllus</em> ‘Electric Blue’</td>
<td><em>Saccanthera</em></td>
<td>Slopes of foothill woodlands, yellow pine forest; 18 – 1600 m elev.</td>
<td>Garden origin; species native to CA, San Diego to Humboldt Co.</td>
</tr>
<tr>
<td>*P. × ‘Pike's Peak Purple’</td>
<td><em>Penstemon</em></td>
<td>Highly adaptable hybrid cultivar</td>
<td>Garden origin</td>
</tr>
<tr>
<td><em>P. rydbergii</em> (White &amp; Blue)</td>
<td><em>Penstemon</em></td>
<td>Mountain meadows, along streams, prefers moist climate; 1402 – 3490 m elev.</td>
<td>CA, OR, WA to MT, WY, CO, NM</td>
</tr>
</tbody>
</table>
Table 4.1 (cont.)

<table>
<thead>
<tr>
<th>Species and cultivars</th>
<th>Subgenus</th>
<th>Habitat</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. tenuis</em></td>
<td><em>Penstemon</em></td>
<td>Wet woodland soils</td>
<td>AR, e.TX, LA, OK</td>
</tr>
<tr>
<td><em>P. venustus</em></td>
<td><em>Saccanthera</em></td>
<td>Dry, open rocky cliffs; 914 – 2210 m elev.</td>
<td>E. OR, WA, w. ID</td>
</tr>
<tr>
<td><em>P. 'Dark Towers'</em></td>
<td><em>Penstemon</em></td>
<td>Hybrid cultivar; *P. 'Husker Red' × <em>P. 'Prairie Splendor'</em></td>
<td>Garden origin</td>
</tr>
<tr>
<td><em>P. 'Prairie Dusk'</em></td>
<td><em>Penstemon/Habroanthus</em></td>
<td>Hybrid cultivar</td>
<td>Garden origin</td>
</tr>
<tr>
<td><em>P. 'Raven'</em></td>
<td><em>Penstemon</em></td>
<td>Hybrid cultivar</td>
<td>Garden origin</td>
</tr>
<tr>
<td><em>P. 'Schoenholzeri'</em></td>
<td><em>Penstemon</em></td>
<td>Hybrid cultivar</td>
<td>Garden origin</td>
</tr>
<tr>
<td>aka P. RUBY</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aka P. FIREBIRD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>P. 'Sour Grapes' (Type 2)</em></td>
<td><em>Penstemon</em></td>
<td>Hybrid cultivar</td>
<td>Garden origin</td>
</tr>
</tbody>
</table>

Classification based upon description from Way and James (2003). Type 2 as described by Way and James (2003).
Table 4.2. Crosses conducted among Penstemon germplasm Spring – Summer 2014 in a glasshouse. Fruit set, seed mass and number of seedlings produced included. First row for each female is the sum of all crosses. Final row in table is total crosses conducted overall.

<table>
<thead>
<tr>
<th>Female parent</th>
<th>Male parent</th>
<th>No. of flowers crossed</th>
<th>No. of fruit set</th>
<th>Seed mass (mg)</th>
<th>No. of seedlings</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Penstemon barbatus</em> 'Elfin Pink'</td>
<td>Total</td>
<td>78</td>
<td>72</td>
<td>29.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. cardwellii</em></td>
<td>2</td>
<td>1</td>
<td>0.5</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. davidsonii ssp. davidsonii</em></td>
<td>23</td>
<td>21</td>
<td>5.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. rydbergii</em> (White)*</td>
<td>10</td>
<td>10</td>
<td>3.8</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. tenuis</em></td>
<td>10</td>
<td>8</td>
<td>3.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. venustus</em></td>
<td>20</td>
<td>18</td>
<td>9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. 'Prairie Dusk'</em></td>
<td>13</td>
<td>14</td>
<td>6.2</td>
<td>0</td>
</tr>
<tr>
<td><em>P. davidsonii</em> ssp. <em>davidsonii</em></td>
<td>Total</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. tenuis</em></td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. davidsonii</em> ssp. <em>menziesii</em></td>
<td>Total</td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. tenuis</em></td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. venustus</em></td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. 'Electric Blue'</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. davidsonii</em> ssp. <em>praetertius</em></td>
<td>Total</td>
<td>26</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. tenuis</em></td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. venustus</em></td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. 'Prairie Dusk'</em></td>
<td>5</td>
<td>4</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>P. digitalis</em> 'Husker Red'</td>
<td>Total</td>
<td>66</td>
<td>12</td>
<td>15.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. davidsonii</em> ssp. <em>praetertius</em></td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. tenuis</em></td>
<td>18</td>
<td>8</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. venustus</em></td>
<td>21</td>
<td>4</td>
<td>2.9</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. 'Prairie Dusk'</em></td>
<td>17</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>P. heterophyllus</em> <em>Electric Blue</em></td>
<td>Total</td>
<td>78</td>
<td>8</td>
<td>14.2</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. davidsonii</em> ssp. <em>menziesii</em></td>
<td>8</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. davidsonii</em> ssp. <em>praetertius</em></td>
<td>37</td>
<td>1</td>
<td>1.3</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td><em>P. rydbergii</em> (White)</td>
<td>12</td>
<td>6</td>
<td>11.8</td>
<td>0</td>
</tr>
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<tr>
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</tbody>
</table>
Table 4.2 (cont.)

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<th>Male parent</th>
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<th>No. of fruit set</th>
<th>Seed mass (mg)</th>
<th>No. of seedlings</th>
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<tbody>
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</tr>
<tr>
<td></td>
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<tr>
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Table 4.2 (cont.)

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<th>Female parent</th>
<th>Male parent</th>
<th>No. of flowers crossed</th>
<th>No. of fruit set</th>
<th>Seed mass (mg)</th>
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<td>0</td>
<td>0</td>
<td>0</td>
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<tr>
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<td>P. tenuis</td>
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<td>1</td>
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<tr>
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<td>P. venustus</td>
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<td>44</td>
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<td>12.6</td>
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<td></td>
<td>P. tenuis</td>
<td>20</td>
<td>0</td>
<td>9.6</td>
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<td>63</td>
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<td>15.8</td>
<td>0</td>
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Table 4.2. 'White' and 'Blue' following *P. rydbergii* refers to flower color. 'Type 2 'Sour Grapes' as described by Way and James, 2003.
Table 4.3. Selfed *Penstemon* taxa with number inflorescences bagged (10 – 30 flowers per inflorescence), number of fruit set, mass of seed recorded, and number of seedlings recovered.

<table>
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<th>Taxon</th>
<th>No. of bagged infl.</th>
<th>No. of fruit set</th>
<th>Mass of seed (mg)</th>
<th>No. of seedlings</th>
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</thead>
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<td>11</td>
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<td><em>P. tenuis</em></td>
<td>3</td>
<td>41</td>
<td>331.4</td>
<td>25</td>
</tr>
<tr>
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<td>1</td>
<td>29</td>
<td>79.2</td>
<td>15</td>
</tr>
<tr>
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<td>1</td>
<td>2</td>
<td>10.1</td>
<td>10</td>
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<tr>
<td><em>P. 'Elfin Pink'</em></td>
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<td>47</td>
<td>24.6</td>
<td>0</td>
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<tr>
<td><em>P. 'Pike's Peak'</em></td>
<td>2</td>
<td>17</td>
<td>15.2</td>
<td>0</td>
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<tr>
<td><em>P. 'Prairie Dusk'</em></td>
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<td>22.8</td>
<td>0</td>
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<tr>
<td><em>P. 'Raven'</em></td>
<td>1</td>
<td>6</td>
<td>12.9</td>
<td>0</td>
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<tr>
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<td>26</td>
<td>39.8</td>
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</table>
Inducing and Evaluating Phenotypic and Cytometric Variation in Landscape Plants: Observations from Acer, Ornithogalum, and Penstemon

Chapter Five: Conclusion

Landscape plants are a lucrative commodity in Oregon and in the nation as a whole as demonstrated by USDA Census data (2016) and ODA data (2015) presented in Chapters 1 and 2. While landscape crops generate a considerable amount of revenue for the nursery industry, social and environmental benefits of ornamental plants provide added value to crops that may be considered as simply aesthetic.

As with all horticultural and agricultural crops, there is always a demand for improvement; whether this be in the form of aesthetic appeal, environmental purpose, or disease resistance. In this thesis, I evaluated or applied several methods available to a traditional plant breeder in the proverbial breeder’s toolbox: genome size and ploidy variation, chemical mutagenesis, and interspecific hybridization.

Genome size and ploidy variation information can be an excellent tool for developing breeding objectives relative to landscape plants. Due to the potential for weediness or invasiveness of exotic or native landscape plants, finding natural variation in ploidy and genome size could provide a means for mitigating the effects of introduced plant materials. In maples, I found that there is indeed genome size and ploidy variation. This knowledge can be used to develop breeding objectives for developing sterile cultivars, while eliminating the need to develop polyploids. This would greatly reduce the number of years it takes to develop a sterile cultivar. Maples have a lengthy juvenile period, as noted in Chapter 2, and while the development of polyploid
germplasm can be valuable, it can also take a considerable amount of time before polyploids can be used in crosses—sometimes 20 years. The 1Cx genome size will be a useful tool in determining hybridity of intersectional crosses. Finally, this is the largest genome size and ploidy survey that has been conducted on this valuable genus, adding to the growing knowledge base of angiosperms.

Chemical mutagenesis through the application of EMS has been a method applied by breeders of agricultural crops for some time now (cite wheat, rice). However, the application in ornamental crops is not as widely published. Through field evaluations and greenhouse experiments, I found that this tool is useful not only for inducing phenotypic variation, but also a potential method of reducing fertility in weedy landscape plants. For weedy herbaceous perennials that have optimized propagation protocols developed, this could be an excellent and simple method of reducing fertility. It should also be noted that the cultural practices applied to herbaceous perennial can have a considerable effect on habit (e.g. lodging) and on germination.

While the interspecific hybridization of garden *Penstemon* in this study was not very fruitful, it did provide some insight for the development of a *Penstemon* breeding program in the Willamette Valley. Factors to consider moving forward with this *Penstemon* breeding program are developing environments for controlled crosses that may yield better results and identifying fertile germplasm. Additionally, this compatibility study identified some species and a cultivar that are self-compatible including P. ‘Dark Towers’ previously reported to be sterile (Lindgren and Schaaf, 2009).
As stated in the General Introduction, phenotypic variation in plants can be attributed to two factors: genetics and environment. In the Acer study, I found genetic variation in the form of genome size and ploidy variation; and in the Ornithogalum and Penstemon studies, I found that environment can play a significant role on the outcome of experiments and the expressed phenotype.

**Common Bibliography**


References:


