Final Report titled

Development of sterile cherrylaurel cultivars

submitted to

Agricultural Research Foundation

for

Oregon Department of Agriculture
Nursery Research and Regulatory Committee

Principle Investigator

Ryan Contreras
Assistant Professor
Department of Horticulture
Oregon State University
contrery@hort.oregonstate.edu
4017 Ag. and Life Sciences Bldg.
Corvallis, OR 97331-7304
Voice: 541-737-5462

Cooperators

Sarah Doane
Oregon Research Station Manager
Landscape Plant Development Center
sdoane@landscapecenter.org
P.O. Box 442
Aurora, OR 97020
Voice: 503-816-6358

Mara Friddle
Faculty Research Assistant
Department of Horticulture
Oregon State University
friddlem@hort.oregonstate.edu
4017 Ag. and Life Sciences Bldg.
Corvallis, OR 97331-7304
Voice: 541-737-5462

Background:

Prunus laurocerasus (common cherrylaurel) and *P. lusitanica* (portugese cherrylaurel), collectively referred to as cherrylaurels, are closely related species that are widely grown in the nursery industry and commonly planted in landscapes of the Pacific Northwest and other regions across the U.S. Using the Oregon Association of Nurseries Directory and Buyers' Guide (OAN-DBG) combined with the Nursery Appraisal Software for the Federal Crop Insurance Corporation for 2011 (http://www.rma.usda.gov/tools/eplpps/) we estimated a range for the number and value of cherrylaurels being grown in Oregon. Cumulatively, the estimated value of cherrylaurels for 2011 in Oregon alone is between $17.1 and $36.4 million.

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. There are more than 45 cultivars in the trade including the popular 'Otto Luyken'. 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.

Prunus laurocerasus is an autopolyploid with a chromosome complement of $2n = 22x = 176$ which is the highest in the genus and is unusually high for woody plants. This high chromosome number has been reported to generate irregularities during meiosis including loss of chromosomes; however, these disturbances are not great enough to reduce the fertility of the pollen or egg cells produced.

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. *Prunus lusitanica* is generally a small tree in the landscape but responds well to pruning and may be used as a dense, evergreen hedge with regular pruning. Portugese cherrylaurel is an octoploid ($2n = 8x = 64$), which is more typical of the genus. 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.

Most cultivars of cherrylaurel were developed/selected in Europe and introduced before the current ban on *Prunus* introduction to the U.S., therefore in order to develop novel forms we began a program to improve cherrylaurel utilizing alternative methods including interspecific hybridization, mutagenesis, and ploidy manipulation. The senior author has no knowledge of breeding programs in the U.S. that are conducting similar breeding work on cherrylaurels. Development of odd-ploidy forms that exhibit reduced fertility has been successfully used to develop triploid forms of food crops such as banana, watermelon, and apple as well as ornamentals such as rose of sharon, trumpet vine, and St. John's wort. Developing plants with odd ploidy levels is a two-step process in which chromosomes of plants are doubled to produce polyploids, $2n = 44x = 352$ in the case of common cherrylaurel, that are then backcrossed to untreated individuals. Developing sterile forms using mutagenesis via gamma radiation has resulted in sterile forms of food crops such as apple, grape, and citrus as well as ornamentals such as crapemyrtle and ruellia. There are also numerous examples of sterile, interspecific or intergeneric hybrid landscape plants including ×*Chitalpa tashkentensis*, *Buddleia* 'Asian Moon', *Buddleia* 'Blue Chip', and *Rhododendron* 'Fragrant Affinity'.

Objective:

The objective of the current research is to use hybridization, ploidy manipulation, and mutagenesis to develop new forms of cherrylaurel that are both sterile and exhibit superior ornamental characters. This research seeks to develop new cultivars of common cherrylaurel (*Prunus laurocerasus*), Portugese laurel (*P. lusitanica*), as well as hybrids of these species that combine the positive characteristics of each resulting in forms that are cold-hardy, shothole disease resistant, and sterile.

Progress:

Hybridization: We attempted to make interspecific crosses between common and Portugese cherrylaurel. In 2010, due to relative timing of flowering we performed crosses in only one direction by emasculating and then pollinating a total of 616 flowers of Portugese cherrylaurel with 3 genotypes of common cherrylaurel. We recovered 8 seed from which 2 seedlings germinated that had the appearance of the seed parent (Portugese cherrylaurel). Analysis showed that these are the result of self-pollination, even though when we self-pollinated 229 flowers of Portugese cherrylaurel we recovered no seed and we emasculated flowers prior to pollination. Apparently, the stigmas of these were inadvertently exposed to seed parent pollen, which resulted in these 2 seedlings. Data from 2010 crosses are summarized in Table 1.

In 2011, we conducted controlled crosses in both directions (Table 1). We emasculated 1,499 flowers of Portugese cherrylaurel and pollinated them with three genotypes of common cherrylaurel. We also emasculated 993 flowers of two genotypes of common cherrylaurel and pollinated them with Portugese cherrylaurel. We collected 90 seed from crosses using Portugese cherrylaurel as the seed parent but recovered no hybrids. I was very encouraged by the substantial amount of fruit set from these crosses. Even though we recovered no hybrids at this time, we confirmed that crosses yield fruit and seed. This has led to the establishment of multiple experiments being conducted by Ph.D. student, Jason Lattier to establish an embryo culture protocol to recover hybrids.

Ploidy manipulation: During 2010, we induced polyploidy in 34 plants of common cherrylaurel using the technique shown in Figure 1. Seedlings at the first true leaf stage were treated for 3, 5, or 7 days with oryzalin solidified with agar. These seedlings were allowed to go dormant during winter 2010-11, and were analyzed for ploidy level during late spring 2011. At this time it was determined that all seedlings had reverted to diploids. It is not clear why or how they reverted; however, this phenomenon has been observed in other taxa in the Contreras Lab (*Acer platanoides*) and in other labs around the country, namely at North Carolina State (*Magnolia* spp., *Acer platanoides*). The assumption is that there are populations of diploid cells that outcompete polyploid cells and we did not detect these during initial flow cytometry analysis.

In 2011, we attempted to use a protocol developed in the Brand Lab at UConn to treat *Berberis thunbergii*. We have successfully employed this technique on Japanese barberry and recovered over 60% polyploids, a rate that we have not observed using any other technique to date. This technique involves treating seed in which the radicle is allowed to emerge and then soaking seed in an oryzalin solution. We believed that treating the shoot apical meristem at this early stage will provide the best chance to recover stable polyploids that will not revert. Seed that were exposed to this treatment were cold stratified but radicles never emerged. It is unclear why this seed lot did not germinate, as we had used this seed source successfully on other experiments. Nevertheless, we used the reversion of polyploid seedlings as an impetus to pursue alternative treatments that would result in homogenous polyploids that would be stable. Since this time we have successfully developed a large population using in vitro treatments.

Irradiation: In 2010, we exposed seeds of common cherrylaurel to gamma radiation at rates of 0, 10, 25, 50, and 75 Krad. The study was conducted to determine the rate at which approximately 50% of seed were killed when compared to the control (0 Krad). We determined that 20 Krad was an appropriate rate. From the 2010 study, we retained 11 plants treated with 10 Krad and 8 plants treated with 25 Krad. These plants were field planted during September 2011. From the 2011 treatment with 20 Krad, we have a total of 66 plants. Of these, 38 were from seed collected from a standard species (wild type) genotype, 6 plants from seed collected from 'Otto Luyken', and 12 plants are from seed collected from 'Zabeliana'. These plants were field planted in 2012. We have continued to monitor plants from 2010 and 2011 treatments for growth, fertility, and overall ornamental impact. None of the plants have flowered yet but there is substantial variability in growth form, leaf shape, and new growth color. Of particular note, there are several forms that exhibit a very ornamental red new growth (Figure 2) but it is clearly affected by environment. We will continue monitoring this trait.

Table 1. Crosses conducted during 2010-11 between *Prunus laurocerasus* and *P. lusitanica* with the goal of developing sterile cherrylaurels; includes self-pollination to determine self-compatibility.

Cross (female x male)	# flowers pollinated	# seed	# germ	% germ
		2010		

P. lusitanica x *P. laurocerasus*	218	0	0
P. lusitanica x *P. laurocerasus* 'Schipkaensis'	200	4	2[z]
P. lusitanica x *P. laurocerasus* 'Otto Luyken'	198	4	0
P. lusitanica self-pollination	229	0	--
2010 Total	**845**	**8**	**2**
		2011	
P. lusitanica x *P. laurocerasus*	1499	46	0
P. lusitanica x *P. laurocerasus* 'Schipkaensis'	460	22	0
P. lusitanica x *P. laurocerasus* 'Otto Luyken'	573	22	0
P. laurocerasus 'Otto Luyken' x *P. lusitanica*	2173	0	0
P. laurocerasus 'Schipkaensis' x *P. lusitanica*	70	0	0
2011 Total	**2,492**	**90**	**0**

[z]Both seedlings resulted from self-pollination.

Figure 1. During 2010-11, we treated seedlings of common cherrylaurel at the first true leaf stage with an oryzalin solution to double chromosome number. Solution was solidified with agar to increase contact with shoot mersitem.

Figure 2. Common cherrylaurel plants resulting from treating seed with 20 Krad gamma radiation. Plant is two years from seed. Red new growth is affected by environment but we hope to identify genotype(s) that consistently expresses this trait.