Comparing Vegetative Propagation of Two 'Schipkaensis' Common Cherrylaurel Ploidy Levels

Justin A. Schulze, Ryan N. Contreras[1,3,4], and Carolyn F. Scagel[2]

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Summary. **'Schipkaensis' common cherrylaurel (*Prunus laurocerasus*) is an important nursery crop across the United States. In our breeding efforts to reduce shothole symptoms and weediness, we have created chromosome doubled forms of this cultivar. Vegetative propagation is an important factor in nursery production, and we have found no studies that have looked at comparative adventitious rooting of stem cuttings using induced polyploids. The objective of this research was to determine if rooting ability varied between these two ploidy levels. Semihardwood stem cuttings from wild-type (22*x*) and polyploid (44*x*) ploidy levels were taken at the end of July 2015 and the beginning of July 2016. Cuttings were dipped in 1030 ppm (0.10%) indole-3-butyric acid (IBA) and 660 ppm (0.066%) 1-naphthaleneacetic acid (NAA) before being set in rooting substrate. After 1 month, cuttings were removed from substrate and data collected. Data included; rooting percentage, root number per rooted cutting, average root length, and total root length. In 2015, 88% of the cuttings from the 44*x* plants and 63% of the cuttings from the 22*x* plants rooted. In 2016, 100% of cuttings from both ploidy levels rooted. In both years, average root length and total root length were similar between ploidy levels; however, cuttings from 22*x* plants generally had more roots than those from 44*x*. Chromosome-doubled 'Schipkaensis' common cherrylaurel rooted effectively, and produce transplantable cuttings similar to the standard ploidy.**

Common cherrylaurel is a species of evergreen stone fruit native from eastern Europe to southwest Asia. In some parts of the Mediterranean, this species is grown for food and medicinal purposes (Kolayli et al., 2003). In the United States and Europe, cultivars of this species are common ornamental landscape plants. Popular compact cultivars include; Mount Vernon, Otto Luyken, Schipkaensis, and Zabeliana. Many cultivars are susceptible to bacterial and fungal pathogens that cause shothole disease [e.g., *Pseudomonas syringae* pv. *syringae*, *Xanthomonas arboricola* pv. *pruni*, *Wilsonomyces carpophilus*, *Microgloeum pruni*, and *Cercospora* sp. (De Boer, 1980; Marchi et al., 2014; Pscheidt and Ocamb, 2014; Williams-Woodward, 1998)]. Shothole disease presents with numerous small circular holes in the leaves of plants, these holes are caused by loss of necrotic leaf tissue in areas killed by pathogens. This can greatly reduce ornamental appeal, and in severe cases, cankers that girdle stems may kill infected plants. Due to abundant fruit production, this species has become naturalized across areas of the North American west coast (U.S. Department of Agriculture, 2006), and in other regions is considered invasive (Hättenschwiler and Körner, 2003).

In attempts to address shot-hole symptoms and the weedy tendencies in this species, we created chromosome doubled forms of the cultivar Schipkaensis. Although many studies have compared morphological variability in ploidy series (Huang et al., 2015; Kermani et al., 2003; Li et al., 1996; Ulrich and Ewald, 2014), we have found none that addressed adventitious rooting of stem cuttings. Successful vegetative propagation is an important consideration in determining plant potential for large-scale production (Hartmann et al., 2011). We wanted to assess if alteration in ploidy level had an effect on vegetative propagation of this plant.

Previous research seeking to optimize vegetative propagation of common cherrylaurel successfully rooted hardwood cuttings at high percentages (Ribeiro et al., 2010; Sülüşoğlu and Çavuşoğlu, 2009; Yazici, 2009). Ribeiro et al. (2010) reported exogenous application of IBA improved rooting percentage compared with a control without IBA, but IBA ranging from 1000 to 7500 ppm (0.1% to 0.75%) produced no statistically significant difference among treatments. Other sources report taking semihardwood cuttings in summer is also effective (Adams, 1983; Dirr, 2009; Sülüşoğlu and Çavuşoğlu, 2010). The cultivar Schipkaensis, in particular, has been reported to root as quickly as 3 weeks (Adams, 1983). Our objective was to determine if rooting percentage and other root traits vary between natural [$2n = 22x = 176$ (Meurman, 1929)] and chromosome doubled ($2n = 44x = 352$) ploidy levels of 'Schipkaensis' common cherrylaurel.

Materials and methods

Plant material. In 2015, standard ploidy level (22*x*) 'Schipkaensis' common cherrylaurel material was

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[1]Oregon State University, Department of Horticulture, 4017 Agricultural and Life Sciences Building, Corvallis, OR 97331

[2]U.S. Department of Agriculture, Agricultural Research Service, Horticultural Crops Research Unit, 3420 NW Orchard Avenue, Corvallis, OR 97330

[3]Associate Professor of Horticulture.

[4]Corresponding author. E-mail: ryan.contreras@oregonstate.edu.

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Units

To convert U.S. to SI, multiply by	U.S. unit	SI unit	To convert SI to U.S., multiply by
3.7854	gal	L	0.2642
2.54	inch(es)	cm	0.3937
25.4	inch(es)	mm	0.0394
0.0254	mil	mm	39.3701
28.3495	oz	g	0.0353
1	ppm	$mg \cdot L^{-1}$	1
(°F – 32) ÷ 1.8	°F	°C	(°C × 1.8) + 32

collected from 3-year-old commercially grown plants (Blue Heron Farms, Corvallis, OR) growing in 3-gal containers. Chromosome doubled (44*x*) plant material was confirmed via flow cytometry (Contreras and Meneghelli, 2016), and collected from three 3-year-old plants growing in 7-gal containers containing a soilless fir-bark substrate. The 44*x* plants received 20N–4.4P–8.3K, controlled-release fertilizer (Apex® Evergreen; J.R. Simplot, Boise, ID) at a rate of 32 g per container annually in April. All 44*x* plants were under overhead irrigation twice per day for 40 min when cuttings were collected. In 2016, cuttings were collected from the previous year's rooted cuttings (1 year old) growing in 3-gal containers, under the irrigation and fertility conditions previously stated. All plants in both years were grown on outdoor container pads in full sun.

Cutting treatments. On 29 July 2015 and 5 July 2016, 24 cuttings from each ploidy level were taken. Cuttings were 7 to 10 cm long with three to five nodes. The three youngest leaves were retained on cuttings and any remaining leaves were removed. Retained leaves were bisected to reduce water loss. The basal 5 cm of cuttings were dipped for 10 s in 1030 ppm IBA (0.1%) and 660 ppm (0.066%) NAA dissolved in 9.8% isopropyl alcohol (Woods Rooting Compound; Earth Science Products, Wilsonville, OR) and the lower 3 to 5 cm of each cutting was inserted into 4-inch square containers (Gauge 400s; Merrill's Packaging, Burlingame, CA) filled with two perlite (Supreme Perlite, Portland, OR): one soilless substrate (Metro-Mix 840PC; Sun Gro Horticulture, Agawam, MA) by volume. Each year, the 48 cuttings were randomly arranged in three 17-inch square flats, with eight replicates from each ploidy level in a flat. Flats were placed in a clear 6-mil polyurethane mist-tent with bottom heat (78 °F) under intermittent mist (30 s every 30 min from 0600 HR to 2000 HR) for 1 month. The mist-tent was located in a glass-house covered with a 50% shadecloth and temperatures set to 75/65 °F day/night. The glass-house was located on the campus of Oregon State University in Corvallis.

Rooting evaluation. Cuttings were removed from the substrate, and rinsed gently with water to remove remaining substrate. Cuttings were placed on a flat surface and the roots were manually spread apart. Roots on each cutting were measured using a standard metric ruler to the nearest millimeter. Number of roots and length of each root on all rooted cuttings were measured if the root was over 2.0 mm long. The total root length and average root length was calculated per cutting.

Data analysis. Data were analyzed using R-studio, version 3.1.1 (R Foundation for Statistical Computing, Vienna, Austria). The experimental design was a randomized complete block design with replication. Due to lack of normality and unequal variance, rooting percentages were compared using the nonparametric Kruskal-Wallis rank sum test. Year and ploidy were evaluated separately, with flats as experimental units. Furthermore, the dependent variables; root number and total root length, failed a Levene's test for homogeneity of variance, and were subsequently transformed using a square root transformation. After transformation, variances were stabilized, and analysis was performed for root length, root number, and total root length using a two-way analysis of variance. This portion of the analysis considered ploidy and year explanatory variables, and experimental units were individual cuttings. Probability values were determined using type III marginal sum of squares to account for unequal sample size resulting from several unrooted 2015 cuttings.

Results and discussion

Ploidy had no influence on the number of cuttings that rooted in either year (Fig. 1A). In 2015, 63% of the 22*x* cuttings rooted and 88% of

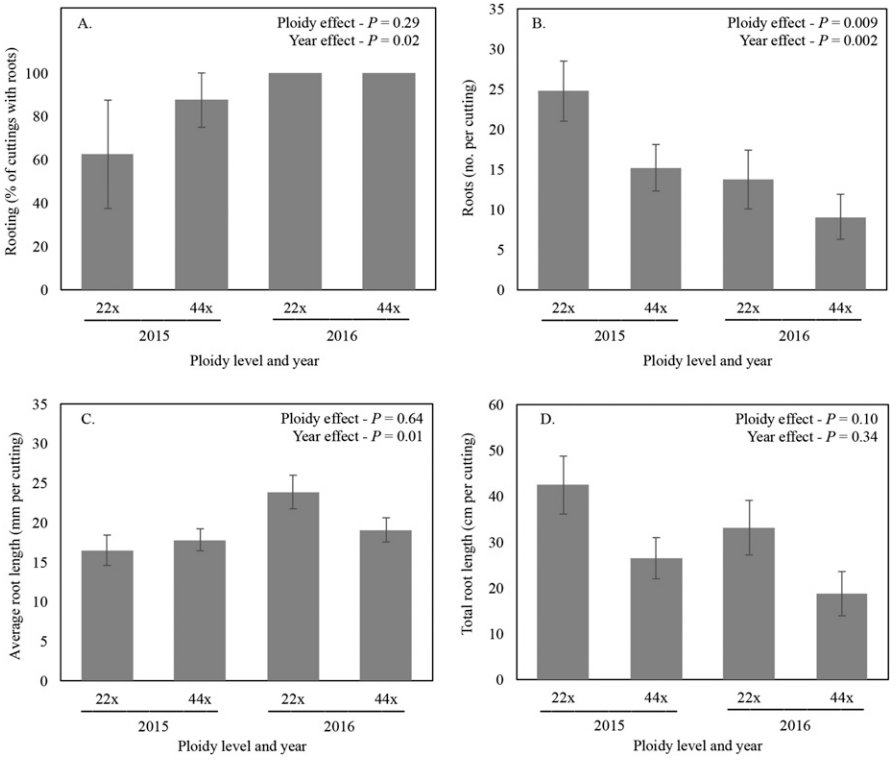

Fig. 1. Adventitious rooting, average root length, root number, and total root length on stem cuttings from 22*x* and 44*x* 'Schipkaensis' common cherrylaurel. Cuttings were taken in late July 2015 and in early July 2016. (A) Column means and SE bars based on Kruskal-Wallis rank sum test (n = 6). (B, C, D) Unrooted samples in 2015 resulting in variable sample size [2015 22*x* (n = 15), 2015 44*x* (n = 21), 2016 22*x* (n = 24), 2016 44*x* (n = 24)]. (B, D) Probability for ploidy and year are for main effects from analysis of variance (ANOVA), using transformed variables. Columns are back-transformed means and error bars are one SE of the mean. (C) Probability values for ploidy and year are for main effects from ANOVA. Columns are least squares (LS) means and error bars are one SE of LS means; 1 mm = 0.0394 inch, 1 cm = 0.3937 inch.

the 44*x* rooted, but the variability among replicates was high resulting in similar rooting between 44*x* and 22*x* plants. All cuttings rooted in 2016. Previous research of wild-type common cherrylaurel cuttings resulted in rooting between 60% and 88%, when IBA was used (Ribeiro et al., 2010; Sülüşoğlu and Çavuşoğlu, 2009, 2010). Our results are consistent with Dirr (2009), who reports up to 100% rooting in semihardwood cuttings taken in summer.

Increased ploidy decreased the number of roots produced by cuttings (Fig. 1B). On average, cuttings from 22*x* plants produced 21 roots and 44*x* plants produced 13 roots, representing a 38% decrease in root number. Having fewer roots could have a detrimental effect on water and nutrient absorption. However, in our study we applied only one concentration of IBA and NAA. Sülüşoğlu and Çavuşoğlu (2010) show an increase in number of roots from 1000 to 2000 ppm (0.2%) IBA, and then subsequent decrease in root number from 2000 to 8000 ppm (0.2% to 0.8%) IBA. The 44*x* plants may have different requirements for optimal rooting compared with 22*x*, and perhaps altering auxin concentrations could improve the root number on 44*x* cuttings. Root number also varied by year, suggesting sensitivity to factors other than auxin-concentration and ploidy, such as collection time. In previous studies, collection time appears to affect root number per cutting in this species. Hardwood cuttings taken in March (Sülüşoğlu and Çavuşoğlu, 2009), and semihardwood cuttings taken in July (Sülüşoğlu and Çavuşoğlu, 2010), both treated with 2000 (0.2%) ppm IBA, produced an average of 55.0 and 22.2 roots per rooted cutting, respectively.

Ploidy did not influence average root length (Fig. 1C). Average root length of cuttings from 22*x* plants (20.2 mm) were similar to that of 44*x* plants (18.5 mm) for both years. However, average root length in 2016 was 25% greater than in 2015. This suggests that collection timing may affect average root length as well as number of roots per cutting. Compared with our results, Sülüşoğlu and Çavuşoğlu (2010) reported longer average root length (42.2 to 62.6 mm) on cuttings of wild-type common cherrylaurel evaluated after 90 d. It is likely that increasing time to evaluation would also increase our average root length.

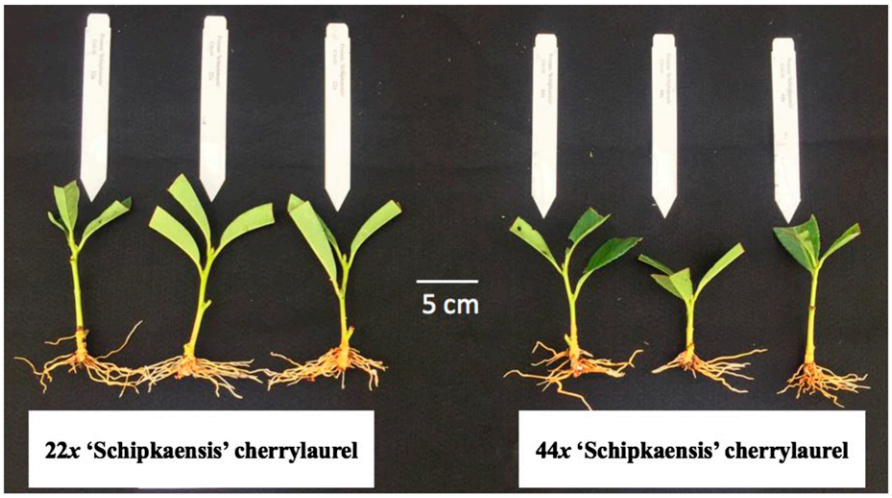

Fig. 2. Rooted stem cuttings from 22*x* and 44*x* ploidy levels of 'Schipkaensis'common cherrylaurel. This photo was taken of 2016 cuttings that measured the highest in the category "total root length" from each block; 1 cm = 0.3937 inch.

Total root length per cutting did not differ between years (Fig. 1D). On average, total root length on cuttings from 22*x* plants (37.8 cm) was greater than total root length on cuttings from 44*x* plants (22.6 cm), but this difference was not statistically significant.

Our study confirmed the chromosome doubled form of 'Schipkaensis' common cherrylaurel roots at a sufficiently high percentage and produces transplantable cuttings (Fig. 2). Compared with the 22*x* ploidy level, the 44*x* produced fewer roots, but this difference is not likely commercially important. Visually, the root systems on cuttings of both ploidy levels were coarse with no branching or fine root development. Roots of 44*x* cuttings did appear slightly thicker, but we did not measure root diameter or determine root dry weight. Future studies should include these factors when assessing differences in rooting among treatments. All rooted cuttings have been successfully transplanted into larger containers, and we have seen no negative effects of reduced root number, even though the 44*x* cuttings produced nearly 10 and five fewer roots per cutting in 2015 and 2016, respectively. As propagation can be a bottleneck for production, it is important to ensure that new cultivars are able to be propagated efficiently. In our study, there were no apparent detrimental effects to rooting cuttings of 'Schipkaensis' common cherrylaurel following induction of higher-level polyploids.

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