# Oregon Department of Agriculture and Oregon Association of Nurseries Nursery Research Project Final Report 2016

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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 in some cases invasiveness. Some crops are being banned in some states or regions (e.g. maples in New England) and others simply are known to produce seedlings in a garden situation (e.g. althea). The Ornamental Plant Breeding Program at Oregon State University has been addressing some of these species over the past six years. A brief background of several ongoing projects that have been previously funded 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. Our goals are to 1) develop sterile forms of common cherrylaurel that exhibit the typical phenotype that consumers are used to and 2) develop sterile hybrids of common cherrylaurel x Portugese cherrylaurel that exhibit shothole disease resistance.

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.

A huge and untapped resource is determining the genome sizes and ploidy levels of available maple species. Little work has been done in this area but we have been conducting a large scale screening of the genus using flow cytometry (FCM) and to date we have screened more than 100 accessions from gardens and arboreta around the country that house the core collection of the North American Plant Collection Consortium (NAPCC). This includes 15 of the 19 sections of the genus and with collections Morton Arboretum and Hoyt Arboretum still to process, we will have 18 of 19 sections represented. We have identified tetraploid forms that we plan to use in crosses without having to induce polyploidy and wait for resulting plants to flower, so we expect we will shorten the duration for cultivar development by several to many years. This work is ongoing and we ultimately plan to report on more than 150 accessions that represent all but one taxonomic group within Acer.

Our goal is to use all tools (induced polyploidy, natural polyploidy identified through FCM) develop sterile triploids from which superior clones may be selected that exhibit various trait combinations such as leaf colors (new growth, growing season, fall color) and growth forms (fastigiate, standard, columnar, etc.).

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.

We have identified ploidy levels of most commercially available cultivars as well as documenting fertility of those same cultivars; however, the work on fertility is ongoing. We also have developed many pentaploid (5x) plants that we will evaluate. Our crossing work on this group has been extensive (Table 1) and we will continue on this scale as we expand our findings on heritability and fertility of our hybrids. Our goal is to use these data we have collected over the last two to three years to develop improved althea (rose-of-sharon) cultivars that are sterile and exhibit new combinations of traits. We also have expanded our work into those cultivars that resulted from crosses between *H. paramutabilis* x *H. syriacus*. In 2015 we made more than 600 crosses using the hybrid cultivars Full Blast ('Resi'), 'Lohegrin', and 'Tosca' in combination with several cultivars of althea including 'Blushing Bride', the "Chiffon series", and the "Smoothie series". We recovered 114 fruits, of which 51 have been sown and we have already recovered 89 seedlings as of 15 September; therefore, I expect that we will recover at least several hundred seedlings to select among for novel traits including flower color and flower size.

#### Methods and Timeline

Cherrylaurels: Our polyploids should flower in 2016, as they again did not flower in 2015. We will assess fertility of our polyploid forms of 'Otto Luyken' and 'Schipkaensis' in comparison to standard genotype at flowering, which we hope to be spring 2016. We have confirmed that polyploids root equally well as untreated plants, which is essential for production (Figure 1). We made nearly 1,000 crosses between common cherrylaurel and Portugese cherrylaurel and have almost 100 seed in tissue culture (Table 2). Seedlings in tissue culture should germinate during winter 2016 and we will test them to confirm hybridity as they emerge. We also developed 4 polyploid forms of Portugese cherrylaurel (16x). After these plants flower they will be extensively crossed (including reciprocals) with common cherrylaurel. We believe the polyploid Portugese cherrylaurel (16x as opposed to wild-type 8x) will give us a better chance of recovering hybrids with common cherrylaurel, which is a 22x. It is unclear if these plants will flower in 2016 but we will force them as much as possible to encourage growth.

Maples: We confirmed that tetraploid norway maples have remained stable over the past nearly 4 years. A number of genotypes have been dug from J Frank Schmidt and interplanted with several industry standards at the Lewis-Brown farm in Corvallis. Four of these plants flowered in 2015 and did not set any seed. It is possible that these tetraploids will exhibit an acceptable level of fertility and we could select among them. Ten genotypes were propagated and we are collaborating with J. Frank Schmidt and Sons who are providing a second location for long-term observation to determine if the tetraploids are sterile.

We had very precocious flowering of tetraploid and mixoploid (with tetraploid pollen and eggs) Acer ginnala. We made interploidy level crosses again in spring 2015 after failing to recover seedlings from 2014 crosses. This year we made 828 interploidy crosses and recovered 78 seed that we are waiting to germinate. If we recover seedlings, we will test them for ploidy level to confirm and at flowering their fertility will be assessed. Acer buergerianum tetraploids have not flowered to date but plants are growing well and there is a great deal of seedling variation among polyploids to develop a diverse group of triploids to select from. Due to the size of our trees, we will field plant polyploids of both amur and trident maples in fall 2015 and make future crosses in the field.

# **Budget Summary**

Salary

| Total  | \$23,403 |
|--|----------|
| Flow cytometry kits                          | \$1,250  |
| Supplies                                     |          |
| Other Payroll Expenses (OPE)                 | \$600    |
| Student workers                              | \$5,000  |
| (OSU health benefits, insurance, retirement) |          |
| Other Payroll Expenses (OPE)                 | \$6,700  |
| Faculty Research Assistant (25% FTE)         | \$9,853  |
| Salary                                       |          |

Table 1. Hibiscus syriacus (rose-of-sharon, althea) crosses conducted between 2012-14 and the number of plants field planted.

| <u>Y</u> ear | Cross combinations performed | # Flowers pollinated | # Field planted |
|--------------|------------------------------|----------------------|-----------------|
| 2012         | 63                           | 487                  | 550             |
| 2013         | 134                          | 1059                 | 631             |
| 2014         | 68                           | >1400                | TBD*            |
| 2015         |                              |                      | - 37.62         |
| Total        | 265                          | >2946                | 1181            |

<sup>\*</sup>Will be field planted during fall 2015.

Table 2. Crosses and seeds collected from crossing *Prunus lusitanica* x *P. laurocerasus* 'Otto Luyken' and put into tissue culture to recover hybrids.

| Date Cultured    | 6/24/15 | 7/15/15 | 8/6/15 | Totals |
|------------------|---------|---------|--------|--------|
| # Crosses        | 215     | 230     | 500    | 945    |
| Seeds in Culture | 22      | 25      | 46     | 93     |

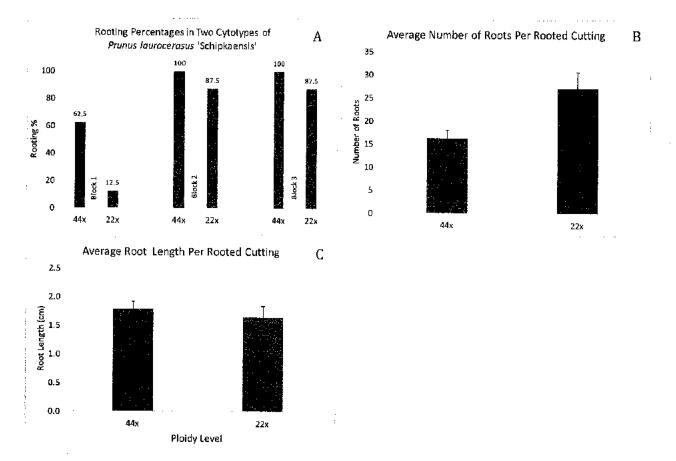


Figure 1. Assessment of rootability and quality of cuttings of polyploid 'Schipkaensis' compared to the standard cultivar including rooting percentages (A), average number of roots (B), and average root length (C).

# **2015 – 2016 UPDATES**

## Cherrylaurels

#### Common cherrylaurel

Unfortunately, none of our induced 44x 'Otto Luyken' or 'Schipkaensis' flowered in 2016. We are continuing to grow the polyploids and waiting for flowering. Six of the mixoploid 'Otto Luyken' flowered this season. We discovered that their pollen is not different than 22x plants, and would not be useful in future crossing to produce 33x plants, so we discarded them. Growth of 'Otto Luyken' appears to be negatively impacted by the increase in ploidy level. These induced polyploid forms are slow growing with high rates of reversion. However, this reversion has also led to five (5) plants that "settled" at the 33x level. These plants are being propagated for further analysis. 'Schipkanesis' polyploids, in contrast, retain much of their vigor. We have over 20 of the 44x 'Schipkaensis' rooted and growing alongside their 22x counterpart. The most notable aspect of this comparison is the reduced amount of shothole symptoms on the leaves of the 44x plants. This may be, in part, due to increased foliar thickness. We will continue to observe these plants in the coming years. We are also in the process of repeating the rooting study which has given evidence of high quality vegetative propagation capabilities of induced polyploids of 'Schipkaensis'. We hope to have sufficient material to distribute to collaborating nurseries in 2016-17 to assess shothole severity under production conditions.

### Portuguese cherrylaurel

We are continuing our observations of the induced polyploid Portuguese cherrylaurel and encouraging vigorous growth in attempts to reduce time to flowering. In the mean time we have been comparing morphological traits of the induced polyploid to the natural forms. The induced ployploids have decreased stomatal density and increased stomatal size (Figure 2). Previous literature has cited this as a potential source of drought tolerance in other woody species. We will continue this work by using physiological measurements to test stomatal conductance and photosynthetic rates of these plants. While portugese cherrylaurel is already a drought tolerant species, perhaps our plants exhibit even higher levels of water use efficiency and/or drought tolerance.

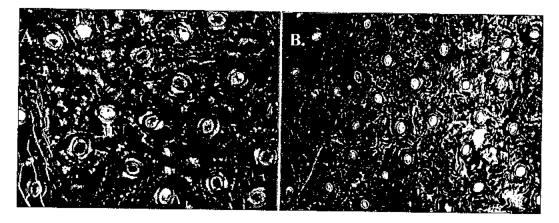


Figure 2 . Stomatal variation between A.) 16x and B.) 8x one-year-old Prunus lusitanica observed under x200 magnification



Figure 3. Morphological variation between and among cytotypes of one-year-old Prunus lusitanica

# Common cherrylaurel x Portuguese cherrulaurel

In 2015, crosses were performed outdoors on a tree on campus. As a result, we had several putative hybrids that appear to have been accidental self-pollinations. In 2016, we performed crosses in a glasshouse under controlled conditions. All of the crosses in the table below yielded only 2 fruit (both from *P. lusitanica* x 'Otto Luyken') which were placed in tissue culture on 7-13-16. We hope we have more luck with the 16x Portuguese cherrylaurel (Figure 3) as the mother in the coming years. Based on the many thousands of crosses we have attempted since 2011 – including 2016 (Table 3) between these species, we are suspending our efforts until the 16x plants flower.

Table 3. 2016 cherrylaurel crossing.

|        | to Luyken' Crosses | P. lusitanica X 'Schipkaensis' Cross |     |  |
|--------|--------------------|--------------------------------------|-----|--|
| 22-Apr | 153                | 22-Apr                               | 0   |  |
| 23-Apr | 141                | 23-Apr                               | ő   |  |
| 24-Apr | 99                 | 24-Apr                               | ő   |  |
| 25-Apr | 204                | 25-Apr                               | 25  |  |
| 26-Apr | 149                | 26-Apr                               | 36  |  |
| 27-Apr | 0                  | 27-Apr                               | 0   |  |
| 28-Арг | 149                | 28-Apr                               | 28  |  |
| 29-Apr | 35                 | 29-Apr                               | 22  |  |
| Total  | 930                |                                      | 111 |  |

#### Maples

Norway maples did not flower in 2016, therefore we do not have an update on the fertility. Trees are all continuing to grow on well and I hope they return to flowering in 2017.

We had five Amur maple and one trident maple 4x plants flower in 2016. We collected seed in early July from these plants, which were interplanted with 2x plants (Table 4). These seed will be sown after stratification and seedlings will be screened for ploidy level. From these two species we collected 4,367 seeds from which I feel confident that we will recover triploids that also exhibit outstanding ornamental and production characteristics. Among the tetraploid parents are several that exhibited impressive new growth traits. One in particular was quite interesting (Figure 4). We will grow out these plants starting winter/spring 2017 and begin ploidy analysis after plants have produced at least three sets of leaves to prevent damage. We also have four (4) triploid amur maples and one (1) triploid trident maple that have yet to flower.

Table 4. Number of seed collected from five Amur maple accessions and one trident maple selection that flowered in 2016.

| Species           | Field location | Ploidy | seed# | into<br>strat | out of strat |
|-------------------|----------------|--------|-------|---------------|--------------|
| Acer ginnala      | 74.19          | 4x     | 1277  | 7/7/16        | 10/7/16      |
| Acer ginnala      | 74.22          | 4x     | 562   | 7/7/16        | 10/7/16      |
| Acer ginnala      | 75.11          | 4x     | 869   | 7/7/16        | 10/7/16      |
| Acer ginnala      | 75.15          | 4x     | 247   | 7/7/16        | 10/7/16      |
| Acer ginnala      | 75.18          | 4x     | 619   | 7/7/16        | 10/7/16      |
| TOTAL             |                |        | 3,574 |               |              |
| Acer buergerianum | 72.15          | 4x     | 793   | 7/7/16        | 10/7/16      |

#### GENOME SIZE DATA

We conducted a large scale evaluation of *Acer* to identify genome size or ploidy level variations. We identified variation among taxonomic groups and a number of polyploids. For convenience, I have attached as an appendix summary of our findings presented to the NAPCC maple core collection collaborative.

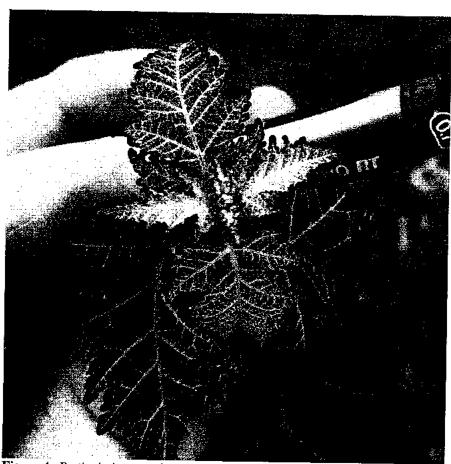


Figure 4. Particularly attractive new growth on a tetraploid *Acer ginnala* (12-0011-011, field location 74.22). We collected 562 seed from this selection in 2016. The goal is to have a large population from which to select excellent phenotypes that are desirable to growers and consumers, in addition to being sterile.

Rose-of-sharon (althea). 2014 intraspecific crosses were not field planted but we are growing them in containers to expedite evaluation. We evaluated them based on flower size, color, and petal number and kept the top 2% (52 out of 2548) of plants with regard to each trait. The criteria for each trait was greater than 3500 mm<sup>2</sup> for petal area, petal number greater than 50, and an "L\*" value less than 58. L\* refers to the depth of color; the lower the value, the richer the color. These were field planted spring 2016.

Eighteen (18) 5x plants currently are in the glasshouse for controlled crossing to test fertility. We will use various fertile parents, as determined from previous years' crossing data, to make crosses using these 5x plants as females. Among the putatively sterile pentaploids resulting from our previous crosses are:

Aphrodite x Pink Giant Bluebird x Pink Giant Diana x Pink Giant Helene x Pink Giant

Woodbridge x Pink Giant Pink Giant x Bali Pink Giant x Bluebird Pink Giant x Blushing Bride Pink Giant x Fiji Pink Giant x Lil Kim Pink Giant x Red Heart

Table 5. Hibiscus syriacus (rose-of-sharon, althea) crosses conducted between 2012-15 and the number of plants field planted or being grown in containers for evaluation.

| Year  | Cross combinations performed | # Flowers pollinated | # Evaluated |
|-------|------------------------------|----------------------|-------------|
| 2012  | 63                           | 487                  | 550%        |
| 2013  | 134                          | 1059                 | 631%        |
| 2014  | 68                           | 1830                 | 2548*       |
| 2015  | 77                           | 561                  | 3095#       |
| Total | 342                          | 3937                 | 6824        |

%Plants were field planted during spring 2013 (2012 crosses) and spring 2014 (2013 crosses), respectively. \*Were grown in containers until selection for floral traits; top 52 performers (top 2%) were field planted spring 2016.

#These plants currently are being grown in containers for floral evaluation and will be planted fall 2016/spring 2017. A similarly stringent selection criterion will be used; I expect to keep no more than 75 of the top performers.

We have more than 800 seedlings (Table 6) that are putative backcrosses of interspecific hybrids either to H. syriacus or H. paramutabilis. These plants are beginning to flower are being evaluated for novel floral traits, improved growth, and fertility. Additionally, Hsuan Chen, Ph.D. student is confirming hybridity using ISSR markers.

Table 6. 2015 backcrosses to *Hibiscus syriacus* or *Hibiscus paramutabilis* using 'Resi' (Full Blast™) and 'Lohengrin', which are cultivars putativesly derived from interspecific hybridization of these parents.

| Female           | Male             | Flowers | Fruit set | Seeds | Seedlings |
|------------------|------------------|---------|-----------|-------|-----------|
| Full Blast       | H. paramutabilis | 3       | 0         |       |           |
| H. Full Blast    | H. Syriacus (4)* | 7       | 1         | 2     |           |
| H. Lohengrin     | H. Lohengrin     | 14      | 0         |       |           |
| H. Lohengrin     | H. paramutabilis | 2       | 0         |       |           |
| H. Lohengrin     | H. Syriacus (1)* | 60      | 1         | 2     | 2         |
| H. paramutabilis | H. Full Blast    | 1       |           |       |           |
| H. paramutabilis | H. Lohengrin     | 1       |           |       |           |
| H. paramutabilis | H. paramutabilis | 3 ,     |           |       |           |
| H. paramutabilis | H. Syriacus (4)* | 15      |           |       |           |
| H. Syriacus (6)* | H, Full Blast    | 53      | 17        | 120   | 83        |
| H. Syriacus (8)* | H. Lohengrin     | 239     | 92        | 1177  | 711       |
| H. Syriacus (9)* | H. paramutabilis | 152     | 5         | 17    | 7**       |
| H. Syriacus      | H. Syriacus      | 56      | 21        | 261   | 194       |
| Total            |                  | 606     | 137       | 1579  | 997       |

<sup>\*</sup>Number in parenthesis indicates the number of cultivars used in that cross direction.



**FIGURE 5.** Extremely large, full-double flower of *Hibiscus syriacus*. Among the improvements we are reaching are larger flowers with the full double form seen in the smaller "Smoothie Series". Also, we are combining these desirable phenotypes with pentaploid chromosome compliments that we predict will be nearly sterile.

# Summary Report of the *Acer* genome size and ploidy survey

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June 2016

The Acer genome size and ploidy survey is coming to a close. Initiated by Oregon State University (OSU) in 2014, this is a collaborative project with botanical gardens and arboreta around the country including: Hoyt Arboretum, Quarry Hill Botanic Garden, Morris Arboretum, Morton Arboretum, US National Arboretum, Arnold Arboretum, and Cornell Plantations. Additionally, material was donated by the nurseries of J. Frank Schmidt, Heritage Seedlings and Whitman Farms. The objective of this study was to determine genome size and ploidy information for a diverse and wide-ranging collection of *Acer* species. This data can be used for determining breeding objectives and/or developing future research objectives regarding *Acer*.

Relative genome size was measured using *Pisum sativum* 'Ctirad' (2C genome size = 8.76 pg) as the internal standard. Nuclear DNA content was stained using the fluorochrome DAPI (4',6-diamidino-2-phenylindole). In order to discern base pair composition, a subset of accessions was analyzed using the fluorochrome PI (propidium iodide). Chromosome number counts were confirmed through traditional cytology.

Plant materials received included cuttings shipped by botanical gardens and arboreta, cuttings collected by investigators, and whole plants donated by Heritage Seedlings. The cuttings received were propagated for use in cytological analyses, and bud tissue was used for cytometric analyses. See the attached Table for accessions that were successfully rooted (rooting success indicated by "y"). A total of 192 accessions were analyzed using the flow cytometer. This includes species, hybrids, and cultivars. Accessions with an asterisk in the "PI Subset" column were analyzed with both DAPI and PI.

Root squashes have been completed for seven accessions representing six sections. We will continue doing root squashes with the goal of representing as many sections as possible. This will be limited by availability of quality root tips. Currently, we have root tips collected from sections Rubra, Palmata, Pentaphylla, Arguta, Macrantha, Platanoidea, Ginnala, Acer, Oblonga, Negundo, Pubescentia, Trifoliata, Macrophylla, and Indivisa.

Our results show that there is genome size and ploidy variation in *Acer rubrum*. The data also indicate that there may be variation in monoploid genome size within sections Arguta, Macrantha, Palmata, Pentaphylla, Platanoidea, Spicata, and Trifoliata. Further statistical analysis is needed to confirm this. Variation in monoploid genome size can be useful for identifying hybrid progeny. Potential natural polyploids include *Acer heldreichii*, *A. pseudoplatanus*, *A. velutinum*, *A. yui*, *A. pilosum*, and *A. pycnanthum*. Polyploid maples developed at OSU were included in the survey.

Vouchers of pressed material are being prepared for deposit in the Oregon State University Herbarium. These vouchers will be included in the Cultivated Flora collection.

A manuscript is being prepared for publication in the Journal of the American Society for Horticultural Science. This manuscript will include genome size and ploidy data, cytological photomicrographs, collection/origin information for each accession when available, and OSU Herbarium accessions.

While the current project is coming to a close, there is still much work that can be done with the genus relative to genome size and ploidy. The objective of this project was to get broad coverage of wild collected material available in North America. Potential areas of research for future projects would include surveys of cultivated material and a deeper investigation into the ploidy levels and genome sizes of taxonomic sections with wide and varied geographic distribution (i.e. Section Rubra).

| Source | Taxa   | Section          | Source Acc. | Rooted           | Cytology   | Pi subset  |
|--------|--|------------------|-------------|------------------|--|--|
| JFS    | Acer rubrum 'October Glory'  | Rubra            | n/a         | <del></del>      | <del> </del> -                                   |  |
| JFS    | Acer rubrum 'Somerset'   | Rubra            | n/a         |                  | <del>- </del>                                    | <del> </del> -                                     |
| JFS    | Acer rubrum 'Columnare'  | Rubra            | n/a         |                  | <del>-</del>                                     | ļ <u> </u>   |
| JFS    | Acer rubrum 'Morgan'   | Rubra            | n/a         | <del></del>      | <del> </del> -                                   | <del></del>  |
| JFS    | Acer rubrum 'Scarsen' (Scarlet Sentinel®)                          | Rubra            | n/a         | <del>-</del>     | ·  | <del>-</del>                                       |
| JFS    | Acer xfreemannii 'Sienna' (Sienna Gien®)                           | Rubra            | n/a         | <del></del>      | -  |  |
| JFS    | Acer rubrum 'Red Rocket'   | Rubra            | n/a         |                  | <del> </del>                                     |  |
| JF5    | Acer xfreemanii 'DTR 102' (Autumn Fantasy®)                        | Rubra            | n/a         | <del></del> -    | ļ <u>.</u>                                       | <del> </del>                                       |
| JFS    | Acer rubrum 'Autumn Flame'   | Rubra            | n/a         | <del>-  </del> - | <del>-</del>                                     | <del>                                       </del> |
| JFS    | Acer rubrum 'Autumn Spire'   | Rubra            | n/a         | -                | <del> </del>                                     |  |
| JFS    | Acer rubrum 'Bowhall'  | Rubra            | n/a         | - <del> -</del>  | +  |  |
| JF\$   | Acer xfreemanii 'Celzam' (Celebration®)                            | Rubra            | n/a         | <del></del>      | ·  | <del> -</del>                                      |
| JFS    | Acer pseudosieboldianum x palmatum 'Hasselkus'<br>(Northern Glow®) | P <b>al</b> mata | n/a         |                  |  |  |
| JFS    | Acer rubrum (Halka)  | Rubra            | n/a         | <del>-</del> -   |  | <u>.                                    </u>       |
| MRS    | Acer caesium   | Acer             | 1994-005    | <del></del> -    |  | <del></del>  |
| MRS    | Acer heldreichii trautvetteri                                      | Acer             | 2004-172    |                  | <del> </del> .                                   |  |
| MRS    | Acer monspessulanum ibericum                                       | Acer             | 2008-189    |                  | +  |  |
| MRS    | Acer saccharum skutchii  | Acer             | 2014-242    |                  | -  |  |
| MRS    | Acer acuminatum  | Arguta           | 1994-009    | <del></del>      | <del> </del> -                                   |  |
| MRS    | Acer tegmentosum   | Macrantha        | 1993-342    | Y                | <del> </del>                                     |  |
| MRS    | Acer pubipalmatum  | Palmata          | 2009-106    | Y                |  |  |
| MRS    | Acer distylum  | Parviflora       | 1995-163    | <u>'</u>         | <del> </del>                                     |  |
| MRS    | Acer yui   | Pentaphylla      | 2005-204    |                  | <del>-</del>                                     | <u> </u>   |
| MRS    | Acer okomotoanum   | Platanoidea      | 1991-080    | _                | <del> </del>                                     |  |
| MRS    | Acer miyabei miaotalense   | Platanoidea      | 1996-395    | <del></del>      | <del>                                     </del> |  |
| MRS    | Acer shenkanense   | Platanoidea      | 2010-158    | <del></del>      | <del> </del>                                     |  |
| MRS    | Acer pilosum stenolobum  | Pubescentia      | 2007-056    | - Y              | <del> </del>                                     |  |

| Source | Taxa   | Section     | Source Acc. | Rooted           | Cytology   | PI Subset |
|--------|--|-------------|-------------|------------------|--|-----------|
| MRS    | Acer rubrum trilobum                               | Rubra       | 1961-382    | <del>-   -</del> |  |           |
| MRS    | Acer wuyuanense                                    | Spicata     | 2009-031    |                  |  | · · · —   |
| MRS    | Acer sp.   | unknown     | 2010-159    | <del></del> -    | <u> </u>   | ·         |
| ARN    | Acer argutum                                       | Arguta      | 640-77B     | у                | <del></del>                                      |           |
| ARN    | Acer campestre leiocarpum                          | Platanoidea | 1053-76A    | <u> </u>         | †  |           |
| ARN    | Acer campestre leiocarpum                          | Platanoidea | 1053-76B    |                  | † <del></del> -                                  |           |
| ARN    | Acer heldreichii macropterum                       | Acer        | 200-85A     | - <del>-</del> - | <del> </del>                                     | <u>-</u>  |
| ARN    | Acer hyrcanum                                      | Acer        | 31-73A      | - · -            |  |           |
| ARN    | Acer maximowiczianum x griseum                     | Trifoliata  | 641-91A     |                  | <del></del> -                                    |           |
| ARN    | Acer mono maryii                                   | Plotanoidea | 12505A      |                  |  |           |
| ARN    | Acer mono okamotoonum                              | Plotanoidea | 1620-81A    |                  | · -  |           |
| ARN    | Acer alivaceum                                     | Palmata     | 249-95A     | Υ                | <del>                                     </del> |           |
| ARN    | Acer pilosum                                       | Pubescentia | 287-2008A   |                  | <del>                                     </del> |           |
| ARN    | Acer pseudosieboldianum koreanum                   | Palmata     | 486-83A     |                  | <del> </del>                                     |           |
| ARN    | Acer pubinerve                                     | Spicata     | 50-90A      | <del></del>      |  | -         |
| ARN    | Acer pubipalmatum                                  | Palmata     | 320-2004A   | Y                |  |           |
| ARN    | Acer saccharinum laciniatum                        | Rubra       | 201-55A     |                  | <b>-</b>   |           |
| ARN    | Acer shenkanense                                   | Platanoidea | 635-2010B   |                  |  | ¬¬        |
| ÄRN    | Acer shenkanense                                   | Platanoidea | 635-2010*A  | <del></del>      |  |           |
| ARN    | Acer tataricum aidzuense                           | Ginnala     | 1852-77A    | y                |  |           |
| ARN    | Acer tatoricum aidzuense                           | Ginnala     | 542-89B     | Y                |  |           |
| ARN    | Acer velutinum                                     | Acer        | 1329-77B    |                  |  | -         |
| ARN    | Acer xdieckii (platanoides x cappadocicum lobelii) | Platanoidea | 181-86A     |                  | 1  |           |
| ARN    | Acer xhillieri (miyabei x cappadocicum)            | Platanoidea | 245-39B     | <u> </u>         | <del>  -</del>                                   |           |
| ARN    | Acer yangbiense                                    | Lithocarpa  | 637-2007    |                  | <u> </u>   |           |
| QHBG   | Acer caesium                                       | Acer        | 1995-051    |                  |  |           |
| QHBG   | Acer acuminatum                                    | Arguta      | 1993-076    |                  |  |           |
| QHBG   | Acer acuminatum                                    | Arguta      | 1993-139    | у                | <del>                                     </del> |           |
| QHBG   | Acer acuminatum                                    | Arguta      | 1993-039    | <del></del>      |  | -         |

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| Source | Тахо                          | Section     | Source Acc. | Rooted          | Cytology   | Pl subset  |
|--------|-------------------------------|-------------|-------------|-----------------|--|--|
| QHBG   | Acer caudatifolium            | Macrantha   | 2002-156    | V               | Cytology   | r i subset                                       |
| QHBG   | Acer forrestii                | Macrantha   | 2003-394    | - 'y            | <del>- -</del>                                   |  |
| QHBG   | Acer laxiflorum               | Macrantha   | 2001-292    | ,<br>y          |  |  |
| QHBG   | Acer morrisonense             | Macrantha   | 2004-176    | У               |  |  |
| QHBG   | Acer morrisonense             | Macrantha   | 2004-185    | 7 y             | <del></del> -                                    | -  |
| QHBG   | Acer albopurpurascens         | Oblonga     | 2003-088    | γ               | · <del> </del>                                   | <del>                                     </del> |
| QHBG   | Acer campbellii flabellatum   | Palmata     | 1994-182    |                 | <del>                                     </del> | -  |
| QHBG   | Acer fabri                    | Palmata     | 2003-087    |                 | <del> </del>                                     |  |
| QHBG   | Acer oblongum                 | Palmata     | 2003-204    |                 |  | <del></del>                                      |
| QHBG   | Acer serrrulatum              | Palmata     | 2004-171    | у               | <del>                                     </del> |  |
| QHBG   | Acer sinense                  | Palmata     | 2003-388    | <u> </u>        |  |  |
| QHBG   | Acer amoenum amoenum          | Palmata     | 2001-087    |                 |  | ·  |
| QHBG   | Acer amplum catalpifollum     | Platanoidea | 2009-235    | y               |  | -  |
| QHBG   | Acer cappadocicum sinicum     | Platanoidea | 1991-129    | <del>-</del> '  | <del>                                     </del> |  |
| QHBG   | Acer cappadocicum sinicum     | Platanoidea | 1998-054    | <del></del>     |  |  |
| QHBG   | Acer cappadocicum sinicum     | Platanoidea | 2001-404    | Α               |  |  |
| QHBG   | Acer miyabei                  | Platanoidea | 1997-131    | γ               | ·  |  |
| QHBG   | Acer pictum macropterum       | Platanoidea | 1996-115    | <u> </u>        | ··-  |  |
| QHBG   | Acer pictum macropterum       | Platanoidea | 1999-087    |                 | ··-  |  |
| QHBG   | Acer pycnanthum**             | Rubra       | 1987-466    |                 |  |  |
| COR    | Acer pensylvanicum            | Macrantha   | 96-175      | Υ               |  |  |
| COR    | Acer shirasawanum             | Palmata     | 01-262      | Y -             |  |  |
| COR    | Acer xfreemanii 'Firefall'    | Rubra       | 08-228      | <del>-</del>  - | <del> </del>                                     |  |
| COR    | Acer opalus                   | Acer        | 03-233      | <del></del>     | · -  |  |
| COR    | Acer pictum                   | Platanoidea | 84-161      | <del>-  -</del> | <del> </del>                                     |  |
| COR    | Acer pseudoplatanus           | Acer        | 83-361      | 1               | <del>  </del>                                    | <u> </u>   |
| USNA   | Acer velutinum                | Acer        | 78548       | -   Y           | <del>                                     </del> |  |
| USNA   | Acer saccharum ssp floridanum | Acer        | 78004       | <del>-</del>    | <u> </u>   |  |
| USNA   | Acer saccharum ssp skutchii   | Acer        | 79379       | ν               |  | <del></del>                                      |

| Source | Таха                               | Section     | Source Acc. | Rooted      | Cytology   | Plsubset   |
|--------|------------------------------------|-------------|-------------|-------------|--|--|
| USNA   | Acer pseudoplatanus                | Acer        | 2836        |             | .,   | *  |
| USNA   | Acer barbinerve                    | Arguta      | 68777       | <del></del> | <del></del>                                      | *  |
| USNA   | Acer diabolicum                    | Lithocarpa  | 562         |             | <del></del>                                      |  |
| USNA   | Acer pensylvanicum                 | Macrantha   | 74456       |             |  |  |
| USNA   | Acer tegmentosum                   | Macrantha   | 64194       |             | <del> </del>                                     | *  |
| USNA   | Acer davidii x tegmentosum         | Macrantha   | 65062       | Υ           |  | *  |
| USNA   | Acer henryi                        | Negundo     | 48987       |             |  |  |
| USNA   | Acer henryi                        | Negundo     | 72459       | <del></del> |  | <del></del>                                      |
| USNA   | Acer wuyuanense                    | Palmata     | 60719       |             |  | *  |
| USNA   | Acer japonicum                     | Palmata     | 62344       | <u> </u>    | <del>                                     </del> | +  |
| USNA   | Acer pubipalmatum                  | Palmata     | 61153       |             | <del> </del> -                                   | *  |
| USNA   | Acer erianthum                     | Palmata     | 67795       | · · · ·     |  | <del>                                     </del> |
| USNA   | Acer ceriferum                     | Palmata     | 64942       |             |  |  |
| USNA   | Acer palmatum matsumurae           | Palmata     | 44905       |             | 1  | *  |
| USNA   | Acer xzoeschense                   | Platanoidea | 15648       | <del></del> |  | *  |
| USNA   | Acer xzoeschense 'Annae'           | Platanoidea | 17443       |             | · <del></del>                                    |  |
| USNA   | Acer truncatum                     | Platanoidea | 45011       |             | ╁  | *  |
| USNA   | Acer cappadocicum sinicum          | Platanoidea | 71950       |             | <u> </u>   |  |
| USNA   | Acer truncatum                     | Platonoidea | 44904       |             | <u> </u>   |  |
| USNA   | Acer rubrum trilobum               | Rubra       | 31022       |             | <u> </u>   |  |
| USNA   | Acer pycnanthum                    | Rubra       | 67194       |             | <del> </del>                                     | *  |
| USNA   | Acer triflorum                     | Trifoliata  | 58016       | <u> </u>    | <u> </u>   | *  |
| MRT    | Acer negundo v. texanum            | Negundo     | 533-96*2    |             |  | *  |
| MRT    | Acer saccharum f. conicum          | Acer        | 354-51*1    |             | <del> </del>                                     | · · · · ·  |
| MRT    | Acer campestre x A. miyabei        | Platanoidea | 65-2007*1   |             | <del> </del>                                     | *  |
| MRT    | Acer grandidentatum                | Acer        | 276-742     | <u> </u>    | ·  | *  |
| MRT    | Acer sterculiaceum ssp. franchetii | Lithocarpa  | 332-2000*3  |             | <del>                                     </del> | *  |
| MRT    | Acer griseum x Acer nikoense       | Trifoliata  | 243-94*1    |             | <del>- </del>                                    | *  |

| Source      | Taxa   | Section     | Source Acc. | Rooted         | Cytology   | Pl subset    |
|-------------|--|-------------|-------------|----------------|--|--------------|
| MRT         | Acer pseudosieboldianum x A. palmatum<br>'Koshimino' | Palmata     | 323-2003*1  |                | -,   | *            |
| MRT         | Acer griseum x A. triflorum                          | Trifoliata  | 70-2011*1   |                | · - · · · · · · · · · · · · · · · · · ·          | <del></del>  |
| MRT         | Acer opalus obtusatum (mixaploid?)                   | Acer        | 326-82*1    | <del>-  </del> |  |              |
| MRT         | Acer platanoides x A. truncatum                      | Platanoidea | 761-50*4    | <del></del> -  | <del> </del>                                     | <del> </del> |
| MRT         | Acer davidii x A. davidii ssp. grosseri              | Macrantha   | 244-2014    |                | <del>                                     </del> | *            |
| MRT         | Acer xfreemanii 'Jenner'                             | Rubra       | 349-2005*1  | ·              | <del>                                     </del> | *            |
| MRT         | Acer stachyophyllum ssp. betulifolium                | Arguta      | 854-2005*2  | -              | <del>                                     </del> |              |
| MRT         | Acer caudatum ssp multiserratum                      | Spicata     | 878-2005*1  |                | <del>                                     </del> |              |
| MRT         | Acer pentapomicum                                    | Pubescentia | 560-2001*2  |                | <del> </del>                                     | *            |
| MRT         | Acer tschonoskii                                     | Macrantha   | 329-2000*3  | <del> </del>   | <del> </del>                                     | *            |
| MRT         | Acer barbinerve                                      | Arguta      | 258-2002*1  |                | <del> </del>                                     | *            |
| MRT         | Acer diabolicum                                      | Lithocarpa  | 1276-55*1   | <del> </del> - | <b>├</b> ·                                       | *            |
| MRT         | Acer crataegifolium                                  | Macrantha   | 220-73*2    |                | <del> </del>                                     | *            |
| MRT         | Acer mono f. subtrifidum                             | Platanoidea | 183-76*1    |                | <del></del> -                                    |              |
| MRT         | Acer hyrcanum  | Acer        | 67-2001*1   |                | ·  | *            |
| MRT         | Acer negundo v. interius                             | Negundo     | 227-86*3    | <del></del> -  | +  | *            |
| OSU         | Acer platanoides 'Emerald Queen'                     | Platanoidea | 10-0033     |                | <del> </del>                                     | *            |
| OSU         | Acer platanoides                                     | Platanoidea | 11-153-139  |                |  |              |
| OSU         | Acer platanoides                                     | Platonoidea | 11-154-079  | <b> </b> -     | <del> </del>                                     | <u> </u>     |
| osu         | Acer buergerianum                                    | Pentaphylla | 12-008-003  |                | <del>                                     </del> | *            |
| OSU         | Acer buergerlanum                                    | Pentaphylla | 12-008-004  | <del>- </del>  | <u> </u>   | *            |
| osu         | Acer buergerianum                                    | Pentaphylla | 12-008-005  | <del> </del>   | <del> </del>                                     | *            |
| <b>OS</b> U | Acer buergerianum                                    | Pentaphylla | 12-008-008  |                | -  | *            |
| osu         | Acer carpinifolium                                   | Indivisa    | 14-0051     |                |  | *            |
| osu         | Acer pauciflorum                                     | Palmata     | 14-0057     |                |  |              |
| osu         | Acer macrophyllum                                    | Macrophylla | 14-0059-01  | <del> -</del>  | <del>                                     </del> |              |
| osu         | Acer negundo   | Negundo     | 14-0089-01  | <del></del>    | <del> </del>                                     | *            |
| OSU         | Acer rubrum 'Vanity'                                 | Rubra       | 14-0131     | -              | *  | *            |

| Source | Taxo                               | Section     | Source Acc. | Roated        | Cytology   | Pisubset   |
|--------|------------------------------------|-------------|-------------|---------------|--|--|
| osu    | Acer triflorum                     | Trifoliata  | 14-0143     |               | *  | *  |
| OSU    | Acer saccharum                     | Acer        | 14-0147     | <u> </u>      |  | <del> </del>                                     |
| OSU    | Acer circinatum                    | Palmata     | 14-0153     |               | <del></del>                                      | _  |
| OSU    | Acer pseudosieboldianum            | Palmata     | 14-0156     | <del></del> - | <del>                                     </del> | · <del></del> -                                  |
| osu    | Acer buergerianum                  | Pentaphylla | 14-0158     |               | *  | <del></del>                                      |
| OSU    | Acer davidii                       | Macrantha   | 14-0162     | <u> </u>      | *  | ├  |
| osu    | Acer palmatum 'Wolff' (Emperor I®) | Palmata     | 14-0166     |               |  |  |
| osu    | Acer palmatum 'Butterfly'          | Palmata     | 14-0168     |               |  | -  |
| osu    | Acer shirosawanum 'Aureum'         | Palmata     | 14-0169     |               | <del> </del>                                     | <del> </del>                                     |
| OSU    | Acer palmatum 'Uki gomo'           | Palmata     | 14-0170     | <del></del>   | +  | <del>                                     </del> |
| OSU    | Acer palmatum 'Ara Kawa'           | Polmata     | 14-0179     |               | <del></del>                                      |  |
| OSU    | Acer palmatum 'Fireglaw'           | Palmata     | 14-0180     |               |  | <del></del>                                      |
| OSU_   | Acer xfreemanii 'Autumn Blaze'     | Rubra       | 14-0181     |               | <del>  -</del>                                   | *  |
| OSU    | Acer rubrum 'Celebration'          | Rubra       | 14-0183     |               | <del> </del> -                                   | *  |
| O\$U   | Acer rubrum 'Brandywine'           | Rubra       | 14-0186     |               |  |  |
| osu    | Acer rubrum                        | Rubra       | 14-0193     |               |  | *  |
| osu    | Acer argutum                       | Arguta      | 14-0194     |               | *  | *  |
| osu    | Acer fabri                         | Palmata     | 14-0195     | 1             | <del> </del> -                                   |  |
| osu    | Acer campestre                     | Platanoidea | 14-0196     |               |  | *  |
| osu    | Acer pectinatum                    | Macrantha   | 14-0197     |               |  |  |
| osu    | Acer buergerianum                  | Pentaphylla | 14-0198     | <del></del> - | <del> </del>                                     | *  |
| OSU    | Acer rufinerve albo-limbatum       | Macrantho   | 14-0200     |               | <del> </del>                                     |  |
| osu 📑  | Acer tataricum                     | Ginnala     | 14-0202     | · · · · · ·   |  | *  |
| osu    | Acer platanoides 'Columnare'       | Platanoidea | FL 76.03    |               | <del> </del>                                     |  |
| osu    | Acer platanoides 'Deborah'         | Platanoidea | FL 75,04    | <del></del>   | <del>                                     </del> | *  |
| osu    | Acer platanoides 'Emerald Queen'   | Platanoidea | FI 76.09    |               |  |  |
| osu    | Acer platanoides 'Royal Red'       | Platanoidea | FL 75.06    | _             | <del> </del>                                     |  |
| OSU    | Acer maximowiczianum               | Trifollata  | 14-0148     | <del>-</del>  | <del>                                     </del> | <del></del> -                                    |
| osu    | Acer maximowiczianum               | Trifoliata  | 14-0149     | <del>- </del> | <del> </del>                                     |  |

| Source | Taxa                                 | Section     | Source Acc. | Rooted   | Cytology   | Pl subset     |
|--------|--------------------------------------|-------------|-------------|--|--|---------------|
| OSU    | Acer maximowiczianum                 | Trifoliata  | 14-0150     |  | -,   | 1100000       |
| СМР    | Acer globrum                         | Glabra      | n/a         |  |  | *             |
| CMP    | Acer macrophyllum                    | Macrophylla | n/a         |  |  | *             |
| CMP    | Acer saccharinum                     | Rubra       | n/a         |  | -  | *             |
| WHIT   | Acer distylum                        | Parvifloro  | n/a         |  |  | *             |
| HYT    | A. sempervirens                      | Acer        | 1993-116    | <del>-   -   -   -   -   -   -   -   -   -  </del> | <del> </del> -                                   | ,             |
| HYT    | A. xcoriaceum                        | Acer        | 1989-047    | 1  | <del>-</del>                                     | <del> </del>  |
| HYŢ    | A. carpinifolium                     | Indivisa    | 1993-118    | <del></del>  |  |               |
| НҮТ    | A. rufinerve                         | Macrantha   | 1963-4000   |  | <del>                                     </del> | <del> </del>  |
| HYT    | A. rubescens                         | Macrantha   | 2003-169    | <del></del>  |  |               |
| НҮТ    | A. cissifolium                       | Negundo     | 1989-056    |  |  | <del></del>   |
| HYT    | A. sieboldianum                      | Palmata     | 1974-3957   | <del>                                     </del>   | "  | <del></del> - |
| НҮТ    | A. circinatum 'Hoyt's Witches Broom' | Palmota     | 2006-073    |  | ·  | <del></del>   |
| HYT    | A. elegantulum                       | Palmato     | 2014-047    | <del>                                     </del>   | -  | <del></del>   |
| НҮТ    | A. oliverianum                       | Palmata     | 2014-271    | _  | <del> </del>                                     |               |
| HYT    | A. palmatum 'Aconitifolium'          | Palmata     | 1989-038    | <del></del>  |  | <del>-</del>  |
| HYT    | A. pentaphyllum                      | Pentaphylla | 2011-12420  |  | <del> </del>                                     |               |
| HYT    | A. saccharinum                       | Rubro       | 1981-028    | <del></del>  | <del>-</del>                                     |               |
| НҮТ    | A. caudatum ukurunduense             | Spicata     | 2014-041    | <del>                                     </del>   | <del>  -</del> ·                                 | *             |
| HYT    | A. spicatum                          | Spicata     | 1989-057    | <del></del>  | <del> </del>                                     | *             |
| НҮТ    | A. nikoense                          | Trifoliata  | 1969-3958   | <del>-</del>                                       | <del>                                     </del> |               |
| HYT    | A. griseum                           | Trifoliata  | 1998-029    | <del></del>  | <del> </del>                                     | -             |

Table 1. Source abbreviations are as follows: JFS = J. Frank Schmidt Arboretum, Boring, OR; MRS = Morris Arboretum, Philadelphia, PA; ARN = Arnold Arboretum, Baston, MA; QHBG = Quarry Hill Botanical Garden, Glen Ellen, CA; COR = Cornell Plantations, Ithaca, NY; USNA = US National Arboretum, Washington D.C.; MRT = Morton Arboretum, Lisle, IL;, OSU = Accessions in the Ornamental Breeding Program of OSU, Corvallis, OR; CMP = material planted on the campus of OSU, Corvallis, OR; WHIT = Whitman Farms, Salem, OR; HYT = Hoyt Arboretum, Portland, OR. "y" in rooted column indicates rooting success. "\*" in cytology and PI subset columns indicate root squashes completed for that accession and/or accession was included in PI subset for analysis of base pair composition. Taxonomic sections in table are based on classifications presented in

| Maples of the World by van Gelderen, de Jong and Oterdoom in addition to the unpublished putative clades developed by the Jianhua Li Lab at Harvard University ( <a href="http://www2.huh.harvard.edu/research/jii/maples.html">http://www2.huh.harvard.edu/research/jii/maples.html</a> ). For species not found in either source, the Flora of China was referenced at <a href="https://www.eFlorgs.org">www.eFlorgs.org</a> . | ! |
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