Oregon Department of Agriculture and Oregon Association of Nurseries
Nursery Research Project Proposal 2014

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Title: Developing sterile forms of economically important nursery crops

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Background:

Oregon is among the nation's leaders in production and export of nursery stock, with approximately 80% being shipped outside the state. Unfortunately, some of the species that the nursery and landscape industries have historically relied on have begun to show signs of invasiveness. The Ornamental Plant Breeding Program at Oregon State University has been addressing some of these species over the past three years. A brief background of several projects follows.

Cherrylaurels. Common cherrylaurel is a handsome evergreen often used as a hedge that is pH adaptable, does well in full sun or deep shade, is salt spray tolerant, and withstands heavy pruning. The estimated wholesale value of cherrylaurels for 2011 in Oregon was between $17.1 and $36.4 million. Common cherrylaurel has several deficiencies that could be addressed through breeding including invasive tendencies, excessive fruit litter, quarantine due to western cherry fruit fly, and leaf shothole disease under production conditions. Groups such as the Native Plant Society of Oregon are giving more attention to common cherrylaurel as an invasive species and currently consider it a medium-high impact species. Portugese laurel shares many of the same outstanding characters as common cherrylaurel such as tolerance to sun and shade and pH adaptability but is more tolerant to heat and drought stress and is not susceptible to leaf shothole disease. Fruit development is also prolific in this species and it has started to receive similar attention as common cherrylaurel regarding invasive potential.

Maples. Oregon is the leading producer of shade trees for the US and maples are among the most commonly produced and planted trees across the country. However, several important maple species have been identified as invasive and some have been banned including amur maple in Connecticut and Norway maple in Connecticut and Massachusetts. Other economically important maple species also produce copious amounts of seed, such as trident maple. This species is not yet regulated but the potential remains unless sterile forms can be identified. I propose that development of sterile forms prior to regulation by government agencies will allow producers to continue to grow and market each of these species.

Rose-of-sharon. The US National Arboretum introduced four rose-of-sharon cultivars described as sterile triploids including 'Diana', 'Minerva', 'Aphrodite', and 'Helene'. These cultivars have since been observed to produce substantial amounts of seed. It is unclear why these cultivars are fertile; however, we have several hypotheses we are testing. We began a breeding program to investigate several aspects of reproductive behavior of these and other cultivars. Of particular interest is 1) what is the actual ploidy level of available cultivars, 2) what is the relative fertility of available cultivars, and 3) how are ornamental traits such as eye spot, double flowers, and flower color inherited? If we can address issues of inheritance it may be possible to utilize targeted breeding to develop sterile forms with specific traits of interest.

Objectives

The objectives of the proposed research are to continue our efforts toward developing improved forms of the species listed above with reduced fertility.

Methods and Timeline

Cherrylaurels. We have developed a large number of polyploid cherrylaurels. In 2010, we successfully developed a number of polyploids but all reverted. In 2012, we received funding through the Agriculture Research Foundation and conducted experiments in tissue culture. We treated 'Otto Luyken' and 'Schipkaensis' in tissue culture and have recovered a large number of polyploids. For 'Otto Luyken', we have 62 mixoploids and 76 homogeneous polyploids (44x). For 'Schipkaensis' we have 64 mixoploids and 23 homogeneous polyploids.

- 2012 Developed polyploids
- September 2013 – Polyploids potted into #3 containers and moved to into a shade house at the Lewis-Brown Farm in Corvallis
- Spring 2014 – Tentatively expect plants to flower in 2014 but it is unclear what effect polyploidy will have on flowering time. If plants flower, we will conduct controlled crosses and lab assays to evaluate male and female fertility and backcrosses will be conducted with 'Otto Luyken' and 'Schipkaensis' if polyploids are fertile.
- Spring 2014 – Independent of flowering, we will determine ploidy level to assess the year to year stability of these induced polyploids

In 2010 and 2011 we irradiated a large number of common cherrylaurel seed to induce sterility. These plants have been planted at the Lewis-Brown Farm in Corvallis where we have observed significant differences among these plants but continued observation is needed to determine if this is due to seedling variation or induced mutation. In 2013, we have noted substantial variation in leaf forms and growth habits as well as two genotypes that have exhibited red new growth, however, this trait appears to be significantly influenced by the environment.

We attempted hybridization during 2010 and 2011, making 4,587 controlled crosses between *Prunus laurocerasus* and *P. lusitanica.* We recovered large amounts of seed but no hybrids. It is clear that these species are difficult to hybridize but there may be several reasons. In addition to being distantly related species, they are different ploidy levels. To overcome the latter issue, we have developed several polyploid Portugese cherrylaurels that we believe may have a greater chance of hybridizing but we cannot predict how long it will take for these individuals to flower. These individuals were developed in 2011-12, and are continuing to grow. In 2013, Ph.D. student Jason Lattier began experiments to optimize ovule culture for cherrylaurels. I am confident that combined efforts of hybridization at the newly induced ploidy level (16x) for Portugese cherrylaurel along with ovule culture will lead to hybrids.

- Spring 2013 – Controlled reciprocal crosses between species
- Spring – Summer 2013 – Collect fruit at varying days after pollination (DAP) and place into tissue culture under varying conditions and media
- Fall 2013 – A number of seed have germinated. We will evaluate the data during winter to identify the optimal media
- Spring 2014 – Controlled crosses and ovules will be cultured on media identified in 2013

Maples. To date, we have identified 113 Norway maple tetraploids, 9 trident maple tetraploids, and 5 amur maple tetraploids. Scion of 15 selections of Norway maple was sent to be propagated in August 2012 in order to expedite the breeding process. These selections were tested again in 2013 to determine if they are remaining stable, homogeneous tetraploids. 14 of 15 selections remained tetraploid. Replicates of these selections will be dug and transplanted to the Lewis-Brown Farm in Corvallis during Fall/Winter 2013-14.

- Fall – Winter 2012 – Continue screening treated seedlings as material is available
- Spring – Summer 2013 – Re-test plants identified as tetraploid to ensure they have not reverted. Send more scion wood for propagation.

Rose-of-sharon. We have made reciprocal crosses between all of our cultivar collection to begin to identify how flower color, double flowers, and eye-spots are inherited. Our cultivars include 'Diana', 'Aphrodite', 'Minerva', 'Lucy', 'Woodbridge', 'Red Heart', 'Pink Giant', 'Blue Bird', 'Blue Chiffon', and 'Blue Satin'. We have reciprocal

seed set between all but those using the double flowered forms ('Blue Chiffon' and 'Lucy') as pollen parents. We will calculate relative fertility of each based on germinated seedlings per pollinated flower and will record resulting flower architecture and color to assess inheritance. We will conduct flow cytometry and cytology screening of our cultivars to identify ploidy levels of each.

Budget Summary

Salary	
Faculty Research Assistant (30% FTE)	$12,134
Other Payroll Expenses	$7,644
(OSU health benefits, insurance, retirement)	
Services and Supplies	
Lab Supplies	$222
Total	**$20,000**

Benefit to Nursery Industry

Invasive plants continue to garner more attention and regulations will become more stringent as time goes on. Couple this situation with the loss of such important nursery crops as ash due to the emergence of emerald ash borer, and it is even more important that we develop sterile forms of these crops. Additionally, no one asks, "what's old", therefore it is important that we maintain ourselves on the cutting edge of new crops. My program is dedicated to delivering not only forms with reduced fertility but also cultivars that have improved ornamental and landscape characteristics. Furthermore, my goal is to offer these cultivars for open licensing to all Oregon Growers without exclusive rights for individual nurseries.

Final Report on Activities
29 December 2014

- ***Cherrylaurels.***
  - None of the polyploids flowered during 2014 so we have not been able to assess fertility. Growth looks similar to standard 'Otto Luyken' and 'Schipkaensis' thus far in #7 containers.
  - Plants resulting from irradiated seeds were all killed in 2013-14 cold event (-2F). This was a disappointing setback and for the near future, we will focus efforts on other methods than mutation.
  - Our 16x portugese cherrylaurel was left unprotected and was killed during 2013-14. This oversight has cost us time but we will repeat the protocol to develop more polyploids to achieve this cross.
  - Sufficient crosses were not made on this project in 2014 due to Jason Lattier's efforts being focused on lilacs and althea.
  - Another graduate student, Justin Schulze, has been hired and his efforts are on developing a 16x portugese cherrylaurel and growing resulting plants. Treatments are ongoing at this time but will end on January 6, 2015.
- ***Maples.***
  - We confirmed that tetraploid norway maples have remained stable. A number of genotypes were dug from J Frank Schmidt and interplanted with several industry standards at the Lewis-Brown farm in Corvallis in 2014. We are waiting for these plants to flower. Additional selections (7) were recently dug and will be spring planted in 2015 at the L-B Farm.
  - We had very precocious flowering of tetraploid and mixoploid (with tetraploid pollen and eggs) *Acer ginnala*. We made interploidy level crosses in spring 2014 and currently are stratifying the seed. We presume that resulting seedlings will be triploid and at flowering their fertility will be assessed. Thus far, seed have not germinated and may not have been viable. During 2015 we expect flowering to be more prolific than in 2014. We hope to pollinate several hundred flowers in 2015, as opposed to the 10's of flowers pollinated in 2014.
  - *Acer buergerianum* tetraploids have not flowered to date.
- ***Rose-of-sharon***
  - We have identified that all cultivars are tetraploid except 'Pink Giant', which is a hexaploid.
  - We determined that there is no reduction in fertility of any cultivars except 'Pink Giant'
  - **This confirms that ploidy manipulation is an effective means to reduce fertility in althea.**
  - We have performed extensive hybridization with 'Pink Giant' to develop pentaploids that we expect to have essentially no fertility. These plants did not flower in 2014, but we expect they will flower in 2015. At flowering, we will conduct an extensive evaluation of fertility of these plants by making reciprocal crosses with 'Pink Giant' as well as several other fertile cultivars.
  - **See attached SNA paper for more complete summary of findings.**
  - **Additionally, the 2014 Farwest issue of Digger contained an article on this work**