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California Sycamore Genetics and Propagation Study

Project #3161-03 and #3754-02

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Section 1. Introduction

1.1 California Sycamore Ecology, Threats, and Restoration

California sycamore (*Platanus racemosa*) is an iconic native tree species found in California and northern Baja California. California sycamore trees are generally found along intermittent streams and floodplains subject to high intensity flooding, and are the dominant trees comprising sycamore alluvial woodland habitat. Sycamore alluvial woodland is a rare habitat type defined as an open to moderately closed canopy, winter-deciduous, broad-leafed riparian woodland dominated by well-spaced California sycamores (Holland 1986).

A current threat to California sycamore populations is a lack of regeneration, particularly of observed recruitment from seed. The reduction in regeneration is likely caused by many factors including hydrologic modifications of creeks that has led to other altered abiotic and biotic conditions (Beagle et al. 2017). For example, with many rivers dammed and regulated decreasing the number of high intensity floods, there may be a lack of freshly deposited alluvium necessary for recruitment and seedling competition with riparian vegetation may have increased. Infection of anthracnose (*Gnomonia platani* and related fungi) may also play a significant role in reducing regeneration (Shanfield 1984).

Another factor limiting California sycamore recruitment is hybridization with a common non-native landscaping tree, London planetree (*Platanus ×hispanica*) (Johnson et al. 2016). Hybridization with non-native trees can dilute native genetics, lead to outbreeding depression, and may threaten the existence of California sycamores as a species (Anttila 1998; Johnson et al. 2016). Additionally, while sycamore hybrids are not currently recognized by the California Invasive Plant Council as invasive (Cal-IPC 2019), hybridization between native and non-native species is a common evolutionary pathway that can lead to invasiveness of non-native species (Schierenbeck and Ellstrand 2009). California sycamore × London planetree hybrids were found to be common along the Sacramento River in northern California (Johnson et al. 2016), but it is not known how common hybrid trees are in Santa Clara County. Additionally, it is not known if hybridization started to occur in Santa Clara County around a certain time, potentially when planted London planetrees became reproductive. If a specific time was known this could allow for a certain size (a proxy for tree age) to be used as a general guideline to identify genetically pure California sycamores.

Habitat restoration and mitigation projects have typically used nursery stock grown from wild-collected California sycamore seed, but this practice is now recognized as risky due of the potential that seeds may have been fertilized with London planetree pollen and thus result in hybrid trees. One approach for reducing risk is to propagate cuttings from verified native California sycamores. However, identifying native California sycamore trees cannot be done by visually evaluating physical characteristics and requires genetic testing (i.e., DNA sequencing). Furthermore, vegetative propagation of California sycamores has been difficult, with agreement between many regionally local native plant nursery practitioners quoting success rates of about 10%

(Beagle et al. 2017). It remains unclear which factors limit the successful propagation of California sycamore from cuttings and whether there are collection or propagation techniques that can increase the rate of success.

1.2 Upper Llagas Creek Flood Protection Project

The Santa Clara Valley Water District (SCVWD), City of Morgan Hill, and U.S. Army Corps of Engineers are finalizing plans to increase flood protection for urban areas of Morgan Hill, agricultural areas of San Martin, and unincorporated areas of Santa Clara County by increasing the flood conveyance capacity of Llagas Creek. Implementation of the project would require grading the creek bank and low-flow channel; installing bank protection, grade control structures, and other permanent fills in the creek channel; and removing riparian trees, including California sycamores. Mitigation for removal of California sycamores was negotiated with the California Department of Fish and Wildlife (CDFW) to include the following requirement (Section 3.4 of the CDFW Lake and Streambed Alteration Agreement):

> *3.4 Sycamore Tree Mitigation. In consideration of the dominance of the hybridization of native sycamore trees with the non-native London plane (Platanus × hispanica) trees in the South Bay, and the challenges of establishing successful, pure genetic stands of replacement sycamores due to soil and hydrologic limitations, loss of sycamore trees within the project area shall be compensated by a combination of in-kind, on-site sycamore planting and out-of-kind mitigation in the form of a propagation and genetic study (CDFW 2017).*

1.3 California Sycamore Genetic and Propagation Study Plans

In 2016, H. T. Harvey & Associates led the preparation of study plans to better understand California sycamore genetics, improve California sycamore propagation to meet the flood protection project's sycamore tree mitigation requirement, and hopefully provide verified native California sycamore planting stock for use in replanting portions of Upper Llagas Creek (H. T. Harvey & Associates 2016a) (H. T. Harvey & Associates 2016b). The objectives of the genetic and propagation study plans are presented below.

1.3.1 Genetic Study Plan

The genetic study plan describes tree sampling, DNA sequencing, and analyses that would be used to determine the ancestry fraction (percent native California sycamore) of each individual tree sampled. It also includes coring trees and measuring trunk diameters to determine if age correlates to size and therefore potentially would allow using size as a proxy for age. The genetic study plan includes the following objectives:

- **Objective 1. Examine the degree of hybridization present in southern Santa Clara County, and compare the results of this study with those of previous hybridization studies conducted in the northern Sacramento Valley to determine the relative degree of hybridization in the regions.**

  To achieve the first objective, H. T. Harvey & Associates sampled California sycamores at five study sites (Pacheco Creek, Upper Coyote Creek, Hunting Hollow, Uvas Creek, and Upper Llagas Creek), as well as London planetrees at four study sites in southern Santa Clara County (Morgan Hill, San Martin,

Gilroy, and Gavilan College) (Figure 1). Leaves collected from each tree were submitted to Dr. Michael Miller's genetics lab at University of California, Davis (Miller Lab) for DNA extraction, genotyping, and statistical analysis. The analysis identified the ancestry fraction (e.g., 25% native, 95% native, 100% native) for each tree sampled to estimate the degree of hybridization present in southern Santa Clara County. Our results and the results of a similar study conducted in northern Sacramento Valley (Johnson et al. 2016) were compared to identify regional differences in the amount of hybridization, if any.

- **Objective 2. Use tree coring and the genetic analysis to determine approximately when hybridization began to occur in southern Santa Clara County. If a point in time can be identified before which hybridization did not occur, then we will identify the minimum tree size (diameter at breast height) that can be used as a "rule of thumb" to select pure California sycamore trees as source materials for propagation. Such a short cut would be a significant advantage for future sycamore restoration projects.**

  To achieve the second objective, San Francisco Estuary Institute (SFEI) cored the trunks of 13 randomly selected California sycamores that were sampled for genetic analysis. SFEI analyzed the tree cores to estimate the age of each sampled tree, estimate the relationship of trunk size to age, and investigate the relationship between flood history and California sycamore regeneration. H. T. Harvey & Associates and SFEI hypothesized that old trees with large trunk diameters would be less likely to be hybrids than young trees with small trunk diameters. This is because older trees were more likely than young trees to have established before London planetrees were introduced to southern Santa Clara Counties. Based on the relationship of trunk size to age, the research team would target determining a minimum trunk diameter that could be used to identify native California sycamores.

- **Objective 3. Identify genetically pure California sycamore "mother" trees for use in the propagation study associated with this project and for use in future habitat restoration projects.**

  To achieve the third objective, results of the genetic analyses were used to select native California sycamores to be used as sources for cuttings for use in the propagation study and for production of native California sycamore nursery stock for the Upper Llagas Creek Flood Protection Project. The same trees can be used for future habitat restoration projects. The proposed approaches to achieving the three objectives are described in further detail in the genetic study plan, provided as Appendix A.

1.3.2 Propagation Study Plan

The propagation study plan describes research that would examine a variety of methods to vegetatively propagate California sycamores as a means of ensuring that genetically pure California sycamores could be grown by native plant nurseries for habitat restoration projects. The study plan describes methods for collecting plant material, treatments that would be tested, an experimental design, and response variables and analyses that could be used to evaluate the treatments.

Figure 1. Vicinity Map

The propagation study plan includes the following objectives:

- **Objective 1. Advance the science of vegetative propagation of California sycamore.**

  To achieve the first objective, the propagation study plan described an experimental design to evaluate the effects of various treatments on California sycamore cuttings in a plant nursery setting (Table 1). Treatments were selected based on general vegetative propagation principles, successes and failures in previous studies conducted on the Platanus genus, and treatments of cuttings that restoration nurseries have applied successfully to other woody native plant species. The proposed treatments would be tested using a blocked factorial experimental design to evaluate the effects of the treatments on various response variables (e.g., survival, vigor, and growth).

Table 1. Treatments and Treatment Levels Proposed in California Sycamore Propagation Study

Treatments	Levels	Definitions
Cutting material	Basal	Root or trunk sprouts within 1 meter of the base of the tree
	Crown	Material from branches of the tree originating above 1 meter on the trunk of the tree; material is 1 year old or less
Cutting preparation	Simple cut	Cut directly below a leaf node along an unbranching stem
	Heal cut	Cut at joining section between a branch and a stem, ideally with first year's growth sprouting from previous year's growth
Willow water presoak	Yes	Cutting material soaked in willow water (concentration and duration tbd) before Dip'n Grow at 1,000 ppm
	No	Cutting material soaked in water only (same duration as willow water soak) before Dip'n Grow at 1,000 ppm
Rooting media	Perlite	Cuttings planted in perlite only
	Rockwool	Cuttings planted in cubes of rockwool in flats filled with perlite
Cutting season	Spring	February and March, the period before bud burst
	Fall	October and November, the period around leaf fall

Notes: ppm = parts per million; tbd = to be determined.

The propagation study included the Watershed Nursery and the Grassroots Ecology Nursery. These nurseries were included in the propagation study because they are native plant nurseries that frequently provide substantial quantities of plant material for restoration projects throughout Santa Clara and surrounding counties. The nurseries would coordinate all collection events so that the vegetative propagation material used by the two nurseries would be as similar as possible. The exact number of cuttings propagated at each nursery in each season, and in total, would vary because of differences in contract obligations and would depend on the availability of cuttings and the configuration of replicates. The approximate number of cuttings to be propagated by each nursery is provided in Table 2.

Table 2. Approximate Number of Cuttings to Be Propagated by Each Nursery in Each Season and Year

	2017		2018		
Nursery	**Spring**	**Fall**	**Spring**	**Fall**	**Total**
Grassroots Ecology Nursery	250	250	250	250	1,000
The Watershed Nursery	375	375	375	375	1,500
Total	**625**	**625**	**625**	**625**	**2,500**

Collections would occur on the same days and would be made at the same sites and from the same trees, using the same collection protocols. The experiment would strive for a fully balanced design so that each experimental condition would have the same number of replicates. The response variables that would be measured are described in Table 3.

Table 3. Summary of Response Variables Proposed for Assessing California Sycamore Propagation Success

Response Variable	When Response Variable Is Measured
Survival	Survival will be assessed at first transplant into individual small containers (expected approximately 6–16 weeks after striking). For February–March material, we will assess in April–June. For October–November material, we will assess in December–February. Survival will be assessed at second transplant into individual larger containers (approximately 3–6 months after initial transplanting). For February–March material, we will assess in September–December. For October–November material, we will assess in May–August. Survival will be assessed at final point in experiment (when plants are ready for outplanting).
Initial vigor	Initial vigor will be assessed at time of initial transplanting from rooting medium and based on a ranking system (Table 4).
Growth	Initial height measurements will be taken at the time of first transplanting. Subsequent height measurements will be taken at the time of next transplanting, and at the completion of the experiment.
Ongoing vigor	Ongoing assessments will be made at the time of transplanting into the final container size and at the completion of the experiment (Table 5).
Photodocumentation	At each data collection point during the study, we will take photographs to visually assist with documentation. Photographs also will be taken outside the data collection points as necessary to visually record items that may be pertinent to the study.

All plant material collection and propagation methods would be reexamined and adapted after the first season if advised based on consultation with the nurseries, H. T. Harvey & Associates, and Phytosphere Research.

- **Objective 2. Improve the cost-effectiveness of vegetative propagation of California sycamore.**

  To achieve the second objective, the results of the propagation study are to be presented in a manner that provides clear direction regarding the most promising collection and propagation techniques.

- **Objective 3. Determine future studies that could be employed to build off the propagation study and further advance the science and efficiency of vegetative propagation of California sycamore.**

  To achieve the third objective, ideas for future studies are to be developed and included with the results of the propagation study.

  The proposed approaches to achieving the three objectives are described in further detail in the propagation study plan, provided as Appendix B.

1.4 Purpose of this Report

This report documents the actual approach, methodologies, and results of the genetic and propagation studies and serves to meet the out-of-kind mitigation requirement for the loss of sycamore trees associated with the Upper Llagas Creek Flood Control Project. The methods and results of each study are presented separately and compared to the objectives described above. This report includes figures that show the location of trees sampled for the genetic study and used for propagule collection for the propagation study. It also includes recommendations concerning future sycamore collection and propagation attempts and further studies that could be performed based on what was learned.

Section 2. California Sycamore Genetic Study

2.1 Introduction

This section provides a detailed description of the genetic study methods and results, and includes a discussion comparing the results to the study objectives. The study involved collecting and analyzing the genetic material of presumed California sycamore and London planetree leaves to examine the degree of hybridization present in southern Santa Clara County. The genetic results were then used to identify California sycamores for use as propagule sources in the propagation study as well as for use in future habitat restoration projects. Tree coring data was also collected to complement the results of the genetic analysis in an attempt to identify a minimum tree size/age for selecting pure California sycamores as source materials for propagation.

2.2 Methods

2.2.1 Study Sites

Nine study sites across southern Santa Clara County were used in the genetic study (Figure 1). The trees at Upper Coyote Creek, Hunting Hollow, Llagas Creek, Pacheco Creek, and Uvas Creek were presumed to be California sycamores due to their location in wildland settings where planting of London planetree was less likely. The trees at Gilroy, Morgan Hill, San Martin, and Gavilan College were presumed to be London planetrees based on their urban landscaping setting in public spaces where the planting of London planetree was likely. However, all of the sample trees that were presumed to be California sycamores were within 10 miles of London planetrees, which is considered to be within the distance at which pollen from both species can travel (Schierenbeck pers. comm. 2016). Therefore, trees that were presumed to be California sycamores may have actually been hybrids.

Pacheco Creek Site. The Pacheco Creek site is located at 12163 Pacheco Pass Highway, Hollister, California. The creek is a non-confined riverine system with a broad floodplain that is subject to somewhat regular inundation. Hydrologic conditions are influenced by an upstream reservoir (Pacheco Reservoir) in its watershed. The site supports large putative California sycamores, including a moderate number of trees that have died in recent years from an undetermined cause. The site has been affected by past land uses, likely including gravel mining and grazing. The California Department of Transportation (Caltrans) owned the site at the time of the study and granted access. The site also included an area that was planted with riparian species, including sycamores, as part of a Caltrans mitigation project.

Upper Coyote Creek Site. The Upper Coyote Creek site is located at 5007 Gilroy Hot Springs, Gilroy, California. The creek is a non-confined riverine system that supports high-quality sycamore alluvial woodland with natural hydrology; intact fluvial geomorphologic processes; and large, healthy putative California sycamores. The site is located in the Santa Clara Valley Open Space Authority's (OSA) Palassou Ridge Preserve,

and is subject to little human disturbance because of its relatively remote location. However, some trespass grazing has been observed. The OSA granted access for the study.

Hunting Hollow Site. The Hunting Hollow site is located in Henry W. Coe State Park at 4826 Gilroy Hot Springs, Gilroy, California. The site's creek is a tributary to Upper Coyote Creek and is confined in a valley. The site supports high-quality sycamore alluvial woodland with natural hydrology; intact fluvial geomorphologic processes; and large, healthy putative California sycamores. The California State Parks owns the site and granted access for the study.

Llagas Creek Site. The Llagas Creek site is located within the footprint of the planned Upper Llagas Creek Flood Protection Project between Morgan Hill and Gilroy, California. The creek is surrounded by urbanized areas. Its hydrology is managed by reservoirs upstream that regulate flows, primarily to feed off-channel percolation ponds designed to recharge groundwater. Despite the highly modified hydrology and geomorphology of Upper Llagas Creek, the creek supports a large number of mature putative California sycamores. Llagas Creek is incised, and most of its historic floodplain is located well above the active channel. California sycamores located outside the creek's bed and banks are no longer subject to regular inundation, which has likely reduced natural recruitment and negatively affected the health of the trees. The SCVWD granted access for the study.

Uvas Creek Site. The Uvas Creek site is located at 3090 Hecker Pass Highway, Gilroy, California. The creek is a non-confined riverine system that is connected to a broad floodplain that supports large putative California sycamores. Hydrologic conditions are influenced by the upstream Uvas Reservoir. The site is surrounded by rural residential development. Santa Clara County owns the site and provided access for the study.

Morgan Hill Site. The Morgan Hill site is located in Morgan Hill, California. The site is urban and supports planted London planetrees along streets and in parks. Samples were collected along Monterey Road, Depot Street, and 2nd Street between Llagas Road and Butterfield Road. Samples were also collected at Morgan Hill Community and Cultural Center.

San Martin Site. The San Martin site is located in the small rural community of San Martin, California between Morgan Hill and Gilroy. Putative London planetrees are present along major roads and in parking lots. Samples were collected from the Sheriff Office, South County Substation, and Santa Teresa Blvd.

Gilroy Site. The Gilroy site is located in Gilroy, California. Samples were collected along 3rd Street, 4th Street, 5th Street, West 6th Street, and Eigleberry Street between Miller Avenue and Monterey Road. London planetrees were commonly planted along streets and in parks at this site. Samples were also collected from the City's government office.

Gavilan College Site. The Gavilan College site is located at Gavilan College, Gilroy, California. London plane trees were planted in the college's arboretum and throughout campus along bike paths and walkways, around buildings, and in parking lots. Samples were collected along Sycamore Lane and from the arboretum.

2.2.2 Plant Material Sampling

H. T. Harvey & Associates' restoration ecologists collected leaf samples from trees at the study sites on October 3–6, 11–13, 17, and 18, 2016 (Appendix C, Table S1). At each site, individual trees were identified and their size (diameter at breast height, dbh) and location was measured and recorded. The dbh of each sampled tree was measured using a dbh tape. The dbh of trees with multiple trunks was calculated by taking the square root of the sum of all squared stem dbhs, in accordance with *Guidelines for Developing and Evaluating Tree Ordinances* (Phytosphere Research 2001). Tree location was measured and recorded using handheld Global Positioning System (GPS). The number of trees selected for sampling at each study site was determined using proportional allocations (Thompson 2002). Individual trees were selected using a generalized random-tessellation stratified sampling design to provide a spatially balanced and representative sample of each population (Stevens and Olsen 2004). Two leaves from each tree were placed in Ziploc bags upon harvest, labeled with a unique identification code, and kept cool in an ice chest containing dry ice and delivered to the Miller Lab for analysis. Three hundred and eighty-four leaf collections were made across each site. For each tree H. T. Harvey & Associates provided the Miller Lab with a sample identifier (ID), latitude, longitude, collection date, dbh, and putative species (Appendix C, Table S1).

2.2.3 Genetic Analysis

The following information and methods for genetic analysis were developed and described by the Miller Lab (O'Rourke and Miller 2017). One sample (MH001) from the Morgan Hill site was inadvertently included twice in a DNA extraction plate (described below), so the replicates were given individual codes (MH001a and MH001b) (Appendix C, Table S2). The original ID of one sample was lost, thus the putative species and collection location was unknown. The sample was coded to reflect this (NN001) (Appendix C, Table S2).

DNA Extraction. The leaves were stored at -80°C before being dried for 3 days in a Virtis freeze drier (catalog number 6203 3006 OL). For each tree, approximately 2 cm^2 of dried leaf tissue was cut into small fragments and placed into one well of a 96 well plate containing two glass beads. The leaf fragments were mechanically disrupted using a 2010 Geno/Grinder (Spex SamplePrep). An Omega Bio-tek E-Z 96 Plant DNA extraction kit (D1086-02) was then used to isolate DNA. DNA yield was quantified with a PicoGreen assay (Invitrogen).

RAD Sequencing. The Miller Lab used 50 nanograms of DNA from each individual and prepared four SbfI restriction site associated DNA (RAD) libraries using a new RAD method (Ali et al. 2016). The libraries were sequenced with 100 base pair reads on an Illumina HiSeq 4000 system at the University of California, Davis DNA Technologies Core. Sequencing reads were demultiplex to individual samples by requiring a perfect match to the plate barcode, well barcode, and partial restoration site (Ali et al. 2016).

De Novo RAD Locus Assembly. Four putative sycamore samples from the Upper Coyote Creek site (CC043, CC078, CC22, and CC252) with relative high numbers of sequencing reads (Appendix C, Table S2) were used to perform a *de novo* RAD locus assembly as previously described (Miller et al. 2012).

Alignments and Subsampling. Sequences were aligned to the *de novo* RAD assembly (Appendix C, Table S3) using the backtrack algorithm of Burrows-Wheeler Aligner (Li and Durbin 2009) with default parameters. SAMtools (Li et al. 2009) was used to sort and remove unmapped reads from the binary alignment map (BAM) files (Appendix C, Table S2). To remove variation associated with variable sequencing depth, three sets of subsampled BAM files (20k, 10k, and 5k) were generated using SAMtools to randomly sample approximately 20,000, 10,000, and 5,000 alignments from each sample. Subsampling to a lower number of alignments allows more individuals to be included in this analysis but reduces the amount of information for each sample.

PCA and Admixture Analysis. Principal component (PC) and admixture analyses were conducted with each of the three subsampled BAM file sets (20k, 10k, and 5k). Single nucleotide polymorphism (SNP) discovery and genotype posterior probability estimation was performed using Analysis of Next Generation Sequencing Data (ANGSD) (Korneliussen et al. 2014) with a minimum mapping quality score (minMapQ) of 20, a minimum base quality score (minQ) of 20, the SAMtools genotype likelihood model (GL 1), identifying polymorphic sites (SNP_pval 1e-6), inferring major and minor alleles (doMajorMinor 1), estimating allele frequencies (doMaf 2), retaining SNPs with a minor allele frequency of at least 0.05 (minMaf), and using a uniform prior (doPost 2). PCAs were performed with the ngsCovar function implemented in ngsTools (Fumagalli et al. 2014) using called genotypes to calculate the covariance matrix. Admixture analyses were performed using ngsAdmix (Skotte et al. 2013) assuming two ancestral populations.

2.2.4 Tree Coring Sampling

On October 26, 2016, SFEI selected nine trees among the Pacheco Creek and Upper Coyote Creek sites for tree coring. The selected trees were a subset of the trees that were identified for plant material sampling (Section 2.2.2). Each tree had a single trunk, to help ensure reliable aging, and were located in stratified geomorphic positions. SFEI used an increment borer to core each tree. The extracted cores were stored and transported in paper tubes to SFEI's lab, where they were glued and sanded on wooden mounts. Tree rings were counted on each core using a compound microscope, and the age of each tree was estimated to the nearest year (SFEI and H. T. Harvey & Associates 2017).

In 2017, SFEI selected four additional trees for coring: two at the Pacheco Creek site and two at the Upper Coyote Creek site. Each tree was selected, cored, and analyzed using the same methods described above (SFEI 2018).

2.3 Results

2.3.1 Plant Material

A total of 384 trees were sampled. The identification code, geographic coordinates, collection date, and dbh of each tree sampled is provided in Appendix C, Table S1.

2.3.2 Genetic Results

Genetics Summary. The Miller Lab performed RAD sequencing and generated a *de novo* assembly to align the sequence reads. A total of 353 tree samples produced sufficient reads to be included in the analysis. Both the PC and admixture analyses clearly distinguished the California sycamore and London planetree reference samples. Both analyses consistently identified the same individuals to their respective species: Forty-two London planetrees were positively identified; Of the 310 presumed California sycamores analyzed in the genetic study, 303 (97.7%) were identified as California sycamores and 7 (2.3%) were identified as hybrids. Two of the hybrid trees were at the Llagas Creek site and five were at the Pacheco Creek site. It was later determined that the hybrid trees at the Pacheco Creek site were likely installed nursery stock plantings as part of a Caltrans mitigation project. The sample from an unknown location was identified as a California sycamore. Detailed results from the sequence reads and alignment statistics, principle component analysis, and admixture analysis are presented below.

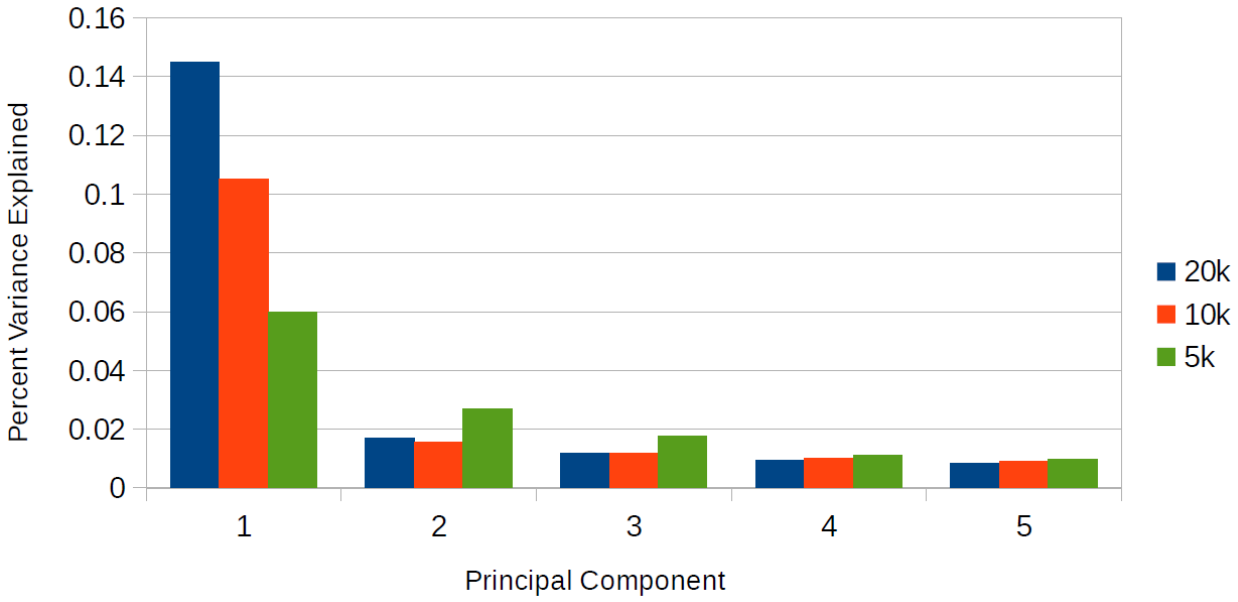
Sequence Read and Alignment Statistics. The mean number of sequence reads across all samples was 381,992. The putative California sycamore and London planetree samples had mean sequence read numbers of 309,867 and 392,205, respectively (Table 4) (Appendix C, Tables S1 and S2). The four putative California sycamore samples used for the *de novo* assembly had greater than 750,000 reads, and the *de novo* assembly produced 6,989 RAD loci (Appendix C, Tables S2 and S3). The mean alignment rate against the *de novo* assembly across all samples was 74.9%, with putative California sycamore and London planetree samples having alignment rates of 70.5% and 75.3%, respectively. Across all samples, 313 (81.5%) had enough alignments to be included in the 20k subsampled analysis, 336 (87.5%) were included in the 10k analysis, and 353 (91.9%) were included in the 5k analysis (Table 4) (Appendix C, Table S2).

Table 4. Sample Sequence Read and Alignment Statistics

Putative Species	Samples	Mean Sequence Reads	Mean Alignments	Samples >=20k Alignments	Samples >=10k Alignments	Samples >=5k Alignments
LPT	50*	309,867	218,319 (70.5%)	34	37	42
SYC	333	392,205	295,478 (75.3%)	278	298	310
Unknown	1	587,488	485,050 (82.6%)	1	1	1
All	384*	381,992	285,925 (74.9%)	313	336	353

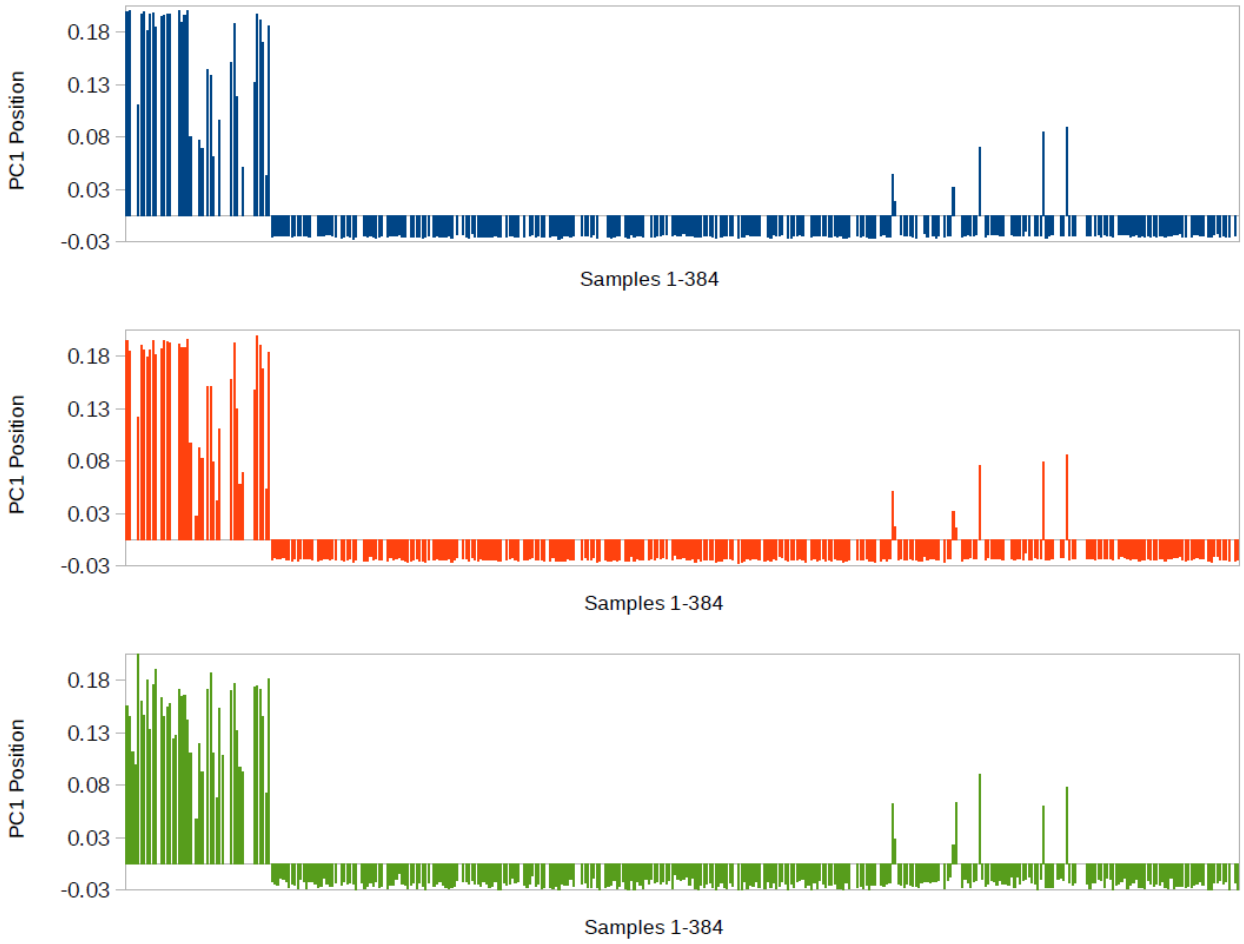
Note: LTP = London planetree, SYC = California sycamore, * denotes that sample included one replicate

Principal Components Analysis. In all three PCAs (20k, 10k, and 5k), the first principal component (PC1) explained a substantially greater proportion of the total genetic variance than the remaining components (Figure 2) (Appendix C, Table S4). The position of samples along PC1 was consistent among the three analyses. All putative London planetree samples included in at least one PCA (n=42) has PC1 positions greater than 0.022 (Figure 3 and Appendix C, Table S5). Of the putative California sycamore samples included in at least one PCA (n=310), the vast majority (n=303) had PC1 positions less than -0.009, while the remaining seven (LL3944, LL4005, PC002, PC004, PC038, PC107, and PC153) had PC1 positions greater than 0.017, indicating potential hybrid individuals (Figure 3) (Appendix C, Table S5).



Source: O'Rourke and Miller (2017)

Figure 2. Principal Component Analysis—Percent Variance Explained by First Five Principal Components for Analyses done with 20k, 10k, and 5k Subsampled Alignments

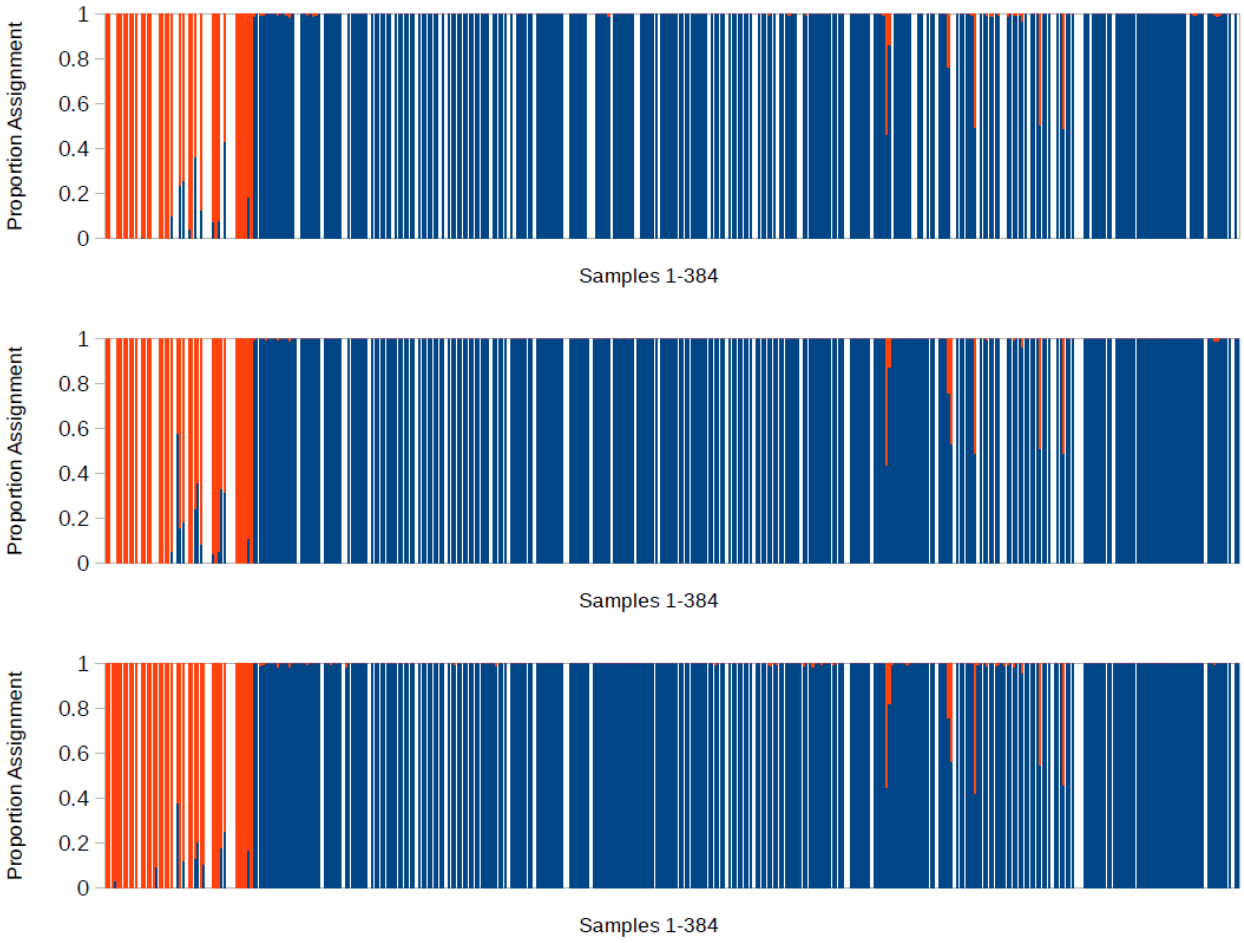


Source: O'Rourke and Miller (2017)

Note: Putative London planetree samples are 1–50, the unknown sample is 51, and putative California sycamore samples are 52–384. Samples without enough alignments for the analysis are included on the x-axis but do not have a bar.

Figure 3. Principal Component Analysis—First Principal Component Position of each Sample for Analyses done with 20k (Top), 10k (Middle), and 5k (Bottom) Subsampled Alignments

Admixture Analysis. The three admixture analyses (20k, 10k, and 5k) produced consistent results that were very similar to the PCAs. All putative London planetree samples included in at least one admixture analysis (n=42) were estimated to have less than 58% ancestry from genetic cluster 1 (Figure 4) (Appendix C, Table S5). Of the putative California sycamore samples included in at least one admixture analysis (n=310), the vast majority (n=303) were estimated to have greater than 95% ancestry from genetic cluster 1, while the remaining seven (LL3944, LL4005, PC002, PC004, PC038, PC107, and PC153) had 41–83% ancestry from genetic cluster 1 (Figure 4) (Appendix C, Table S5).



Source: O'Rourke and Miller (2017)

Note: Red represents genetic cluster1. Blue represents cluster 2. Putative London planetree samples are 1–50, the unknown sample is 51, and putative California sycamores are 52–384. Samples without enough alignments for the analysis are included on the x-axis but do not have a bar.

Figure 4. Admixture Analysis—Proportion Assignment to each Genetic Cluster in an Admixture Analysis done with 20k (Top), 10k (Middle), and 5k (Bottom) Subsampled Alignments and Assuming Two Ancestral Populations (k=2)

2.3.3 Size by Genetically Verified Species

California sycamores had an average dbh of 20.5 inches (± 0.8 standard error of the mean), London planetrees had an average dbh of 17.7 inches (± 1.14 standard error of the mean), and hybrids had an average dbh of 6.4 inches (± 0.9 standard error of the mean). The maximum dbh of a hybrid tree in this study was 9.7 inches (24.6 centimeters). See figures 5–7 for histograms of California sycamores, London planetrees, and hybrids, respectively, plotted by size. Additionally, the average dbh of California sycamores, London planetrees, and hybrids is provided by site, and across sites, in Table 5.

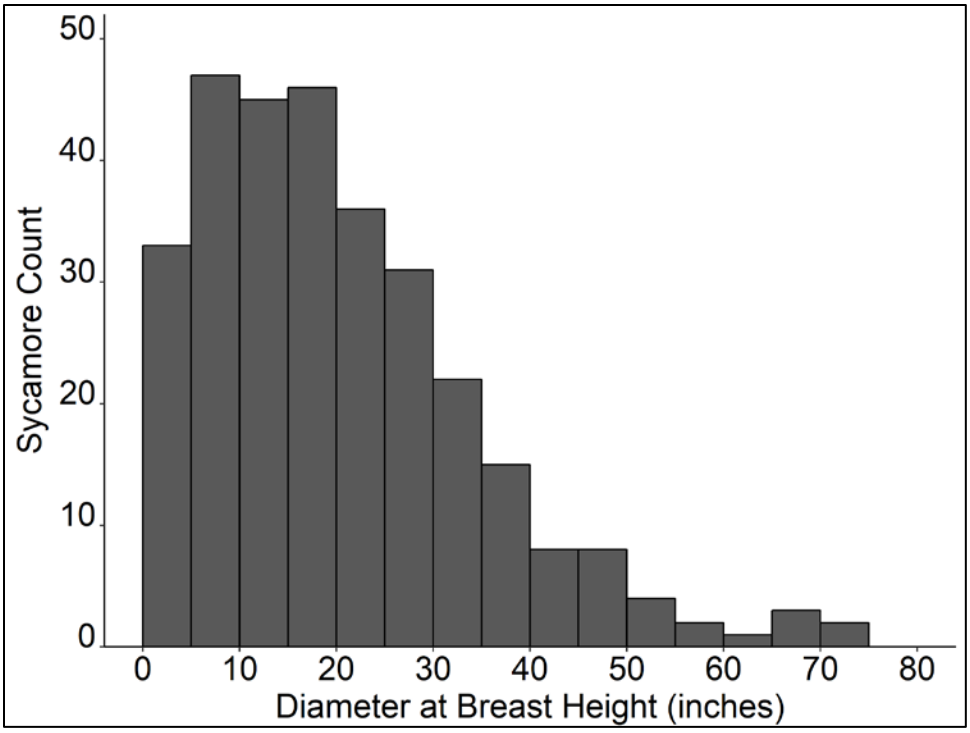


Figure 5. Histogram of Genetically Verified California Sycamores by Diameter at Breast Height

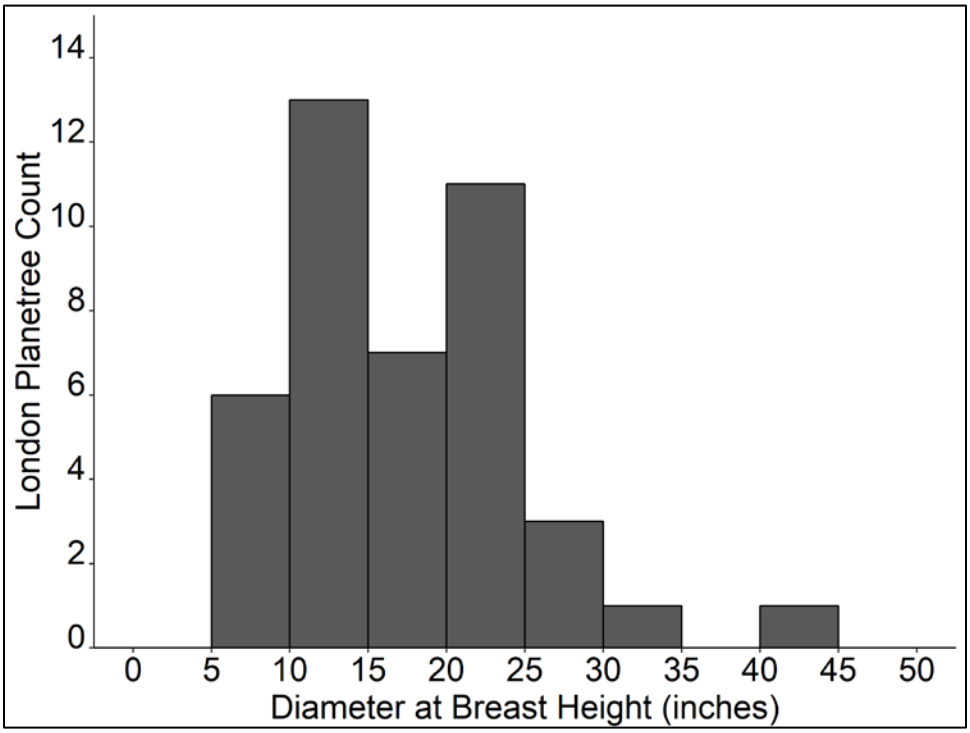


Figure 6. Histogram of Genetically Verified London Planetrees by Diameter at Breast Height

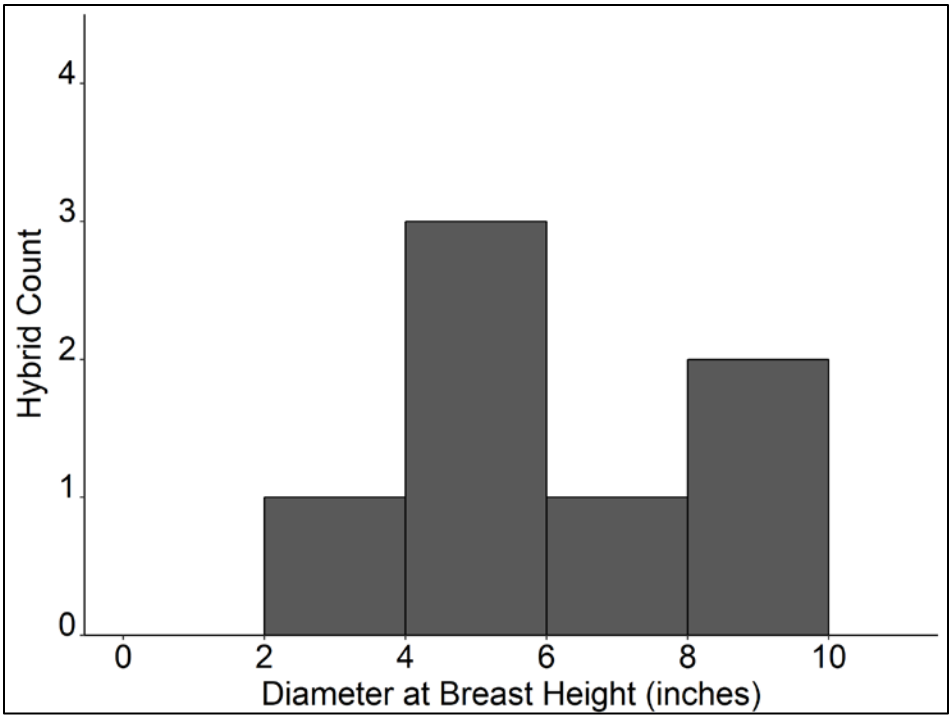


Figure 7. Histogram of Genetically Verified California Sycamore x London Planetree Hybrids by Diameter at Breast Height

Table 5. Average Trunk Diameter at Breast Height of California Sycamores, London Planetrees, and Hybrids by Study Site

	Average dbh (inches)		
Study Site	**California Sycamore**	**London Planetree**	**Hybrid**
Gavilan College	—	21.5 (n=11)	—
Gilroy	—	21.6 (n=10)	—
Hunting Hollow	23.7 (n=43)	—	—
Llagas Creek	14.3 (n=82)	—	6.8 (n=2)
Morgan Hill	—	14.0 (n=18)	—
Pacheco Creek	8.9 (n=38)	—	6.3 (n=5)
San Martin	—	12.6 (n=3)	—
Uvas Creek	23.4 (n=49)	—	—
Upper Coyote Creek	27.8 (n=91)	—	—
Overall Average	**20.5 (n=303)**	**17.7 (n=42)**	**6.4 (n=7)**

Note: dbh = diameter at breast height.

2.3.4 Tree Coring

Thirteen California sycamores selected for genetic sampling were cored to estimate tree age and the relationship between tree age and trunk diameter (Table 6). Four of the trees that were cored had their genetic material

tested and were identified as having 95% or more native California sycamore genetic material (CC009, CC252, PC092, and PC103). Four of the cored trees had heart rot (CC041, CC055, CC065, and CC252), and as a result, complete cores could not be collected, and their age could not be estimated. The dbh of two trees was not recorded (PC043 and PC093); however, each tree was assigned to the medium size class (7–39 inch dbh) during the habitat mapping and regeneration study (SFEI and H. T. Harvey & Associates 2017). Tree age ranged from 13 to 99 years old (Table 6). The average growth rate was estimated to be 0.6 inch dbh per year; however dbh and number of tree rings were not correlated (p=0.15; Figure 8). The lack of a relationship between dbh and number of rings may be due to the low sample size in the tree coring study.

Table 6. Tree Coring at Upper Coyote Creek and Pacheco Creek Sites

Tree ID	Heart Rot	Complete Core	Length of core (inches)	Number of Rings	dbh (inches)	Estimated Year Established	Error (± years)	% Error
CC009[a]	No	Yes	7.2	67	21.7	NA	6	9
CC027	No	Yes	17.6	99	29.9	1917	15	15
CC041	Yes	No	5.0	32	28.0	NA	6	19
CC041	Yes	No	5.9	42	28.0	NA	5	12
CC055	Yes	No	3.5	33	9.8	NA	3	9
CC058	No	Yes	7.5	47	11.4	1970	4	9
CC065	Yes	No	4.8	35	33.1	NA	4	11
CC077	No	Yes	7.8	54	18.9	1963	9	17
CC252 [a]	Yes	No	4.8	35	35	NA	4	11
PC009	No	Yes	4.4	19	7.9	1998	4	21
PC043	No	Yes	6.8	20	NA	1996	3	15
PC092 [a]	No	Yes	10.1	65	66.9	1952	12	18
PC093	No	Yes	5.6	13	NA	2003	5	38
PC103 [a]	No	Yes	3.9	18	6.0	1998	3	17

Source: SFEI and H. T. Harvey & Associates 2017, SFEI 2018

Note: CC = Upper Coyote Creek site, NA = not available, PC = Pacheco Creek site. CC058, CC077, PC009, and PC092 cores were collected and analyzed in 2017; all other cores were collected and analyzed in 2016. Two tree cores were taken for tree CC041, and both had heart rot.

[a] Estimated to have greater than 95% native California sycamore genetic material.

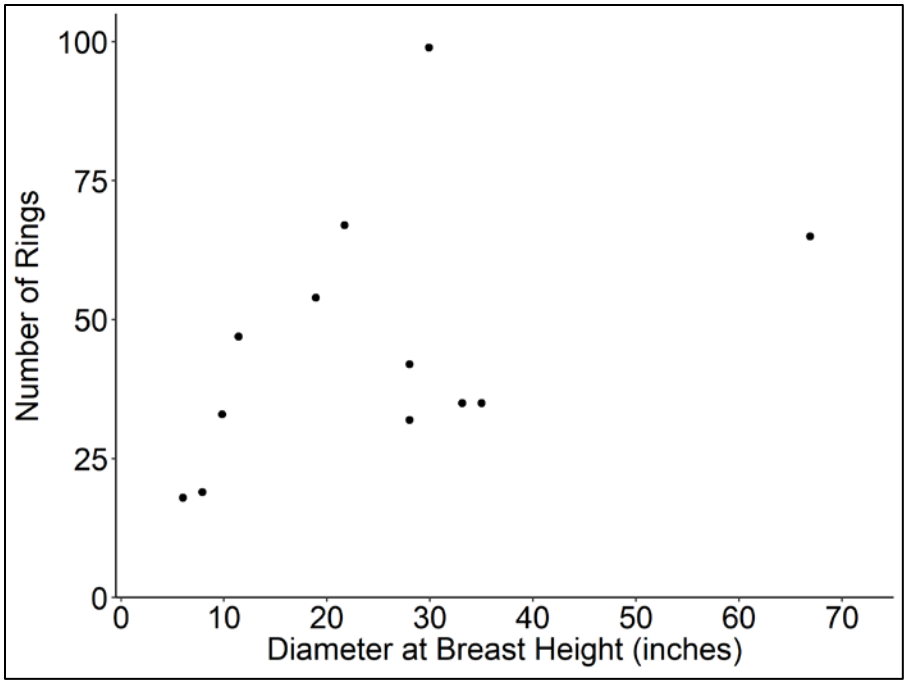


Figure 8. Number of Rings by Diameter at Breast Height of California Sycamore Trees

Due to the poor results of the tree coring effort, the SCVWD and SFEI reallocated funding for this work to include an observational study of sycamore regeneration at the Pacheco Creek site, following the high winter flows of winter 2016/2017. Results of this study are presented in SFEI's *Observational Study of Sycamore Regeneration at two sites in Santa Clara County after the 2016-2017 Water Year* (SFEI 2018).

2.4 Discussion

2.4.1 Objective 1—Degree of Hybridization in Southern Santa Clara County

Seven (2.3%) of the 310 putative sycamores were identified as hybrids. The hybrids were relatively young, having an average dbh approximately three times less than the California sycamores, and five were likely planted as Caltrans mitigation plants. These limited findings indicate that hybridization may be a relatively recent occurrence in southern Santa Clara County and possibly not as widespread as in other areas, such as observed along the Sacramento River. Hybrids accounted for between 5% and 25% of the sampled population along the Sacramento River, depending on age class, and hybrids were found with a dbh up to 50 inches (Johnson et al. 2016). However, the small sample size of hybrids in this study necessitate caution as hybrids may be more common in areas outside of the specific areas used in this study.

2.4.2 Objective 2—Minimum Tree Size to Identify 100% Native California Sycamores

Due to the relatively small spatial range of collections used in this study, the inconclusive results from the tree coring data, and the fact that only seven hybrids were identified (five of which were likely planted nursery stock), it is not possible to make a preliminary recommendation of a general size threshold to be used in

southern Santa Clara County to help identify genetically pure California sycamores. This is especially true based on data collected along the Sacramento River showing hybrids with a dbh of 50 inches (127 centimeters) (Johnson et al. 2016). While environmental and historic sociocultural conditions (i.e. when London planetrees were planted) likely vary between northern California and southern Santa Clara County, the documented chance that larger trees can be hybrids combined with the low sample size in southern Santa Clara County means that a substantial amount of additional data would be required to make any recommendation on a potential size threshold for determining genetically pure California sycamores.

2.4.3 Objective 3—Locations of 100% Native California Sycamores

A total of 303 genetically pure California sycamore "mother" trees were identified and mapped. Many of these trees were used for the propagation study associated with the Upper Llagas Creek Flood Protection project and could be used in future habitat restoration projects. The number of verified California sycamore are presented by study site in Table 7. The geographic coordinates are provided in Appendix C.

Table 7. Number of Genetically Pure California Sycamore Trees by Study Site

Study Site	Number of California Sycamores
Hunting Hollow	43
Llagas Creek	82
Pacheco Creek	38
Uvas Creek	91
Upper Coyote Creek	49
Total	**303**

Section 3. Propagation Study

3.1 Introduction

Due to the observed low level of natural recruitment of native California sycamores, the ability to have genetically pure native nursery stock has become essential to ensuring sycamores continue to be a component of riparian woodland and sycamore alluvial woodland habitat restoration projects (Beagle et al. 2017). Restoration and mitigation projects have typically used California sycamores propagated from wild-collected seed. However, the potential for inadvertently collecting hybrid seed makes that approach risky. Unfortunately, propagation from cuttings remains uncommon as it has proven to be difficult and typically results in low rates of success. Currently, it is unclear what factors limit the successful vegetative propagation of California sycamores and whether there are collection techniques or treatments that can increase the rate of success. To reduce the risk of propagating hybrids, techniques for propagating California sycamores from cuttings collected from known genetically pure trees need to be improved.

Many propagation techniques have been used to increase the survival, vigor, and growth of hard-to-propagate species including collecting cuttings during different seasons, using rooting hormones, changing the location on a plant where cuttings are collected from, and using different cutting techniques (MacDonald 1986, Hartmann et al. 2002). Other treatments that are less frequently documented in scientific literature, but are used in the horticultural industry, may increase the success of vegetatively propagating hard-to-propagate species on a scale that is appropriate for restoration. For example, perlite is a commonly used rooting medium for most species, but other rooting media types, such as rockwool, may benefit species that require longer lasting moisture or are susceptible to root disturbance. Another less well-known practice that can increase plant growth and nutrient transport is presoaking cuttings in willow water before striking them in a rooting media (Hayat and Ahmad 2007). The benefit from willow water is thought to come from salicylic acid that is found in the leaves and twigs of willow species. Overall, the effectiveness of various individual treatments, as well as combinations of treatments, on the vegetative propagation of California sycamores has not been well-documented.

Laboratory experiments on vegetative propagation of California sycamore may not be representative of conditions in the native plant nurseries that are likely to provide plant material on a scale that is appropriate for restoration. Therefore, horticultural treatments applied in-situ in native plant nurseries incorporating the horticultural expertise of nursery staff holds promise to advance the science of vegetative propagation, as well as the practical ability for restoration practitioners and native plant nurseries to vegetatively propagate California sycamore in a cost-effective manner.

This study was conducted to identify techniques that may increase the rate of successful propagation of California sycamores from cuttings, improve the cost-effectiveness of propagating California sycamores for

restoration, and identify future studies to further advance the knowledge and efficiency of vegetatively propagating California sycamores.

H. T. Harvey & Associates collaborated on this study with The Watershed Nursery and Grassroots Ecology Nursery, two native plant nurseries that frequently provide plant material for restoration projects in Santa Clara County. The study investigated how the following treatments affected the survival, health, and growth rate of California sycamore cuttings:

- season of cutting collection (winter vs. spring),
- cutting material (basal vs. crown)
- cutting preparation (simple vs. heal)
- type of presoak (willow water vs. tap water)
- type of rooting media (perlite vs. rockwool)

These treatments were investigated in-situ at each native plant nursery to replicate the conditions likely to be encountered in native plant nurseries and incorporate the horticultural expertise of nursery staff during practical application of California sycamore propagation. The study was deliberately not conducted under laboratory conditions in order to investigate treatments that could be practically replicated on a sufficient scale by native plant nurseries for future restoration with genetically pure California sycamore. As a result of conducting the propagation study in-situ at real-world restoration nurseries, some treatments are not directly comparable between nurseries because of differing conditions and associated horticultural practices at these nurseries. The variability in study rigor is a trade-off for advancing practical, cost-effective California sycamore propagation methods that are informed by horticultural treatments.

The study approach was based on the proposed plan outlined in the California Sycamore Propagation Study Plan (H. T. Harvey & Associates 2016b, Appendix B). The following sections provide a detailed description of the propagation study methods and results, a discussion of whether or not the propagation study objectives (Section 1.3.2) were met, and recommendations for future vegetative propagation attempts.

3.2 Methods

3.2.1 Nurseries

The Watershed Nursery and Grassroots Ecology Nursery collected, processed, and propagated all of the California sycamore cuttings, and measured and recorded all of the data presented in this study. Utilizing two separate nurseries to conduct this study intentionally increased replication across differing nursery conditions. The Watershed Nursery is a moderately sized, for-profit nursery based in Richmond, California. The climate at The Watershed Nursery is mild; freezing is infrequent in the winter and daily high temperatures in the summer are generally below 80°F (27°C) (Benner pers. comm. 2018). The Grassroots Ecology Nursery is a small, non-

profit nursery based in the hills of Palo Alto, California. The Grassroots Ecology Nursery has a more variable and extreme climate; freezing conditions are common in the winter and temperatures are occasionally higher than 100°F (38°C) in the summer (Giuliano, pers. comm. 2018). Both nurseries are at the forefront of using best management practices to reduce the risk of cultivating and spreading *Phytophthora* spp. and other plant pathogens that are known to be in nurseries and can spread to sites where nursery stock are planted.

3.2.2 Plant Material Collection Timing and Location

The Watershed Nursery and the Grassroots Ecology Nursery planned collection events so that the vegetative propagation material used by the two nurseries was comparable. Specifically, collections were coordinated so both nurseries collected on the same days from the same sites and trees, and so that the timing and methods used during propagation were similar, to the extent feasible, to reduce variance between nurseries.

H.T. Harvey accompanied nursery staff and used the results from the genetics work to guide the collections and ensure all cuttings used in this propagation study were collected from verified, genetically pure California sycamores. All collections followed the Santa Clara Valley Water District's Phytosanitary Best Management Practices (Swiecki and Bernhardt 2016). Dr. Tedmund Swiecki (Phytosphere Research) assisted during the initial collection effort to train the team in identifying the presence of pathogens (i.e., *Phytophthora* spp. and anthracnose), in the field.

The first round of collections occurred in spring 2017, on March 29 and 30, which was before the results of the genetic study were released by the Miller Lab on May 30, 2017. The nurseries collected cuttings from the largest trees to increase the probability that the plant material came from California sycamores and subsequently the genetics data was used to confirm all collections were from verified native California sycamores. The cuttings came from sycamores along Upper Coyote Creek, Uvas Creek, Pacheco Creek, and Llagas Creek (Figure 1). During the first round of collections, the project's plant pathologist Dr. Swiecki, Ph.D., observed *Phytophthora* lesions and severe anthracnose infections on almost all available plant material at Upper Coyote Creek. Under direction of Dr. Swiecki, all cuttings were soaked in a hot water bath (120 degrees Fahrenheit for 30 minutes) the evening after each collection event to systemically disinfect them prior to moving them into the nurseries. A hot bath was used instead of a 5% bleach solution, proposed in the study plan, because Dr. Swiecki informed the nurseries that the bleach solution would kill pathogen infestations on the surface, but not the below the surface of the plant material. Dr. Swiecki recommended the heat treatment to address concerns of both *Phytophthora* and anthracnose infection. Therefore, based on Dr. Swiecki's recommendation and the strong desire by both nurseries to not knowingly bring pathogens into their facilities and put their existing inventory at risk, the nurseries soaked all cuttings in a hot water bath the night after they were collected. The second round of collections occurred the following winter, on January 22 and 24, 2018. Collections on January 22 were from Hunting Hollow, Upper Coyote Creek, and Uvas Creek, and cuttings taken on January 24 were from Pacheco Creek and Llagas Creek (Figure 1). Cuttings were collected from trees that were identified as being pure California sycamore by the genetic study. The original study plan included 2 collection windows for each year, 2017 and 2018. However, following the results for the spring 2017 collection and propagation effort it was determined by consensus between H.T. Harvey, the nurseries and Dr. Sweicki that a likely critical

component for successful collection and propagation was having plant material that was fully dormant to reduce the effects of the recommended sterilization treatment. Collection during full dormancy would also likely increase the potential of having verified native nursery stock available for the SCVWD to install for the Upper Llagas Flood Protection Project in fall 2018. Therefore, the "Fall 2017" collection window was pushed to winter 2018 and the "Spring and Fall 2018" collections were abandoned. While this resulted in no replication for timing of collection, the initial findings during the spring 2017 effort made it clear that collecting while trees had live foliage was not preferred as the sterilization process would likely kill the living tissue and impair rooting. As the project was targeting nursery stock for planting in 2018, there was also no replication of the winter/dormant collection timing, as it would result in plant material being collected after the target installation date. However, the nurseries did make efforts to maximize the number of cuttings each could handle in an attempt to compensate for the decrease in collection events. Grassroots Ecology Nursery was able to triple their collection during the second round, compared to the number proposed, by creating additional capacity in a dedicated greenhouse; whereas The Watershed Nursery's greenhouse that was dedicated to the project was able to accommodate only slightly greater quantities than was proposed. These efforts resulted in collecting nearly 2 times the number of cuttings for Winter 2018 and, even with missing 2 complete collection windows, still capturing 78% of the total number of cuttings originally proposed for the study.

Table 8 summarizes the original proposed and actual number of cuttings for each nursery by collection time.

Table 8. Number of Cuttings Proposed to be Propagated Compared to Actual Number of Cuttings Collected

	2017		2018		
Nursery	**Spring**	**Fall (Actual is Winter 2018)***	**Spring**	**Fall**	**Total**
Proposed Number of Cuttings to be Propagated					
Grassroots Ecology Nursery	250	250	250	250	1,000
The Watershed Nursery	375	375	375	375	1,500
Total	**625**	**625**	**625**	**625**	**2,500**
Actual Number of Cuttings to be Propagated					
Grassroots Ecology Nursery	281	792	0	0	1,073
The Watershed Nursery	432	433	0	0	865
Total	**713**	**1,225**	**0**	**0**	**1,938**

*As described above, timing was altered to collect from fully dormant trees. Collection occurred in January 2018.

During the first round of collection, The Watershed Nursery processed most plant material in the field, rather than at their nursery. Plant material collection methods were modified for the second round to optimize the fitness of the material for treatment: plant material was collected while the material was dormant, and minimal processing occurred in the field (less trimming of side branches). Cuttings were processed and placed into a rooting media the day after they were collected. Consistent with the study plan, during both collection events,

all cuttings had approximately 0.25–0.50-inch diameters and were cut down to lengths of approximately 6–8 inches. The final diameter and length of each cutting was not recorded as proposed in the study plan because such measurements were deemed unnecessary; the variability in the size of the material was minimal and such an effort would have been unproductive. Additional details regarding collection and propagation techniques are provided below.

3.2.3 Treatments

Cutting Material. Cuttings were taken from either the base or crown (i.e. canopy) of a tree. Basal cuttings were made from below 3 feet (1 meter) above the ground and crown cuttings were collected from 1-year-old or less branches that originated from 3–15 feet (1–5 meters) above the ground.

Cutting Preparation. Cuttings were collected using either simple or heal cuts. Simple cuts were cuts made directly below a leaf node along an unbranched stem. Heal cuts were cuts made at the joining section between a branch and a stem, often where new growth was sprouting from the previous year's growth.

Willow Water and Tap Water Presoak. The day after collection and disinfecting hot water bath, cuttings were either presoaked in a willow water bath or a tap water bath for 1–3 hours. The willow water was brewed at the Grassroots Ecology Nursery by boiling tap water, removing the water from heat, then steeping overnight with 3–4 inch cuttings of young willow twigs that were stripped of leaves. The twigs were strained from the willow water the following morning. The willow water was then divided between the Grassroots Ecology Nursery and The Watershed Nursery to standardize the willow water contents and concentrations between the nurseries. The tap water used at the Grassroots Ecology Nursery was the same water that was used to make the willow water. The Watershed Nursery used their own tap water for the tap water treatment. After presoaking, a 1:9 (1,000 parts per million) indole-3-butyric acid dilution (Dip'n Grow) liquid rooting hormone was applied to the basal part of each cutting for 10 seconds. After the presoak and rooting hormone application, the cuttings were struck into a rooting medium, described below.

Rooting Media. Two rooting media were used in this study: perlite and rockwool. Perlite is a volcanic glass that has been expanded into lightweight porous particles. Rockwool is a mineral-based medium made of basalt and chalk rocks baked together at 2912ºF (1,600ºC) into a fluffy, moderately-well-draining medium that can be cut into cubes. Cuttings rooted in perlite were struck directly into 2.5-inch (6.4-centimeter)-deep trays filled with perlite. Rockwool rooted cuttings were struck into individual rockwool cubes. Cuttings rooted in rockwool cubes were initially suspended in beds of perlite for the 2017 propagation effort. However, the rockwool rooted cuttings were soon moved to empty nursery flats due to drainage problems caused by the rockwool being in contact with the perlite. Rockwool rooted cuttings were placed in empty nursery flats (i.e., without perlite) for the 2018 propagation effort. Nursery flats of perlite and rockwool were placed in an alternating fashion to prevent microclimate conditions from systematically affecting the cuttings rooted in each media (Photo 1). All cuttings were initially placed on heating mats for two weeks. Irrigation of the cuttings differed slightly between the nurseries as a result of microclimates and infrastructure capabilities. At The Watershed Nursery, perlite and

rockwool cuttings were irrigated using a drip system that ran for 30 seconds four times a day. However, due to the water holding qualities of the rockwool, fewer drip lines were used than for the perlite. At the Grassroots Ecology Nursery the perlite cuttings were irrigated using a dripline system that ran for 3 minutes four times a day; the rockwool cuttings were hand-watered when they were observed to be dry (typically every 2–3 days). Once cuttings were transplanted into soil, both nurseries hand-watered the cuttings when they were observed to be dry (typically every 2–4 days).

Photo 1. Alternating Trays of Rockwool and Perlite Based Cuttings

3.2.4 Response Variables

Response variables included survival, initial vigor, ongoing vigor, and growth rate. Each cutting was assessed twice, with measurements made each time a cutting was transplanted into a larger pot so that the stems, leaves, and roots could be assessed simultaneously. Both nurseries first transplanted the cuttings from rooting media to 3.4 inch (8.6 centimeter) square by 5.0 inch (12.7 centimeter) deep treeband pots, filled with sterilized potting soil filled. Subsequently, each live cutting was later transplanted into 4.0 inch (10.2 centimeter) square by 10.0 inch (25.4 centimeter) deep treepots, also filled with sterilized potting soil. The decision of when to transplant cuttings into larger pots was based on qualitative observations by nursery staff of the growth and development of individual cuttings. Thus, transplanting and measuring dates varied among cuttings. At The Watershed Nursery, cuttings were transplanted to treeband pots on March 29, March 30, April 24, April 27, and June 3, 2018. The cuttings were then transplanted to treepots on June 3, June 15, and July 5, 2018. At the Grassroots Ecology Nursery, cuttings were transplanted to treeband pots on March 28, April 20, and June 20, 2018; they were then transplanted to treepots between August 15 and August 22, 2018. Raw data are provided in Appendix D.

Survival. Survival was recorded for each cutting each time it was transplanted. Any dead cuttings were immediately culled to prevent the potential spread of disease.

Initial Vigor. Initial vigor was assessed when each cutting was first transplanted from rooting media into soil in treeband pots (Photo 2). The Watershed Nursery and Grassroots Ecology Nursery ranked initial vigor using the collaboratively developed ranking system described in Table 9.

Ongoing Vigor. Ongoing vigor was assessed when cuttings were transplanted for the second time, from treeband pots to treepots (Photo 3). Similar to initial vigor, the ranking system for ongoing vigor was jointly developed by the two nurseries (Table 9).

Growth. The height of each cutting was measured at the time of each transplanting. Growth rate was calculated as the difference between the first and second height measurements divided by the number of days between measurements. Growth rate was used instead of the absolute amount of growth in order to standardize for the number of growth days between measurements for each cutting.

Photo 2. Perlite Based Cutting with an Initial Vigor Rating of 3 (Highest Rating)

Photo 3. Cutting with an Ongoing Vigor Rating of 3 (Highest Rating)

Table 9. Vigor Ranking System for California Sycamore Cuttings

Vigor Rank	Qualifications
Initial Vigor Values	
1	Rooting is asymmetrical, and roots are emerging from only one point on the stem. If roots have emerged from more than one point, they are unbranched OR the root ball has not reached 1 inch in diameter. Leaves and shoots may or may not have developed.
2	Roots are emerging symmetrically or from at least two points on the cutting's stem. Root ball is about 1 inch in diameter, with some degree of root branching. Cutting may or may not have leafed out at one or more nodes, but no shoot development is apparent.
3	Roots are emerging symmetrically or from at least two points on the cutting's stem. Root ball is about 2 inches in diameter, with most individual roots branching at least once. At least one node on the cutting has an elongating shoot, as well as new leaves.
Ongoing Vigor Values	
1	Stressed (e.g., wilted leaves, signs of pest damage, signs of disease, stunted growth, necrotic/yellowing parts of leaves)
2	Stable (e.g., no visible sign of damage or stress, but no signs of new/active growth)

3	Actively growing (e.g., new buds and leaves visible, new shoot growth indicated by bright green color and flexible texture)

3.2.5 Statistical Analyses

Staff from the Watershed Nursery and Grassroots Ecology Nursery collected all of the data and H. T. Harvey & Associates compiled and analyzed the data. Statistical analyses were not conducted on data from cuttings collected in March 2017 because of the aforementioned extremely low survival (i.e., only 1 cutting developed roots). All analyses of cuttings collected during January 2018 were conducted independently by nursery due to the different dates of transplanting and data collection, and differences in nursery conditions. All data analyses were conducted using the R Statistical Software (R Core Team 2018). Interactions were included in a statistical model if that model had the lowest Akaike Information Criterion. A posthoc Tukey-HSD test (using aov and TukeyHSD, base R) was performed if an interaction was significant or marginally significant (i.e. $p<0.1$) and if data was numeric and normally distributed to test for significant differences within the interacting treatments.

Survival. Survival was analyzed using a generalized linear model (using glm, base R and Anova, car, Fox and Weisberg 2011) with data categorized as binomial (i.e. a logistic linear regression) with survival as the response variable, and cutting material, cutting preparation, presoak type, rooting media, and their interactions as predictor variables.

Initial and Ongoing Vigor. Initial vigor and ongoing vigor were analyzed using generalized linear models (using glm, base R and Anova, car, Fox and Weisberg 2011) with data categorized as Gaussian. In these models, initial vigor and ongoing vigor were the response variables, and cutting material, cutting preparation, presoak type, rooting media, and their interactions were the predictor variables.

Growth Rate. Growth rate was analyzed using a generalized linear model (using glm, base R and Anova, car, Fox and Weisberg 2011) with the data categorized as Gaussian. Growth rate was the response variable, and cutting material, cutting preparation, presoak type, rooting media, and their interactions were the predictor variables.

3.2.6 Photodocumentation

Photographs were taken at each data collection point to visually document the study. Photographs were also taken outside the data collection points as necessary to visually record items that may be pertinent to the study. Representative photographs are provided in Appendix E.

3.3 Results

Of the 433 cuttings collected by The Watershed Nursery and 281 cuttings collected by the Grassroots Ecology Nursery in March 2017, only one survived. Because of this extremely low survival rate, statistical analyses were not conducted on the 2017 collections. However, the sole survivor was a basal cutting, prepared using a simple cut, presoaked in tap water, started in rockwool and was propagated at the Grassroots Ecology Nursery.

The 2018 collections resulted in producing 296 genetically verified California sycamores that are ready to be used as mitigation plantings for the SCVWD's Upper Llagas Creek Flood Protection Project.

A summary of results from Winter 2018 collections at The Watershed Nursery is provided in Table 10. A summary of results from Winter 2018 collections at the Grassroots Ecology Nursery is provided in Table 11. The results of each treatment and response variable are further described below.

3.3.1 Survival

Across both nurseries, 296 (24.2%) of the 1,225 cuttings collected in January 2018 were alive at the time of the second transplanting and survival assessment (July for The Watershed Nursery and August for Grassroots Ecology Nursery).

Watershed Nursery. At the time of the first transplanting, 149 of the original 433 cuttings (34.4%) were alive. Cuttings grown in rockwool had significantly lower survival than those grown in perlite ($\chi^2_{1, 432} = 174.94$, $p<0.0001$; Table 10). Perlite based cuttings had 62.7% survival whereas rockwool based cuttings had 6.0% survival. Crown cuttings had a 3.6% percent higher survival rate than basal cuttings, but this difference was only marginally significant ($p=0.08$; Table 10). Neither presoak type ($p=0.34$; Table 10) nor cutting preparation ($p=0.19$; Table 10) affected survival at the time of first transplanting.

At the time of the second transplanting, 90 of the original 433 cuttings (20.8%) were alive. All cuttings that were rooted in rockwool died, which resulted in perlite based cuttings having a significantly higher survival rate ($\chi^2_{1, 432} = 148.10$, $p<0.0001$; Table 10). The percent survival of the cuttings originally struck in perlite was 41.5%. Cutting material ($p=0.77$), cutting preparation ($p=0.72$), and presoak type ($p=0.68$) did not affect survival at the time of the second transplanting (Table 10).

Grassroots Ecology Nursery. At the time of the first transplanting, 241 of the 792 cuttings (30.4%) were alive. There were no main effects of any treatment on cutting survival. However, there was a significant interaction between cutting material and presoak type ($\chi^2_{1, 819}=6.31$, $p<0.05$; Table 11), which indicated that basal cuttings had higher survival when soaked in willow water compared to tap water (Figure 9). The difference in survival by presoak treatment for crown cuttings was minimal (Figure 9).

At the time of the second transplanting, 206 of the 792 initial cuttings (26.0%) were alive. Similar to the results from the first transplanting, there were no main effects of any treatment on the survival of cuttings, and there was a significant interaction between cutting material and presoak type ($\chi^2_{1, 899}=5.72$, $p<0.05$; Table 11). Basal cuttings on average had 9.7% higher survival when presoaked in willow water compared to tap water (Table 11, Figure 10). However, the difference in survival among presoak treatments for crown cuttings was minimal (Figure 10).

Table 10. Summary of Results at The Watershed Nursery

Treatment		Survival at First Transplanting	Survival at Second Transplanting	Average Initial Vigor	Average Ongoing Vigor	Average Growth Rate (inches/day)
Cutting material	Basal	32.0%	22.7%	1.9	2.8	0.079
	Crown	35.6%	20.0%	1.8	2.8	0.077
	p-value	*0.08*	0.77	0.6	0.43	0.78
Cutting preparation	Simple	35.0%	21.0%	1.9	2.9	0.083
	Heal	33.1%	20.2%	1.6	2.5	0.064
	p-value	0.19	0.72	*0.06*	**<0.0005**	**<0.05**
Presoak type	Tap Water	36.8%	20.8%	1.8	2.8	0.078
	Willow Water	31.9%	21.1%	1.9	2.8	0.078
	p-value	0.34	0.68	0.54	0.79	0.62
Rooting media	Perlite	62.7%	41.5%	1.9	2.8	0.5
	Rockwool	6.0%	0.0%	1.2	NA	NA
	p-value	**<0.0001**	**<0.0001**	**<0.001**	NA	NA

Note: NA = not applicable. Values that are bold represent statistically significant results. Values that are in italics represent marginally significant results

Table 11. Summary of Results at the Grassroots Ecology Nursery

Treatment		Survival at First Transplanting	Survival at Second Transplanting	Average Initial Vigor	Average Ongoing Vigor	Average Growth Rate (inches/day)
Cutting material	Basal	37.8%	28.8%	1.7	1.4	0.047
	Crown	32.3%	25.3%	1.8	1.5	0.042
	p-value	0.22	0.2	**<0.01**	0.46	0.72
Cutting preparation	Simple	35.0%	26.3%	1.8	1.4	0.046
	Heal	32.3%	26.8%	1.8	1.5	0.039
	p-value	1	0.43	0.21	0.78	0.16
Presoak type	Tap Water	34.7%	25.8%	1.4	1.4	0.047
	Willow Water	33.4%	27.1%	1.5	1.5	0.041
	p-value	0.74	0.69	0.81	*0.06*	0.1
Rooting media	Perlite	35.5%	27.2%	1.9	1.5	0.040
	Rockwool	32.6%	25.7%	1.7	1.4	0.048
	p-value	0.42	0.64	**<0.05**	0.46	**<0.05**
Cutting material* willow water/tap water soak	Basal + Tap Water	33.0%	22.7%	1.8	1.2	NA
	Basal + Willow Water	42.7%	34.9%	1.6	1.5	NA
	p-value	**<0.05**	**<0.05**	*0.09* (p=0.65*)	*0.06* (**<0.05***)	NA

Note: NA = not applicable. Values that are bold represent statistically significant results. Values that are in italics represent marginally significant results. Asterisks indicate p-values from post-hoc Tukey-HSD tests

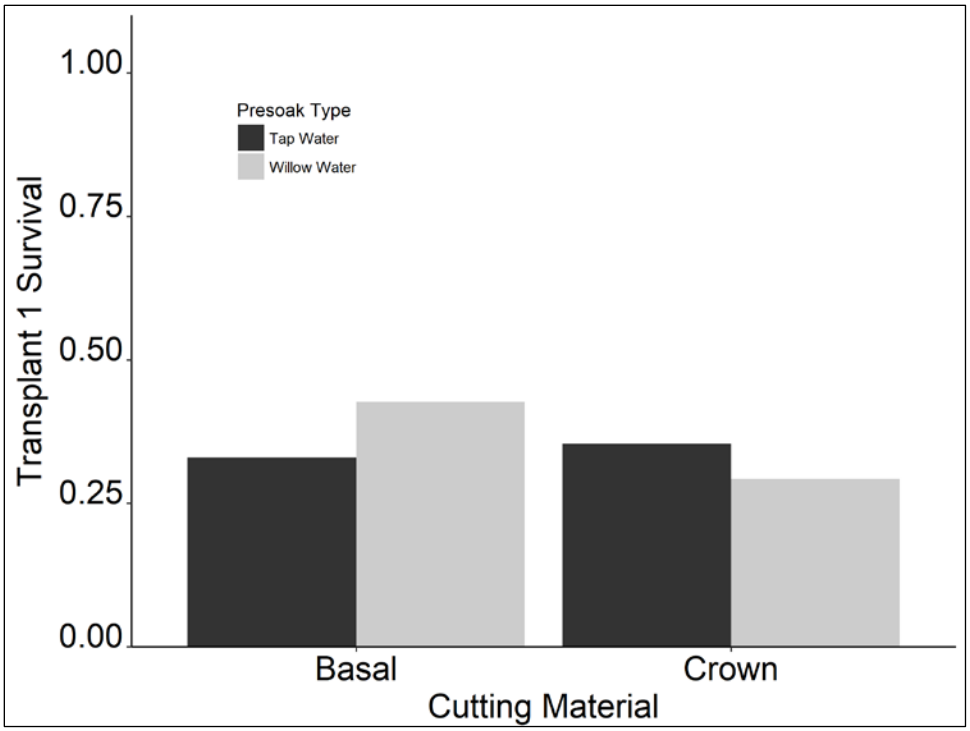


Figure 9. Average Survival during the First Transplanting at the Grassroots Ecology Nursery by Cutting Material and Presoak Type

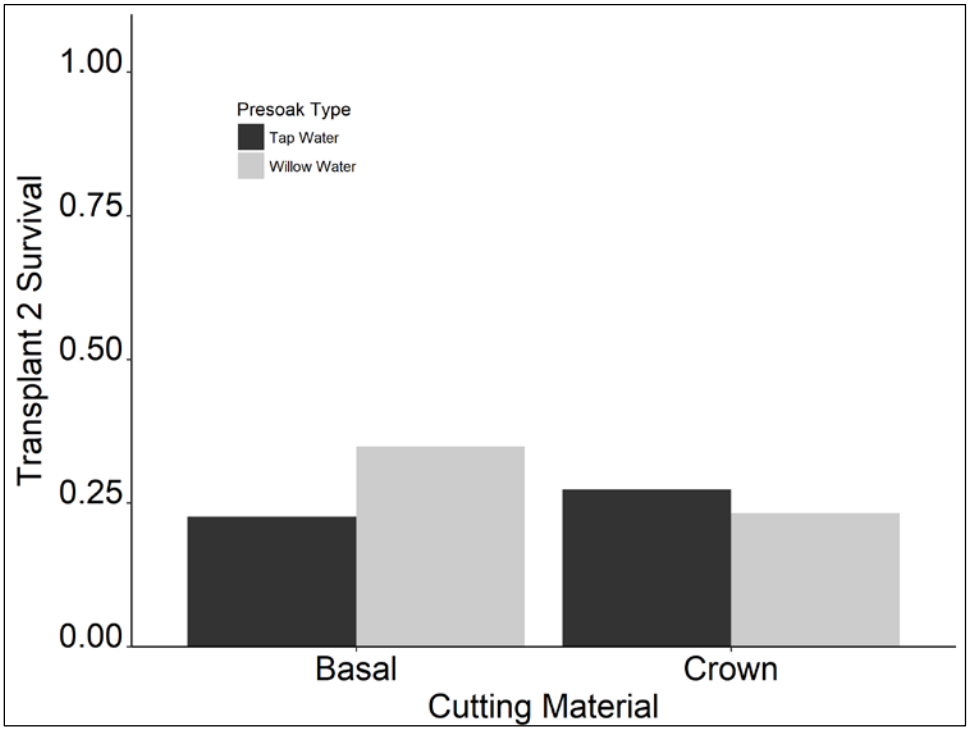


Figure 10. Average Survival during the Second Transplanting at the Grassroots Ecology Nursery by Cutting Material and Presoak Type

3.3.2 Initial Vigor

Watershed Nursery. The average initial vigor ranking across treatments was 1.82 (± 0.28 standard error of the mean). Cuttings rooted in perlite had significantly higher initial vigor ratings than those rooted in rockwool ($\chi^2_{1, 148}$=12.01, p<0.001; Table 10). Initial vigor ratings of cuttings made with simple cuts were marginally significantly higher than those made with heal cuts by 0.25 ($\chi^2_{1, 148}$=3.60, p=0.06; Table 10). Cutting material (p=0.60) and presoak type (p=0.54) did not influence initial vigor (Table 10).

Grassroots Ecology Nursery. The average initial vigor of cuttings across treatments was 1.78 (± 0.04 standard error of the mean). Cuttings based in perlite had significantly higher average initial vigor ratings than those in rockwool, but only by 0.16 ($\chi^2_{1, 320}$=5.06, p<0.05; Table 11). Additionally, crown cuttings had significantly higher average initial vigor rankings than basal cuttings, although only by 0.1 ($\chi^2_{1, 320}$=4.38, p<0.05; Table 11). Neither cutting preparation (p=0.14) nor presoak type (p=0.99) affected the initial vigor of cuttings (Table 11).

3.3.3 Ongoing Vigor

Watershed Nursery. The average ongoing vigor rating at the time of the second transplanting was 2.83 (± 0.05 standard error of the mean). Rooting media type was removed from this model because the only living cuttings at the time of the second transplanting were originally rooted in perlite. Cuttings made with simple cuts had significantly higher ongoing vigor ratings than those made with heal cuts ($\chi^2_{1, 87}$=13.02, p<0.0005; Table 10). Neither cutting material (p=0.43) nor presoak type (p=0.79) had an effect on ongoing vigor (Table 10).

Grassroots Ecology Nursery. The average ongoing vigor rating at the time of the second transplanting was 1.44 (± 0.04 standard error of the mean). Cuttings presoaked in willow water had marginally significantly higher ongoing vigor rankings than those that had been presoaked in tap water ($\chi^2_{1, 203}$=3.62, p=0.06; Table 11). Additionally, while there was no main effect of cutting material to ongoing vigor (p=0.46), there was a marginally significant interaction between cutting material and presoak type, which indicated that the effects of presoak types varied by cutting material ($\chi^2_{1, 203}$=3.49, p=0.06; Table 11). A post-hoc tukey-HSD test shows that the trend of higher ongoing vigor ratings of cuttings soaked in willow water was driven by basal cuttings; basal cuttings presoaked in willow water had significantly higher ongoing vigor ratings than basal cutting presoaked in tap water (p<0.05; Figure 11), but there was no difference in the average ongoing vigor ranking between crown cuttings presoaked in willow water versus crown cuttings presoaked tap water (p=0.97, Figure 11). Neither rooting media (p=0.29), nor cutting preparation (p=0.55) influenced the ongoing vigor rankings (Table 11).

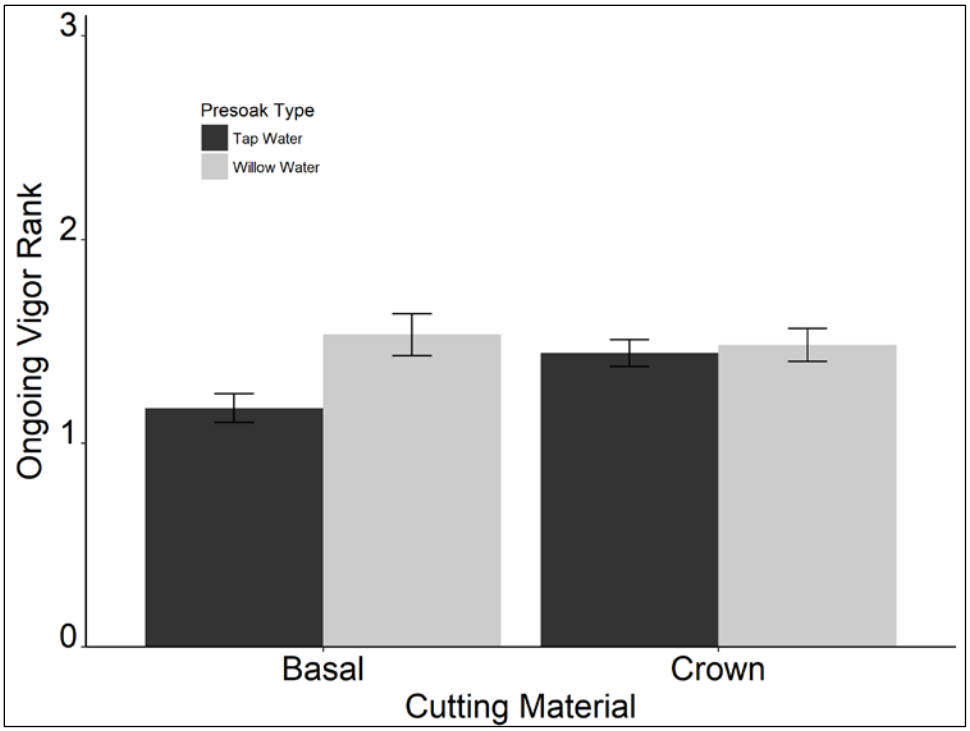


Figure 11. Average Ongoing Vigor Ranking (± Standard Error of the Mean) during the Second Transplanting at the Grassroots Ecology Nursery by Cutting Material and Presoak Type

3.3.4 Growth

Watershed Nursery

The average growth rate of cuttings was 0.079 inches per day (± 0.004 standard error of the mean). Rooting media type was removed from this model because no cuttings initially rooted in rockwool survived to the second transplanting. Cuttings made with simple cuts had significantly faster growth rates than those made from heal cuts ($\chi^2_{1, 87}$=5.14, p<0.05; Table 10). Neither cutting material (p=0.78) nor presoaking treatment (p=0.62) affected growth rate (Table 10).

Grassroots Ecology Nursery

The average growth rate was 0.043 inches per day (± 0.0002 standard error of the mean). Cuttings initially rooted in rockwool had significantly faster growth rates than those rooted in perlite ($\chi^2_{1, 197}$=4.46, p<0.05; Table 11). Cuttings that were presoaked in tap water grew slightly faster (i.e., 0.02 centimeters more per day) than those presoaked in willow water, but this difference was not significant ($\chi^2_{1, 197}$=2.71, p=0.10; Table 11). Neither cutting preparation (p=0.16) nor cutting material (p=0.72) affected the growth rate of cuttings (Table 11).

3.4 Discussion

3.4.1 Objective 1—Advance the Science of Vegetative Propagation

Although the propagation of California sycamores from cuttings is known to be difficult, results from this study are promising for increasing the likelihood of success. Yet, the nearly 100% failure of the spring 2017 collections is representative of the challenges of vegetative propagation. The low survival of the 2017 effort may be attributable to multiple factors, including the degree of dormancy of cuttings, the presence of pathogens, and the disinfection treatment. At the time of the spring 2017 collections, cuttings were showing some degree of active growth (i.e. not dormant), and *Phytophthora* lesions and severe anthracnose infections were observed on almost all available plant material by the plant pathologist Dr. Swiecki, likely as a result of warm, wet spring conditions in 2017. As described above (Section 3.2.2), Dr. Swiecki recommended a hot water bath treatment to address concerns of both *Phytophthora* and anthracnose infection to reduce the risk of introducing them to The Watershed Nursery and Grassroots Ecology Nursery; however, soaking the cuttings in the disinfecting hot water bath may have killed the growing tissue and associated meristems leaving depleted energy reserves for root development and additional sprouting. Ideally, the hot water bath treatment would have been tested on a subset of plant materials to ensure that the plant propagules would tolerate the treatment without substantial damage or loss of viability; however this separate test was not part of the original project and would have entailed a completely separate experiment, thus delaying the contracted experiment. Therefore, the nurseries proceeded using the hot water bath as recommended by the project's plant pathologist.

Survival of cuttings collected in winter 2018 (24.2%) was higher than typically observed at plant nurseries and is promising for increasing the rate and efficiency of successful vegetative propagation of California sycamores. In winter 2018, the California sycamore trees were fully dormant when cuttings were collected and subsequently placed in the hot water bath. Thus, damage to the cutting's tissue was minimized and likely contributed to the higher survival rate. Additionally, the available genetic data enabled collections from smaller, younger trees without concern that they may be hybrids. The nursery practitioners that collected the cuttings qualitatively observed that cuttings from smaller, younger trees seemed healthier and more vigorous than those from larger, older trees (Benner pers. comm. 2018) (Giuliano pers. comm. 2018). Additionally, signs of pathogens appeared to be less abundant, potentially increasing the success of propagation. Overall these factors contributed to the much more successful propagation of winter collections compared to the spring collections.

Across both nurseries, cuttings rooted in perlite had higher survival than those rooted in rockwool. However, when separated by nursery, significant differences in survival between the rooting media treatments were only detected at The Watershed Nursery were all cuttings initially rooted in rockwool died; there was no significant difference in the survival of cuttings rooted in perlite and rockwool at the Grassroots Ecology Nursery. Differing rockwool irrigation techniques may have caused these disparate results. At the Grassroots Ecology Nursery, rockwool was hand-watered once every 2–3 days, whereas at The Watershed Nursery a dripline irrigation system was used and four times a day. The specific reasons for the different irrigation techniques include the vastly different microclimates at the two nurseries, the limited infrastructure capabilities at

Grassroots Nursery as compared to The Watershed Nursery, and the desire by the practitioners to utilize the most appropriate techniques, within given constraints, to increase the likelihood of success. Controlling moisture levels in rockwool was found to be difficult, and the frequent, automated irrigation at The Watershed Nursery may have caused root rot and contributed to mortality. Additionally, a stretch of very warm weather during the study period caused rockwool cuttings to completely dry out, regardless of the automated irrigation, and may have also contributed the high levels of mortality (Benner pers. comm. 2018). Although it was speculated that rockwool would provide better moisture holding capabilities and cause less root disturbance during transplanting than perlite, the only positive effect that was detected from rockwool was a slightly faster (0.008 inches more per day [0.02 centimeters more per day]) growth rate at Grassroots Ecology Nursery. Staff at both nurseries agreed that the moisture was easier to control in perlite (Benner pers. comm. 2018) (Giuliano pers. comm. 2018). Indeed, 41.5% of cuttings rooted in perlite at The Watershed Nursery survived, the highest survival rate out of all of the treatments. Further, initial vigor ratings were higher for cuttings rooted in perlite at both nurseries.

The effect of cutting preparation varied between nurseries. At The Watershed Nursery, initial vigor, ongoing vigor, and growth rate were all significantly higher for cuttings made with simple cuts compared to those made from heal cuts. However, there were no discernable differences for any response variable made between cutting preparations at the Grassroots Ecology Nursery. Nonetheless, the nursery practitioners at both nurseries agreed that the locations to make simple cuts were more common and were easier to make than heal cuts (Benner pers. comm. 2018) (Giuliano pers. comm. 2018).

At the Grassroots Ecology Nursery, presoaking basal cuttings in willow water resulted in higher survival during both transplanting events and higher average ongoing vigor ratings compared to basal cuttings presoaked in tap water. It is unclear why a similar pattern was not observed at The Watershed Nursery. However, the mortality of all rockwool based cuttings potentially made the sample size too small to detect this interaction at The Watershed Nursery. On average, basal cuttings presoaked in willow water had the highest survival rate out of any propagation technique at the Grassroots Ecology Nursery. Additionally, basal cuttings were much more efficient to collect than crown cuttings, and anecdotally appeared to be less prone to disease (Benner pers. comm. 2018) (Giuliano pers. comm. 2018). Thus, basal cuttings will be more efficient to collect and may have higher survival and vigor if presoaked in willow water.

3.4.2 Objective 2—Improve the Cost-Effectiveness of Vegetative Propagation

Based on the results of this study, the following recommendations may increase the rate of successful propagation of California sycamores from cuttings and thereby improve the cost-effectiveness of propagating California sycamores for restoration projects.

General Recommendations

- Collect cuttings from genetically verified California sycamore trees. By collecting from verified trees the future costs of genetic testing of multiple cuttings is bypassed and the need to remove and replace planted hybrids is avoided.
- Treat all cuttings with a hot water bath or similar systemic disinfecting process before bringing cuttings into a nursery or similar area. This approach will limit the potential spread of pathogens and/or disease to the nursery and locations where nursery stock are planted.
- In addition to disinfecting processes, nursery material should be grown with best management practices to limit the spread *Phytophthora* spp. and other plant pathogens, such as the *Guidelines to Minimize Phytophthora Pathogens in Restoration Nurseries* (Working Group for Phytopthoras in Native Habitats 2016) and Santa Clara Valley Water District's Phytosanitary Best Management Practices (Swiecki and Bernhardt 2016).

Recommendations Based on the Results from the Propagation Study

- Collect cuttings during the winter, when trees are dormant. This will likely increase the success of propagation, allow cuttings to tolerate the necessary disinfecting treatments (i.e., hot water bath), and will limit the spread of pathogens.
- Target using simple cuts when collecting cuttings. Simple cuts were more common because they do not depend on having substantial branching points along a sprout and were more efficient to collect than heal cuts. Additionally, at The Watershed Nursery, cuttings made with simple cuts had higher ongoing vigor ratings and faster growth rates than heal cuts.
- Use perlite rather than rockwool as the rooting media for cuttings. Both nurseries found moisture in rockwool difficult to control. This difficulty, along with a run of very warm weather at The Watershed Nursery, likely led to the death of all rockwool rooted cuttings there. Using perlite allows for better control of moisture levels and is expected to increase the overall success rate of propagation.
- When possible, focus collecting cuttings that can be made from the ground (basal cuttings), which were more efficient to collect than crown cuttings and anecdotally harbored less signs of pathogens. However, the study did not show and other clear benefits to collecting basal cuttings over crown cuttings. Therefore, healthy crown cuttings that are relatively easy to collect should still be considered for collection.

3.4.3 Objective 3—Determine Future Studies

This study provides important information regarding techniques to improve the overall success rate of propagating California sycamores from cuttings that should be implemented, although further research is warranted. Additional studies will further advance the knowledge and efficacy of vegetative propagation of

California sycamore trees. A few ideas for future studies, in descending order of importance, include the following:

1. Continue genetics work to expand the database of genetically verified California sycamores with a secondary benefit of further identification of the locations of hybrids and London planetrees. This will increase the geographic diversity and number of source trees for propagule collection and further the degree of understanding the extent of hybridization in Santa Clara County.
2. Assess the effect of source tree size and age on cutting survival and performance.
3. Replicate experiments in this study where treatments only had significant effects at one of the two nurseries (i.e. cutting materials, presoak type, and cutting preparation) to provide further insight into which treatments are most effective, or whether the patterns that were detected occurred solely by chance.

Section 4. References

Ali O. A., S. M. O'Rourke, S. J. Amish, M. H. Meek., G. Luikart, C. Jeffres, and M. R. Miller. 2016. RAD Capture (Recapture): Flexible and efficient sequence-based genotyping. Genetics 202:389–400.

Anttila, C. K., C. C. Daehler, N. E. Rank, and D. R. Strong. 1998. Greater male fitness of a rare invader (*Spartina alterniflora*, Poaceae) threatens a common native (*Spartina foliosa*) with hybridization. American Journal of Botany 85:1597–1601.

Beagle, J., A. Richey, S. Hagerty, M. Salomon, R. A. Askevold, R. M. Grossinger, P. Reynolds, C. McClain, W. Spangler, and M. Quinn. 2017. Sycamore Alluvial Woodland: Habitat Mapping and Regeneration Study. SFEI Contribution No. 816.

Benner, Diana. Nursery Director. The Watershed Nursery, Richmond. November 13, 2018—conversation with Ryan Hegstad of H. T. Harvey & Associates regarding the collection and propagation techniques used in this study, and the climate and conditions at The Watershed Nursery.

California Department of Fish and Wildlife. 2017. Final Lake or Streambed Alteration Agreement, Notification No. 1600-2015-0356-R3, Upper Llagas Creek Flood Protection Project.

Fox, J., and S. Weisberg. 2011. An {R} Companion to Applied Regression, Second Edition. Sage Publications, Thousand Oaks, California. <http://socserv.socsci.mcmaster.ca/jfox/Books/Companion>.

Fumagalli, M., F. G. Vieira, T. Linderoth, and R. Nielsen. 2014. ngsTools: methods for population genetics analyses from next-generation sequencing data. Bioinformatics Oxford, England 30:1486–1487.

Giuliano, Deanna. Nursery Director/Botanical Consultant. Grassroots Ecology Nursery, Palo Alto. November 6, 2018—conversation with Ryan Hegstad of H. T. Harvey & Associates regarding the collection and propagation techniques used in this study, and the climate and conditions at the Grassroots Ecology Nursery.

Grolli, P. R., S. Morini, and F. Loreti. 2005. Propagation of *Platanus acerifolia* Willd. by cutting. The Journal of Horticultural Science and Biotechnology 80:705–710.

Grubb, P. J. 1977. The maintenance of species richness in plant communities: The importance of the regeneration niche. Biological Review 52:107–145.

Hartmann, H. T., D. E, Kester, F. T. Davies, and R. Geneve. 2002. Kartmann and Kester's Plant Propagation: Principles and Practices. Seventh Edition.

Hayat, S., and A. Ahmad. 2007. Salicylic Acid: A Plant Hormone. Springer, Netherlands. <https://doi.org/10.1007/1-4020-5184-0>.

Holland, R. 1986. Preliminary Descriptions of the Terrestrial Natural Communities of California. California Department of Fish and Game, Natural Heritage Division, Sacramento.

H. T. Harvey & Associates. 2016a. Upper Llagas Creek California Sycamore Genetic Study Plan. September. Los Gatos, California.

H. T. Harvey & Associates. 2016b. California Sycamore Propagation Study Plan. November. Los Gatos, California.

Johnson, M. G., K. Lang, P. Manos, G. H. Golet, and K. A. Schierenbeck. 2016. Evidence for genetic erosion of a California native tree, Platanus racemosa, via recent, ongoing introgressive hybridization with an introduced ornamental species. Conservation Genetics 17:593-602.

ICF International. 2012. Santa Clara Valley Habitat Plan. Prepared for County of Santa Clara, City of San Jose, City of Morgan Hill, City of Gilroy, Santa Clara Valley Water District, and Santa Clara Valley Transportation Authority.

Korneliussen, T. S., A. Albrechtsen, and R. Nielsen. 2014. ANGSD: Analysis of Next Generation Sequencing Data. BMC Bioinformatics 15 doi: 10.1186/s12859-014-0356-4.

Li H., and R. Durbin. 2009. Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics Oxford, England 25:2078–2079.

MacDonald, B. 1986. Practical Woody Plant Propagation for Nursery Growers, Volume 1. Timber Press. Portland, Oregon.

Miller, M. R., J. P. Brunelli, P. A. Wheeler, S. Liu, C. E. Rexroad 3rd, Y. Palti, C. Q. Doe, and G. H. Thorgaard. 2012. A conserved haplotype controls parallel adaptation in geographically distant salmonid populations. Molecular Ecology 21:237–249.

Navarez, Kathleen. Seed Specialist. Suncrest Nurseries, Watsonville, California. November 2016—email to Diana Benner regarding experience with propagating *Platanus racemosa.*

O'Rourke, S. M., and M. R. Miller. 2017. RAD sequencing identifies trees that area product of hybridization between California sycamore (*Platanus racemosa*) and London planetree (*Platanus* × *acerifolia*). May 30. Department of Animal Science, University of California, Davis.

Phytosphere Research. 2001. Guidelines for Developing and Evaluating Tree Ordinances. October 31. Vacaville, California. <http://www.phytosphere.com/tree8ord/index.htm>. Accessed September 2015.

Plume, C. A. 1990. Vegetative Propagation of Arizona Sycamore (*Platanus wrightii*) by Cuttings. Thesis. University of Arizona, Tucson.

R Core Team. 2018. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.

[SFEI] San Francisco Estuary Institute and H. T. Harvey & Associates. 2017. Sycamore Alluvial Woodland: Habitat Mapping and Regeneration Study. February. Richmond, California and Los Gatos, California. Prepared for the California Department of Fish and Wildlife, Local Assistance Grant Program. Sacramento, California.

[SFEI] San Francisco Estuary Institute. 2018. Observational Study of Sycamore Regeneration at Two Sites in Santa Clara County after the 2016–2017 Water Year. June. Richmond, California. Prepared for Santa Clara Valley Water District. San Jose, California.

Schierenbeck, K. A. & Ellstrand, N. C. 2009. Hybridization and the evolution of invasiveness in plants and other organisms. Biol Invasions. 11:1093.

Schierenbeck, Kristina. Professor of Biological Sciences, California State University, Chico. April 25, 2016—email correspondence with Charles McClain of H. T. Harvey & Associates regarding California Sycamore Genetic Study.

Shanfield, A. 1984. Alder, cottonwood and sycamore distribution and regeneration along the Nacimiento River, California. Pages 196–201 in R. Warner and K. M. Hendrix, editors. California Riparian Systems. University of California Press. Berkley, California.

Skotte, L., T. S. Korneliussen, and A. Albrechtsen. 2013. Estimating Individual Admixture Proporations from Next Generation Sequencing Data. Genetics 195:693–702.

Stevens, D. L., and A. R. Olsen. 2004. Spatially balanced sampling of natural resources. Journal of the American Statistical Association. 99:262–278.

Swiecki, T. J., and E. A. Bernhardt. 2016. Best Management Practices for Producing Clean Nursery Stock. Phytosphere Research. Support Provided by Santa Clara Valley Water District, California Native Plant Society, and Phytosphere Research. <http://phytosphere.com/BMPsnursery/Index.htm>.

Thompson, S. K. 2002. Sampling. Second edition. John Wiley & Sons, New York, New York.

Working Group for Phytopthoras in Native Habitats. 2016. Guidelines to Minimize Phytophthora Pathogens in Restoration Nurseries. September 22.

Zencirkiran, M., and K. Erken. 2012. The effect of different times collecting cutting and auxin treatments of the rooting in *Platanus orientalis* (Oriental plane tree - Cinar). Journal of Animal and Plant Sciences 22:764–767.

Appendix A. Upper Llagas Creek California Sycamore Genetic Study Plan

Appendix B. California Sycamore Propagation Study Plan

Appendix C. California Sycamore Genetic Study Results Tables

Appendix D. California Sycamore Propagation Study Raw Data

Appendix E. California Sycamore Propagation Study Photodocumentation

Photo 1. Collecting Cuttings from Upper Coyote Creek on March 29, 2017

Photo 2. Collecting Cuttings from Upper Llagas Creek on January 24, 2018

Photo 3. Organized Cuttings at Upper Llagas Creek on January 24, 2018

Photo 4. Disinfecting Hot Water Bath Preparation on March 29, 2017

Photo 5. Implementation of the Willow Water and Tap Water Presoak Treatments at The Watershed Nursery on March 31, 2017

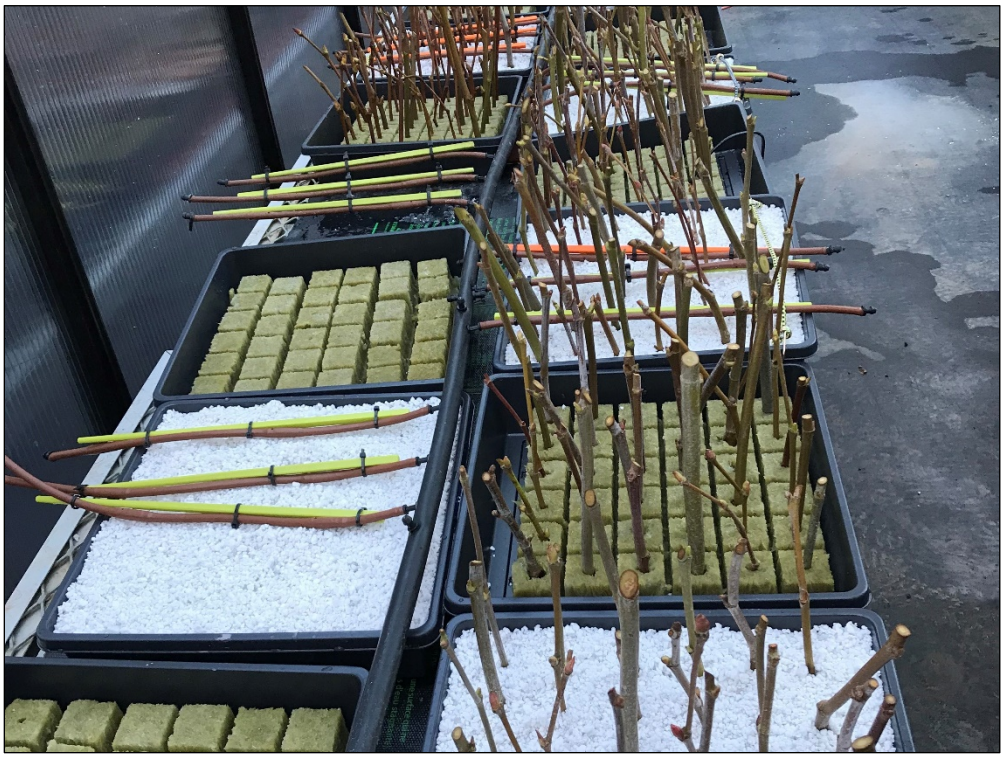


Photo 6. Cuttings the Day of Striking into Rooting Media at Grassroots Ecology Nursery on January 25, 2018

Photo 7. Rockwool Rooted Cutting the Day of First Transplanting and Initial Vigor Ranking at The Watershed Nursey on March 29, 2018

Photo 8. Cutting During the Second Transplanting and Ongoing Vigor Ranking at Grassroots Ecology Nursery on August 21, 2018

Photo 9. Cuttings Ready for Planting at Grassroots Ecology Nursery on November 6, 2018

Photo 10. Cuttings Ready for Planting at The Watershed Nursery on November 13, 2018