Oregon Department of Agriculture and Oregon Association of Nurseries
Nursery Research Project Final Report 2013

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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 weediness or even 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 hedge plant native to Southeastern Europe and Asia Minor that is pH adaptable, does well in full sun or deep shade, is salt spray tolerant, and withstands heavy pruning. Cumulatively, the estimated value of cherrylaurels for 2011 in Oregon alone was between $17.1 and $36.4 million. Foley and Raulston (1994) reported that most cultivars perform well in USDA Zones 7-8 but hardier selections, which are often the small-leaved forms such as 'Otto Luyken', extend into Zone 6 and possible Zone 5. 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. In addition to being weedy, the fruit litter is unsightly and either falls or is deposited by birds, presenting a management problem in urban and suburban settings. Furthermore, quarantine regulations due to cherry fruit fly (*Rhagoletis indifferens*) do not allow fruiting plants to be shipped into California, limiting its marketability. Leaf shothole can be extensive on common cherrylaurel, impacting salability. The problem occurs under overhead irrigation and is caused by the bacterium *Pseudomonas syringae* var. *syringae* and several fungi. Effective sprays are available but result in increased cost of production and can have ecological impacts. Portugese laurel is native from Portugal and Spain to the Canary Islands and 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 can be prolific in this species as well and has begun to receive similar attention as common cherrylaurel with regard to its potential as an invasive species.

Maples. Oregon is the leading producer of shade trees for the US. Much of this material is shipped to New England and upper Midwestern states. Maples are among the most commonly produced and planted trees across the country. Several economically important maple species have been identified as invasive in various regions of the country, particularly New England states. Several of these species have been banned or otherwise regulated due to their weediness including amur maple (*Acer ginnala* syn. *A. tataricum* ssp. *ginnala*) in Connecticut, Norway maple (*A. platanoides*) in Connecticut and Massachusetts, and sycamore maple (*A. pseudoplatanus*) in Connecticut and Massachusetts (USDA State and Federal Noxious Weed Lists; http://plants.usda.gov/java/noxComposite). Other economically important maple species, not yet identified as invasive or noxious weeds, also produce copious amounts of seed. These include trident maple (*Acer buergerianum*) and hedge maple (*A. campestre*). These species are not yet regulated but have potential to become banned or otherwise regulated 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. While at the US National Arboretum, Dr. Egolf developed four rose-of-sharon cultivars that were released 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 have become fertile. It is possible that due to the methods used that these plants were not homogeneous triploids and have reverted, or it could be that triploidy in rose-of-sharon does not reduce fertility as much as other species. We have begun 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. Specifically, developing improved forms of cherrylaurels through interspecific hybridization, ploidy manipulation, and mutagenesis and sterile maples via ploidy manipulation and backcrossing. Furthermore, we will address the questions raised regarding rose-of-sharon cultivars while simultaneously breeding for improved forms with reduced fertility.

Methods and Timeline

Cherrylaurels. We have made significant progress toward developing polyploidy cherrylaurels. In 2010, we successfully developed a number of polyploids; however, these all reverted. We have since received funding through the Agriculture Research Foundation and have begun experiments in tissue culture. We have initiated 'Otto Luyken' and 'Schipkaensis' in tissue culture and have been conducting extensive experiments to induce polyploids using oryzalin at varying rates and duration. Thus far, we have identified many (40-50) explant groups with polyploid cells and are in the process of isolating individual homogeneous polyploid explants. We will use funding to continue this promising work.

- January 2013 – All treatments should be finalized
- February 2013 – Explants will be transferred to new media and begin to be screened for polyploidy
- April 2013 – Isolation of individual polyploids
- May – August 2013 – Growing and rooting of polyploids
- August – December 2013 – Greenhouse growing of polyploids

We utilized mutagenis in 2010 and 2011 by irradiating a large numbers of common cherrylaurel seed to induce sterility and varying phenotypes. These plants have been planted at the Lewis-Brown Horticulture Research Farm in Corvallis and we have been observing them. We are beginning to observe significant differences among these plants, however, it is unclear if this is due to seedling variation or if we have affected some level of induced mutation. Continued observation and comparison to untreated controls should determine if our treatment was effective and will identify any improved and/or sterile forms.

In addition we have employed Hybridization during 2010 and 2011 making 4,587 controlled crosses between *Prunus laurocerasus* and *P. lusitanica.* We recovered large amounts of seed but few seedlings germinated and those that did were determined to be the result of self-pollination. 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, 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. In addition, we have begun attempts to use ovule culture to recover hybrids. We hope to expand these efforts in 2013.

- Spring 2013 – Controlled reciprocal crosses between species
- Spring – Summer 2013 – Collect fruit at varying days after pollination (DAP) and place into tissue culture
- Summer – Fall 2013 – Observe and grow out any resulting seedlings

Maples. To date, we have identified 113 Norway maple tetraploids, 9 trident maple tetraploids, and 5 amur maple tetraploids. From our 2012 treatmets, we have over 100 plants left to screen, so we expect our number of tetraploids to increase. Scion of 15 selections of Norway maple were sent to be propagated in August 2012 in order to expedite the breeding process. Once other tetraploids have adequate material available they will also be sent for propagation

- 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.

UPDATE ON THE PROGRAM

Cherrylaurels: We developed a number of polyploid forms of both *Prunus laurocerasus* 'Otto Luyken', *P. l.* 'Schipkaensis', and *P. lusitanica* (Table 1). These plants are in #3 containers and are growing well, although we sustained damage due to the early December freeze and snowstorm. It is not likely that the common cherrylaurel polyploids will flower in 2014; however, the portugese cherrylaurels are larger and show promise that they may flower. If so, they will be crossed with untreated common cherrylaurel.

Irradiated plants have been planted at the Lewis-Brown Farm in Corvallis since spring 2012, 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 (Figure 1). These plants were severely damaged from the December 2013 storm and xylem appears to be dead in many plants that sampled by cutting stems. Plants will be observed during spring 2014 to determine if they survived and will emerge from the crown. At a minimum, this project will be substantially delayed due to damage.

Ph.D. student, Jason Lattier, has made significant strides toward developing an optimized protocol for embryo rescue of cherrylaurel hybrids. Seed were collected at various stages of development and placed on a variety of media. Several media and collection times have shown great promise for seedling germination (Figure 2) and we will select one after data are analyzed.

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 J. Frank Schmidt for propagation 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 January 2014. We were not able to screen the amur maples in time to have them budded in 2013, they will be propagated in 2014. Similarly, the trident maples will be rooted from stem cuttings in 2014, as they are difficult to graft.

Rose of sharon: We made reciprocal crosses among 9 cultivars in 2012 and 19 cultivars in 2013, to determine relative fertility of each (Table 2). A portion of these progeny (approximately 585 seedlings) were field planted in spring 2013. We have collected height and width data on these to assess how each parent contributed to plant size in the next generation. We will also make observations on flower morphology and color to assess heritability of these traits. In 2014, we will plant an additional 500 to 600 seedlings. We also evaluated ploidy levels and have determined that all 9 cultivars evaluated in 2012 are tetraploid with the exception of ‘Pink Giant’ (Table 3). This is in contrast to what was reported for ‘Diana’, ‘Minerva’, and ‘Aphrodite’. The fact that ‘Pink Giant’ has reduced fertility and was observed to be a hexaploid confirms that ploidy manipulation remains a viable means to produce new sterile cultivars.

Table 1. Polyploids of *Prunus laurocerasus* and *P. lusitanica.*

Taxon	Homogeneous	Mixoploid
P. l. 'Otto Luyken'	76[z]	62
P. l. 'Schipkaensis'	64[y]	23
P. lusitanica	2	2

[z]Polyploids of common cherrylaurel are $2n = 44x = 352$

[y]Polyploids of portugese cherrylaurel are $2n = 16x = 128$

Table 1. Results of crossing study conducted during 2012 and 2013 to estimate the relative fertility of rose of sharon (*Hibiscus syriacus*) cultivars.

Cultivar	Flowers pollinated (No.)	Seedlings (No.)	Seedlings per poll. flower (No.)
	2012		
As female parent			
'Aphrodite'	42	423	10.1
'Blue Bird'	36	66	1.8
'Blue Satin'	59	318	5.4
'Diana'	97	155	1.6
'Lucy'	34	27	0.8
'Minerva'	24	44	1.8
'Pink Giant'	39	3	0.08
'Red Heart'	74	212	2.9
'Woodbridge'	70	180	2.6
Mean	52.8	158.7	3.0
As male parent			
'Aphrodite'	74	55	0.7
'Blue Bird'	54	212	3.9
'Blue Satin'	44	443	10.1
'Diana'	55	186	3.4
'Lucy'[z]	--	--	--
'Minerva'	66	222	3.4
'Pink Giant'	50	25	0.5

‘Red Heart’	66	154	2.3
‘Woodbridge’	66	131	2.0
Mean	59.4	178.5	2.9
	2013[y]		
As female parent			
‘Aphrodite’	51	271	5.3
‘Bali’	124	1,284	10.4
‘Blue Bird’	163	1504	9.2
‘Blue Chiffon’	91	622	6.8
‘Blue Satin’	31	439	14.2
‘Blushing Bride’	20	0	0
‘Diana’	61	258	4.2
‘Fiji’	71	326	4.6
‘Hawaii’	23	131	5.7
‘Helene’	29	0	0
‘Lavendar Chiffon’	4	0	0
‘Lil Kim’	78	272	3.5
‘Lucy’	35	0	0
‘Minerva’	30	82	2.7
‘Pink Giant’	127	96	0.8
‘Red Heart’	40	116	2.9
‘Tahiti’	63	35	0.6
‘White Chiffon’	17	0	0
‘Woodbridge’	68	553	8.1

Mean	**53.6**	**315.2**	**4.2**
As male parent			
'Aphrodite'	42	291	6.9
'Bali'	126	857	6.8
'Blue Bird'	62	381	6.1
'Blue Chiffon'	60	338	5.6
'Blue Satin'	45	166	3.7
'Blushing Bride'	7	15	2.1
'Diana'	70	325	4.6
'Fiji'	118	604	5.1
'Hawaii'	59	33	0.6
'Helene'	16	0	0
'Lavendar Chiffon'	3	1	0.3
'Lil Kim'	97	959	9.9
'Lucy'[z]	--	--	--
'Minerva'	31	95	3.1
'Pink Giant'	155	97	0.6
'Red Heart'	46	183	4
'Tahiti'	106	881	8.3
'White Chiffon'	30	38	1.3
'Woodbridge'	69	627	9.1
Mean	**54.4**	**327.3**	**4.3**

[z]'Lucy' is a double-flowered cultivar that does not produce pollen, therefore could not be assessed as a staminate parent.

[y]Data on germination for 2013 have not been collected and analyzed, therefore the data for fertility in 2013 are number of seeds and seeds per pollinated flower, not seedlings as in 2012.

Table 2. Mean relative holoploid genome size (2C) estimates ± SEM and inferred ploidy levels of nine cultivars of rose of sharon (*Hibiscus syriacus*). Estimates were performed by analyzing DAPI-stained nuclei using flow cytometry using *Solanum lycopersicum* 'Stupicke' (2C = 1.96 pg) as an internal standard.

Cultivar	2C	Ploidy level
'Aphrodite'	4.7 ± 0.04	4*x*
'Blue Bird'[z]	4.6 ± 0.04	4*x*
'Blue Satin'	4.6 ± 0.03	4*x*
'Diana'	4.7 ± 0.06	4*x*
'Lucy'	4.6 ± 0.01	4*x*
'Minerva'	4.6 ± 0.05	4*x*
'Pink Giant'	6.8 ± 0.05	6*x*
'Red Heart'	4.7 ± 0.00	4*x*
'Woodbridge'	4.6 ± 0.06	4*x*

[z]'Blue Bird' is also sold in the trade as 'Oiseau Bleu' and 'Bluebird'.

Figure 1. A seedling of *Prunus laurocerasus* growing at the Lewis-Brown Horticultural Research Farm in Corvallis showing red new growth. This seedling resulted from treating seed with 20Krad gamma radiation in 2010.

Figure 2. In vitro germination of a portugese cherrylaurel seedling as part of an experiment to optimize media for embryo culture of interspecific hybrids between common cherrylaurel and portugese cherrylaurel.