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Drought Resistance of Grapevine Root Systems

By

SAM DUDLEY
DISSERTATION

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in the

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Abstract

Increased severity of drought due to climate change in mediterranean climate regions around the world is expected to threaten viticulture by decreasing yield and quality of grapes. Grapevine rootstocks can provide a level of drought resistance to their scion to mitigate these effects. Grape root systems avoid drought stress by deep rooting, effective exploration of the soil profile for water, and root responses to drought to prevent severe drought stress. While these traits exist to a certain extent in commercially available rootstocks, breeding for new drought tolerant rootstocks has not been prioritized until recently. Breeding has the potential to improve drought resistance in new rootstock varieties beyond what is currently available. This dissertation discusses topics related to improving drought resistance of grapevine rootstocks. In chapters 1, 2, and 3, respectively, we explored comparison of propagation method on root system architecture and mapping quantitative trait loci (QTL) for root traits, predicting drought resistance of rootstocks from root traits in small plants, and root traits alongside drought stress responses in the field. In Chapter 1, we genotyped and phenotyped a population of novel rootstocks produced from a cross between a drought resistant and drought sensitive parent (110R and 101-14), originating from the three most common *Vitis* ancestors to commercial rootstocks: *Vitis riparia*, *Vitis berlandieri*, and *Vitis rupestris*. We identified two QTL for root system traits that have biological significance to drought resistance. We also found clear phenotypic differences between propagation methods, and genotype by propagation method interaction that shows root architecture in young plants is dependent on the mode of propagation. These results identify limitations and a scope of interpretation for each method of propagation for future studies. In Chapter 2 we used a subset of 14 novel genotypes from the population used in chapter 1, and along with seven commercial rootstocks with varying drought resistance, we connected root traits to water relations and

drought resistance. Using neutron radiography, we measured root growth and architecture on the selected genotypes and rootstocks. Using both raw trait values and indices that integrated multiple trait values, we demonstrated that root traits were predictive of drought physiology and observed agronomic drought resistance. We identified that steeper root angles, roots that tended to grow downward, and roots that grew preferentially with moisture were associated with drought resistance in commercial rootstocks. In the novel genotypes, we found long and fast-growing 2nd order roots were the best predictors of root traits and drought performance in a greenhouse assay, indicating a potentially advantageous trait for drought resistance. In chapter 3 we measured root distributions, soil moisture, and drought stress responses for a drought susceptible (101-14) and resistant (110R) rootstock in a randomized field trial. We found that the drought resistant rootstock had less severe physiological responses to soil moisture, maintained higher photosynthesis, stomatal conductance, and predawn water potential under severe drought. We also found that the drought resistant rootstock had a deeper and sparser root distribution with roots that were thicker and more steeply angled when compared to the drought susceptible rootstock. Together, these results connect root traits to rootstock drought physiological responses in the field. Altogether, this project provides information at multiple scales to support the improvement of grapevine rootstock drought resistance.

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Chapter 1: Grapevine Adventitious Root Traits Vary Based on Propagation Method

1.1. Abstract

Development of new rootstocks is an effective strategy to continue grape production despite increased abiotic stress in a changing climate. We measured an F1 population derived from a cross between a drought resistant (110R) and drought susceptible (101-14 MGT) rootstock to identify quantitative trait loci (QTL) for root system architecture traits. We also compared traits and QTL between two propagation methods: hardwood and herbaceous cuttings. Plants were grown for 35 days and measured by destructive harvest and imaging, which provided 17 traits related to root system architecture. We identified two significant QTL for root system architecture traits for root thickness and root angle. Generally, hardwood cuttings had significantly larger root systems, thicker roots, and a higher ratio of lateral roots to adventitious roots, however genotypes did not have consistent phenotypes across propagation methods. This finding indicates that the interpretation of experimental results should depend on the propagation method used. We also found complex, multigenic control of root system architecture traits, which supports the use of genomic selection models for breeding. We expect these results to guide future research using young grapevine root systems and provide insight into the genetic control of root architecture in grapevine.

1.2. Introduction

In 2016, grapes were estimated to be worth \$68 billion in the world market, making it the third most valuable horticultural crop in the world (Cantu & Walker, 2019). Mediterranean climate zones are renowned for their viticulture, however increased drought in these areas poses a threat to future production, with drought affecting both yield and grape quality (Del Pozo et al., 2019; McLain & Castro, 2017; Van Leeuwen & Darriet, 2016). While initially introduced for the purpose of controlling the pest grape phylloxera, grapevine rootstocks can also contribute to improved drought resistance (Ollat et al., 2016), which may allow farmers to keep growing grapes despite periodic water shortages. Rootstocks have been utilized for their drought resistance since their widespread adoption in the 19th and 20th century, however breeding specifically for drought resistance has only started recently, creating an opportunity for continued progress (Chen et al., 2024). Traditional breeding of grapevines takes roughly 25-30 years from cross to release (Eibach & Töpfer, 2015). Moreover, quantitative traits controlled by many genes combined with the difficulty of phenotyping root systems makes it difficult to select root traits. This study aims to assist breeding for grapevine root traits by providing information on their genetic basis and identifying molecular markers for these traits.

Drought resistant rootstocks are defined as those that produce adequate growth, yield, and fruit quality under water-limited conditions (Zhang et al., 2016). Grape rootstocks generally achieve drought resistance through an avoidance rather than tolerance strategy, where avoidance is defined as the ability to prevent tissues from experiencing stress (i.e. maintain high water potentials) and tolerance as the ability to function despite stress or damage from stress (Levitt, 1980). In typical viticultural systems, grapevines rarely reach critically low water potentials, and

maintaining an adequate water supply to the canopy is more important to drought performance than the ability to tolerate low water potentials (Alsina et al., 2011; Blum, 2005; Charrier et al., 2018; Pouzoulet et al., 2020). This tendency towards drought avoidance could also reflect the evolutionary history of wild *Vitis* species, which may have evolved deep and exploratory root systems to avoid direct competition with host trees for resources (Morano, 1995; Morano & Walker, 1995; Smart et al., 2006).

Improving root system architecture (RSA), defined as the spatial distribution of roots throughout the soil, can improve resource acquisition in crops (Lynch, 2013; Osmont et al., 2007; Uga et al., 2013). Due to the complexity of the grape root system, there is not a single drought resistant ideotype, however numerous traits have been identified as important (Bernardo et al., 2025). Extensive, deep roots that explore deeper soil layers, as well as the efficient utilization of fine roots have been shown to be important traits for whole root system drought resistance (Atkinson et al., 1999; Smart et al., 2006; Swanepoel & Southey, 1989). The inability of roots to grow through hard soil is also often a limiting factor in root system development, so root penetration ability, coupled to root thickness, plays a pivotal role in root system development and drought resistance (Clark et al., 2002; Kolb et al., 2017; Materechera et al., 1992; Smart et al., 2006). Root branching frequency and survivorship of roots over multiple years affects the woody structure of the root system (Puhe, 2003; Wells & Eissenstat, 2001), while lateral branching frequency, length, and growth rate affect the ability of fine roots to take up water and nutrients (Bauerle et al., 2008; Song et al., 2020; White & Kirkegaard, 2010). In general, drought resistant rootstocks have been observed to have deeper and more extensive root systems, thicker fine roots, steeper rooting angles, and more root growth throughout the year when compared to

drought sensitive rootstocks (Bauerle et al., 2008; Fort et al., 2017; Reingwirtz et al., 2021; Smart et al., 2006, 2012).

So far, studies have shown that genetic control of RSA in grapevine is complex, with many small effect genes contributing to the overall phenotype (Alahakoon & Fennell, 2023). However, genes with agronomic significance have been identified in other crops, such as the DRO1 gene, which controls rooting depth in rice, and the TaMOR (MORE ROOT) transcription factor in wheat, which produces more roots when overexpressed (Khalid et al., 2024; Kulkarni et al., 2017; Li et al., 2016; Uga et al., 2013). In grapevine, multiple studies have been published recently on the genetic determination of root system architecture (Alahakoon & Fennell, 2023; Bert et al., 2013; Blois et al., 2023; Mohtasebi, 2023; Smith et al., 2013; Tandonnet et al., 2018; Thapa, 2022). However, markers identified in these studies have yet to be integrated into breeding programs, so progress lags behind other crops. Previous studies in other plants have shown that RSA is heavily determined by hormone signaling pathways that regulate root growth and development (Jung & McCouch, 2013; Overvoorde et al., 2010), so most candidate genes proposed in grapevine quantitative trait loci (QTL) studies have been those related to hormone signaling pathways (Alahakoon & Fennell, 2023; Tandonnet et al., 2018). The clearest candidate genes in grapevine came from a genome-wide association study (GWAS) study for root traits in *V. berlandieri* from Blois et al. (2023), that found markers in genes regulating integral membrane components, metal ion binding proteins, a UMP kinase protein, nuclear organization, and alkaloid pathways. Other transcriptomic studies into adventitious root formation in grapevine identified transcriptomic factors related to the effect of light on rooting and two novel genes coding for proline rich proteins (PRP) involved in rooting, however the exact function of the PRPs is unknown (Thomas et al., 2003; Yuan et al., 2024). Furthermore, root system development and persistence over

multiple years can affect long term root system architecture (Lyford & Wilson, 1964; McCormack et al., 2015; Wells & Eissenstat, 2001), and genes governing root function and lifespan have been discovered in other perennial species (Bagniewska-Zadworna et al., 2014).

Grapevines can be propagated as hardwood cuttings from dormant lignified canes or softwood herbaceous cuttings from actively growing shoots. Research on grapevine root systems has used both hardwood and herbaceous propagation (Barrios-Masias et al., 2015; Bartlett et al., 2022; Cuneo et al., 2021; Fichtl et al., 2024; Gambetta et al., 2013), but there is no published research directly comparing the two. Instead, previous research has focused on other factors such as different genotypes or propagation media (Farooq et al., 2018), differences between cutting size (Larrey et al., 2025), or grafted and non-grafted plants (Bert et al., 2013), so direct comparisons between hardwood and herbaceous propagation may provide novel information on how to interpret results from different propagation methods. Hardwood cuttings are cheaper and easier to process at a large scale, robust to damage, and storable for months, so hardwood propagation is the primary technique used by commercial nurseries (Smart et al., 2002; Waite et al., 2015). Using hardwood cuttings in research could make the results easier to compare to commercial settings, but herbaceous propagation also has several advantages that make it a common approach in root biology research (Barrientos-Sanhueza et al., 2024; Cuneo et al., 2016; Gambetta et al., 2013; Reingwirtz et al., 2021). Herbaceous cuttings can be taken from greenhouse-grown mother plants any time of the year, allowing experiments to be conducted year-round, and potted plants do not need induced dormancy cycles for propagation, unlike hardwood cuttings. Additionally, actively growing greenhouse populations may be easier to control environmental factors when compared to field populations used for hardwood cuttings.

The difficult nature of root phenotyping may dissuade selection for root traits in traditional breeding pipelines, however integration of genetic tools for root trait selection can work to close the gap and allow selection for root traits cheaply and easily without complicated root phenotyping systems. Genetic breeding tools such as marker assisted selection (MAS) and genomic selection (GS) have already been integrated into grapevine breeding pipelines to select for traits such as powdery and downy mildew resistance, cluster traits, and berry sugar content (Brault et al., 2024; Viana et al., 2016). MAS has been used to shorten breeding time and provides the possibility of introgression of loci into breeding gene pools (Eibach & Töpfer, 2015). GS models are a more recent advance in grapevine breeding and may perform better than MAS for selecting complex traits governed by many small effect genes (Bhat et al., 2016; Viana et al., 2016).

While research in the genetic determination of RSA in grapevine has increased in recent years, research is scarce compared to other agronomic crops. The goals of this study are to use a cross between a drought resistant (110R) and drought susceptible (101-14) rootstock to identify QTL for RSA traits and to determine how propagation methods influence trait values and QTL results. 110R and 101-14 also vary in root system architecture including angle, thickness, and growth rates, and thus we expect their progeny to provide a wide diversity of root traits (Fort et al., 2017; Reingwartz et al., 2021). To our knowledge, this is the first study to directly compare the root architecture of grapevine using both hardwood and herbaceous propagation methods and the first QTL mapping study to include parentage from all three of the most used rootstocks for breeding (*Vitis. riparia*, *Vitis berlandieri*, and *Vitis rupestris*). Our results will provide new molecular markers and information for rootstock breeding, as well as provide context to interpret studies from each propagation method.

1.3. Materials and methods

1.3.1. Plant material and growing conditions

This study examined root system architecture across a subset of an F1 population of 129 progeny derived from a cross between two commercial rootstocks with different root traits: drought sensitive 101-14 Millardet et de Grasset (101-14, *V. riparia* x *V. rupestris*) and drought resistant 110 Richter (110R, *V. rupestris* x *V. berlandieri*) (Dry & Coombe, 2005; Fort et al., 2017; Keller, 2020; Rahemi et al., 2022).

All experiments were conducted at the University of California, Davis between January and May 2024. We rooted herbaceous cuttings from mother vines that were actively growing in a greenhouse and hardwood cuttings from dormant field-grown vines, following standard procedures for grapevine propagation (Waite et al., 2015). Field grown vines were 12 years old when cuttings were taken, growing in a Yolo silt loam soil (SoilWeb) on a California sprawl trellis system. Average daily high temperatures range from 13.3 °C in December, to 34 °C in July, with 80 cm of rain throughout the year, mainly between November and March (National Weather Service). Greenhouse mother vines were actively growing plants that had not gone through winter dormancy, between six months and one and a half years old. Three plants per genotype were planted in two-gallon pots with two to five actively growing shoots per plant. The shoots were trained up vertical wooden stakes to a height of approximately 1 meter and watered and fertilized with micro and macronutrients with drip irrigation. Supplemental lighting above the vines provided an artificial daylength of 16:8 hour light:dark cycle during the winter and spring.

In January 2024, we took 10 herbaceous cuttings from each genotype in the greenhouse and 20 cuttings from each parent. The cuttings were trimmed to a standard length (5-10 cm) and leaf

area (~10 cm²), dipped in liquid rooting compound for 10 seconds (0.05 % indol-3-butyric acid, 0.025 % naphthalene acetic acid, Earth Science Products Corp., Wilsonville, OR), and planted in rooting bins (15 x 40 x 60 cm). In February 2024, we took 10 hardwood cuttings from a similar set of genotypes from dormant field-grown vines, and 20 cuttings for each parent. We pooled and weighed the 10 cuttings to get an average mass for each genotype, then put the cuttings into cold storage to meet chilling requirements. In March 2024, we removed the cuttings from storage, rehydrated them in water for 24 hours, treated them with liquid rooting compound, and planted them in rooting bins. Including parental genotypes, 101 genotypes successfully rooted for the herbaceous cuttings and 101 for the hardwood cuttings, producing 93 shared genotypes. Rooting bins were filled with fritted clay (Profile Products LLC, Buffalo Grove Ill). We selected clay for its porosity, to prevent excess water retainment, hypoxia, and mold growth around the root crowns, and higher mechanical resistance to root growth than typical growth media (e.g., perlite), to simulate more realistic growing conditions. In total, we planted 10 replicates of each genotype and 20 replicates of each parent, split into 2 blocks. Within each block, five reps of each genotype were planted together in rooting bins, and their location in the block was randomized. All cuttings were grown in a humidified greenhouse under shade cloth, with natural day length and temperature between 10 and 27°C. Benches provided bottom heat, and water was provided with sprayers that misted the plants every 10 minutes throughout the duration of the experiment. We removed any dead plant material weekly to prevent mold.

1.3.2. Destructive harvest and imaging

We destructively harvested all cuttings after 35 days of growth. We excavated and washed the root systems in water to remove soil, working carefully to avoid root breakage. Excavated plants

were stored in humidified plastic bags with their roots in water until imaging later the same day. We imaged all plants using a backlit imaging setup adapted from Seethepalli et al., (2020). The imaging setup consisted of a box containing a backlight and a translucent background to provide a homogenous background for near binary silhouette images. We suspended root systems in front of the backlight at a consistent height and photographed the root systems at a consistent distance and angle with a Canon Rebel T3i DSLR camera. We included an object of known size in each image for scale. We imaged each cutting twice to average traits for two faces. The root system was first positioned so that the largest spread of roots was visible, then the cutting was rotated 90 degrees to obtain the second image. After imaging, the longest root was pulled straight and measured with a ruler. For hardwood cuttings, all roots and shoots for each genotype were cut off at the crown and bud and were dried at 70°C for at least 48 hours to measure biomass.

1.3.3. Image analysis and trait extraction

All image analysis was carried out in Python 3.9.20. First, we cropped each image to a consistent stem length, excluding the plant label. Then, we segmented images with an automated script using Gaussian adaptive thresholding from the OpenCV module, to produce binary images of each root system (Figure 1.1). The binary image was then skeletonized on the midline of each root, and a graph of the root system was created with the NetworkX package. Each node in the graph corresponds to a pixel on the midline in the binary image and has information on location, thickness, and connection to other nodes. During network creation, any roots with a total length shorter than approximately 1.2 mm were removed during the network creation to remove non-root artifacts such as bumps or curves on individual roots, and nodes with a diameter wider than 4.8 mm for hardwood cuttings and 4.2 mm for herbaceous cuttings were removed to eliminate the thick stem nodes from the root measurements.

We distinguished pixels of primary roots (adventitious roots originating from the cutting) from lateral root (daughters of the primary roots) based on user defined root thickness thresholds similar to other root image analysis software (WinRHIZO, RhizoVision Explorer) (Himmelbauer et al., 2004; Seethepalli et al., 2020). For 10 random individuals of different genotypes for each propagation method, we measured root thicknesses at 5 random points across multiple roots from each root order. The bottom quartile of the primary root thickness overlapped with the top quartile of the lateral root thickness for both hardwood and herbaceous cuttings, so we set a threshold width halfway between the minimum thickness for the primary roots and maximum thickness for the lateral roots (i.e., 0.82 mm for herbaceous cuttings and 0.90 mm for hardwood cuttings). Using the data from the 10 random individuals, we calculated the ratio of nodes that would have been miscategorized from the threshold, which varied from 0.059 to 0.116 (Supplementary Figure S 1.2).

We then used a custom automated script to measure root traits for the final networks. Using length, radius, and angle information for all the nodes in the root system network, the script estimated 12 traits: total length (sum of length for all nodes), total surface area (sum of length * π * diameter for all nodes), and total volume (sum of π * radius² for all nodes), primary and lateral root length, primary to lateral ratio (ratio of lateral to primary root length), whole root system median root diameter, primary and lateral median root diameter, number of root tips (number of terminal nodes), and root system fibrousness (number of root tips/total length) (Table 1.1). The average initiation angle was estimated by measuring the average angle of all nodes in a 4 cm range at the top of the image to include only the top portion of the root system, where zero degrees is vertical, and 90 degrees is horizontal. We also measured the rooting rate of each genotype (number of rooted cuttings/number of planted cuttings), where rooting was defined as

formation of roots longer than 3 cm determined from the length of the longest root. Altogether, this produced 14 or 17 traits for each individual root system, depending on the propagation method (Table 1.1).

1.3.4. Genetic map generation

Genomic DNA was extracted from young leaf samples at the University of Minnesota Genome center, and genotype by sequencing (GBS) was performed at the University of California, Davis Genome Center, using the Illumina NovaSeq 6000 platform with paired end (PE) 150 bp reads and an average sequencing depth of 10x, resulting in many markers with > 20x coverage.

TASSEL (Glaubitz et al., 2014) was used for variant calling with haplotype 1 of 110R as the reference genome (Minio et al., 2022). We removed markers that had less than 20x coverage, more than 2 alleles, were monomorphic, and were distorted as tested with chi square test at significance level of $p < 0.10$. Two genetic maps, one for 110R and another for 101-14, were constructed using a pseudo-test cross linkage mapping strategy (Grattapaglia & Sederoff, 1994) in R using the BatchMap and ABHgenotypeR packages (Schiffthaler et al., 2017).

Recombination fractions were estimated with rf.2pts, and genetic distances were obtained with the Kosambi mapping function, which was used throughout map construction. Markers were coded as lm×ll, nn×np, or hk×hk and passed to a custom imputation function as marker types D1.10, D2.15, and B3.7. The imputation function operates in two main steps. First, it builds approximate parental maps using BatchMap: it bins markers, estimates recombination fractions (rf.2pts), assigns markers to linkage groups (group), identifies the two largest pseudo-testcross groups, and orders markers with record.parallel and map.overlapping.batches to obtain preliminary marker order and linkage phase. Second, it uses these approximate phases to recode progeny genotypes into A/B states for a single parent, exports them in ABH format, and applies

ABHgenotypeR (imputeByFlanks, correctStretches) to impute missing data and correct spurious recombination events. After this correction, the cleaned genotypes are filtered for missing data and segregation distortion and then used for the final map calculation. Within our custom imputation routine, the BatchMap functions were executed using LOD = 8 and max.rf = 0.1 in rf.2pts and group, and max.dist = 10, size_batches = 10, and overlap = 5 when ordering markers via map.overlapping.batches. For the final map construction outside the imputation step, BatchMap was run with LOD = 10 in both rf.2pts and group, and marker ordering employed record.parallel(times = 10) followed by map.overlapping.batches(max.dist = 10, size = 15, overlap = 10, phase.cores = 10). All remaining parameters in these functions were kept at their default values. The two resulting parental maps were merged into a composite map for QTL mapping using LPmerge in R, using the LPmerge (maps) function with the default parameters (max.interval = 1:3 and equal weights for both maps).

1.3.5. Statistical analysis

All phenotype analysis was carried out with Python 3.9.20 using the packages *scipy stats*, *statsmodels api*, and *sklearn*. Heritability and QTL analysis was carried out in R and *R/QTL* (Broman et al., 2003). We cleaned the data by checking outliers and removing individuals with clear measurement error, which we defined as any individual with a trait value more than three z-scores away from the population mean. To test the assumptions for ANOVAs, we tested normality with the Anderson-Darling test and for homogeneity of variances with Levene's test. There were some deviations from the assumptions for ANOVA. Due to plant death the data were unbalanced, but for all traits either the ratio of variances for the largest and smallest group was within an acceptable range (< 3), or the variance was coupled with group size, which would

reduce statistical power rather than inflating type I error (Schielzeth et al., 2020). As a result, we continued with ANOVA analysis.

Unless specified otherwise, we conducted all analyses for all traits, and separately for the herbaceous and hardwood cuttings ($N = 113$ unique genotypes, 103 for the herbaceous cuttings, and 103 for hardwood cuttings). We inspected the effect of root system size, measured with total length, on each trait for each propagation method visually and with Pearson correlations and found the responses were similar across propagation methods (Supplementary Figure S 1.1, Table S 1.1, and Table S 1.2), so we continued with our analysis comparing propagation methods for all traits. We performed a Type III ANOVA with the propagation method as the main effect and genotype as second effect to test for differences between propagation method, and we included an interaction term. For the same traits, we performed T tests between parental genotypes for both propagation methods. To test associations between propagation methods for the shared genotype set, we calculated the mean value for each genotype and regressed the hardwood values on the corresponding herbaceous values for all traits.

We used pairwise linear regressions to test for trait correlations across genotypes. We found many significant correlations between traits, so we used principal component analysis (PCA) and unsupervised K-means clustering with three clusters to further interpret collinearity. Three clusters were chosen with the “elbow” test, by identifying the value of K where within-cluster-sum-of-squares values stopped decreasing quickly.

To estimate best unbiased linear predictions (BLUPs) and broad sense heritability for each propagation method we fit the following mixed effect model using the package lme4 in R

$$y_i \sim N(\alpha_{j|i} + \beta_1(block), \sigma^2)$$

$$\alpha_j \sim N(\mu_\alpha, \sigma_\alpha^2), \text{ for genotype } j = 1, \dots, j$$

{Equation 1}

where y_i is the measured trait value for the i^{th} individual, $\alpha_{j|i}$ is the random intercept associated with genotype j , σ^2 is the residual variance, μ_α and σ_α^2 are the mean and variance of the genotype random effects, and $\beta_1(block)$ is a fixed effect to account for the experiment blocking. We estimated broad sense heritability for each trait, H^2 , defined as the ratio of genotypic variance to phenotypic variance, excluding fixed effects (Visscher et al., 2008). Phenotypic variance, σ^2_P , can be split into the sum of genotypic (σ^2_G) and environmental (σ^2_E) variances, resulting in the calculation for broad sense heritability (H^2): $\sigma^2_G / (\sigma^2_G + \sigma^2_E)$. σ^2_P and σ^2_G were estimated from random effect variance and residuals of the model. We estimated confidence intervals for broad sense heritability using the `confint()` function on the mixed effect model output in R.

There were deviations in the normality of BLUPs for some traits, however data were not transformed because deviations were generally small, and traditional parametric methods are still effective despite some non-normality (Rebaï, 1997). For QTL mapping, we used BLUPs estimated from the random effects of the mixed effect model in Equation 1. To identify QTL, the whole genome logarithm of odds (LOD) significance threshold ($p < 0.05$) was determined with permutation ($n = 1000$). Each trait was mapped to a composite map of both parents using the `scanone` function in `r/QTL` with Haley Knott regression as a four-way cross. We used the 110R reference genome browser (Minio et al., 2022) and BLAST (Priyam et al., 2019; Altschul et al., 1997, Altschul et al., 2005) to identify the closest gene to the QTL peak and any associated proteins.

1.4. Results

1.4.1. Population and parental traits are significantly different across genotypes and propagation methods

All the root architecture traits were significantly different across genotypes in the F1 population, for both the hardwood and herbaceous cuttings (ANOVA, $p < 0.001$) (Table 1.2). There were significant interactions between root system architecture and propagation methods for all measured traits (Type III ANOVA, $p < 0.001$), and 7 out of 14 traits showed significant differences between propagation methods ($p < 0.05$, Table 1.2). Hardwood root systems were generally more fibrous, and had higher surface area and volume, more root tips, thicker primary roots, and shallower angles (Figure 1.2). Contrary to expectation, none of the traits were significantly correlated between the hardwood and herbaceous cuttings ($r^2 = 0.0004 - 0.03$, $p > 0.05$, $N = 93$) (Table 1.3).

1.4.2. Differences between parental phenotypes varies between propagation methods

Greater differences between 110R and 101-14, the parental genotypes, were seen with herbaceous cuttings ($p < 0.05$ - t-test). The only significant difference between parental phenotypes for hardwood root systems was primary root thickness, with 110R having thicker primary roots ($p < 0.001$). Herbaceous root systems had 7 traits with significant differences between parents ($p < 0.05$). For herbaceous cuttings, 110R had less fibrous root systems with shorter total lateral root length, steeper rooting angles, thicker primary roots, and thinner lateral roots. (Table 1.2, Figure 1.3)

1.4.3. Most root architecture traits were correlated within each propagation method

Most of the root traits were significantly but weakly correlated across genotypes, in both the hardwood and herbaceous treatments ($|r|$ 0.19 – 0.99, $p < 0.05$) (Supplementary Tables S 1.1 and S 1.2, Figure S 1.1). Positive significant trait correlations ($r = 0.19 - 0.99$), accounted for most of the correlations, except for negative correlations between root thickness traits and plant size traits (total length, total area, number of root tips, length of the longest root, primary root length, and lateral root length) as well as lateral to primary ratio ($r = -0.22 - -0.83$). The strongest relationships ($|r| > 0.7$) were positive correlations between a similar set of traits related to plant size (total length, total area, total volume, number of root tips, length of the longest root, primary root length, and lateral root length), and between traits that were functions of each other. Rooting rate was the least collinear with the other traits, with the fewest number of significant associations compared to all other traits.

Principal component analysis (PCA) allowed for closer investigation of collinearity of measured traits. The first four principal components accounted for 84% and 91% of total variance for hardwood and herbaceous cuttings, respectively (Figure 1.4). Principal component loadings for PC1 and PC2 were similar across propagation methods (Supplementary Figure S 1.3).

PC1 showed strong loadings for plant size traits such as total length, number of root tips, and length of the longest root, while other traits contributed to a lesser extent. PC2 was mainly characterized by median diameter, median diameter of primaries, fibrousness, and lateral to primary ratio, however fibrousness contributed positively to hardwood root systems and negatively to herbaceous root systems. In general, PC3 and PC4 differed more significantly between propagation methods with limited contributions of total plant size. For herbaceous root systems, PC3 showed a higher contribution from average angle and root thicknesses, while PC4

was almost entirely dominated by rooting rate. For hardwood root systems, PC3 and PC4 showed contributions mainly from median diameter of laterals and rooting rate, and PC3 also had significant contributions from fibrousness (Supplementary Figure S 1.3). K-means cluster analysis of the first two principal components with three clusters placed F1 parents in the same cluster for hardwood cuttings (2), and in different clusters for herbaceous cuttings (0, 1) (Figure 1.4)

1.4.4. Environmental and genotypic variance differs between propagation methods

We calculated broad sense heritability for 13 root traits across both propagation methods. Broad sense heritability was generally low, and H^2 ranged from 0.08 to 0.34 for hardwood cuttings and 0.12 to 0.33 for herbaceous cuttings. Traits closely related to overall plant size had higher heritability than traits related to root thickness and angle (Table 1.4). Heritability stayed relatively constant across propagation methods for all traits. While heritability was estimated to be similar across propagation type, both genotypic and environmental variance was higher in the hardwood population.

1.4.5. Quantitative trait loci map to a variety of traits

Genotype by sequencing yielded 304,741 raw markers before filtering. Initial quality control, filtering, and binning redundant markers produced 1017 and 1001 unique markers for 110R and 101-14, respectively. After merging the parental maps from 367 common anchor markers, the final composite map contained 925 markers. The average distance between markers was 0.921 cM, the largest gap was 14.01 cM, the total map size was 834.31 cM, and the mean chromosome length was 43.91 cM.

Across both propagation methods, we identified two QTL for two unique root traits ($p < 0.05$) (Table 1.5). QTL were located on chromosomes 3 and 4 (Figure 1.5). We detected one QTL for each propagation method. We identified a QTL for average angle on chromosome 3 for herbaceous cuttings with a peak at 8.12 Mb and a LOD score of 4.50. We also identified a QTL for median diameter on chromosome 4 for hardwood cuttings with a peak at 2.1 Mb and a LOD score of 5.48. After examining the nearest gene to each peak for chromosome 3 and 4 respectively, we identified genes and associated protein.3167340 (8,122,329 Mb, Chr 3) and protein.3168957 (2,052,887 Mb, Chr 4), uncategorized proteins in the Vitis Interspecific cross - 110R V1.0 protein database (Minio et al., 2022; Priyam et al., 2019). There were closely related proteins identified in *V. vinifera* for both chromosome 3 (hypothetical protein VITISV_009065; 96.1% similarity), and chromosome 4 (hypothetical protein VITISV_008123; 93.5% similar), though both were identified as hypothetical proteins (Altschul et al., 2005).

1.5. Discussion

Results from the current study show novel QTL in grapevine root traits with useful genetic backgrounds for breeding. We also observed unexpected differences in root phenotypes between propagation methods, which provide more context for interpretation of root studies in the future. This data may assist breeding as well as guide future studies on grapevine root systems.

1.5.1 Novel QTL carry biological significance

Of the two identified significant QTL, we did not find any colocalization with previously mapped QTL or genes related to root traits from the literature, suggesting that the QTL are novel (Alahakoon & Fennell, 2023; Bert et al., 2013; Blois et al., 2023; Mohtasebi, 2023; Smith, 2010; Tandonnet et al., 2018; Thapa, 2022). The traits with associated QTL, median diameter and average angle, are both traits that carry biological significance.

Root thickness, measured as median root diameter, may indirectly impart drought resistance through increased growth rates and penetration ability. Root thickness is associated with improved soil penetration that allows for continued root growth despite dry or hard soil (Materechera et al., 1991, 1992). Fast growing, exploratory roots also tend to be thicker, which may assist in creating an extensive, more drought resistant root system (Lynch, 2013; Materechera et al., 1991; Richards, 1983). In grapevine, root thickness has been shown to be significantly greater in drought resistant 110R than susceptible 101-14 in pots (Reingwirtz et al., 2021), which our results corroborated (Table 1.2, Figure 1.3). Though previous studies have found relationships between thick roots and drought resistance, especially in annual crops (Klein

et al., 2020; Lynch, 2013), the effect of root thickness on long term root architecture and drought resistance in grapevine is still unknown.

Rooting angle has been extensively studied in other crops as a means of improving drought resistance (Guo et al., 2024; Lynch, 2013; Trachsel et al., 2013; Uga et al., 2013) and in a previous study of grapevine, the drought resistant rootstocks Ramsey and 110R showed steeper root growth angles in rhizotrons than the susceptible varieties Riparia Gloire and 101-14 (Fort et al., 2017). These results indicate rooting angle is likely an important factor in drought resistance, however, the effects of root initiation angle from cuttings on long-term development of RSA of field-grown grapevine is also still unknown. The effect that root angle has on grapevine is likely more complicated than in annual crop plants due to long lived root systems and root system plasticity from year to year (Smart et al., 2006; Soar & Loveys, 2007).

After investigating the closest gene to each peak, we found they were associated with uncategorized proteins in the 110R protein database. After using BLAST to explore potential protein function, we found the identified proteins were similar (93.5% and 96.1%) to hypothetical proteins present in *V. vinifera*. Hypothetical proteins are proteins that are expected to be expressed from the sequence, however there is yet to be any experimental evidence of translation (Desler et al., 2009). To robustly identify candidate genes, more statistical power and populations that capture more recombination events are needed (e.g. genome-wide association studies) to reduce confidence intervals (Myles et al., 2009), so we did not expand our analysis beyond identification of the closest genes to the QTL peak.

1.5.2. Differences between propagation methods guides results interpretation of future studies

Both propagation methods have been used extensively in scientific studies, and our results more clearly highlight the scope of each propagation method. Significant differences and interaction between genotype and propagation method mean that studies using herbaceous cuttings may not be able to be directly applied to management or breeding for rootstocks. More research must be done to determine if herbaceous traits translate to hardwood propagation.

Because nurseries use hardwood propagation for commercial production of plant material, studies focused on direct management or the selection for advantageous root traits should use hardwood propagation whenever possible. While hardwood propagation may be preferable in many situations, we observed higher environmental variance in hardwood cuttings, likely due to differences in carbohydrate stores in stems affecting root system size (Larrey et al., 2025; Smart et al., 2006). Noisy data in the current study reduced power for QTL mapping and comparisons between genotypes and propagation methods, so care should be taken when developing a mapping population to reduce environmental variance wherever possible. Maintaining greenhouse populations for hardwood cuttings that are rotated in and out of dormancy may better control differences in cane size and development and control for variable soil, weather, or pest damage.

While interpretation of hardwood propagation is easier, despite similar heritability between propagation method, herbaceous cuttings showed clearer differences between the parent genotypes (Table 1.2). These genotypes are known to have different drought performance in the field and different root system architecture in adventitious root systems (Cuneo et al., 2021; Hnizdor, 2023; Reingwirtz et al., 2021; Zhang et al., 2016). This agrees with the results of the PCA K-means clustering, which placed parents in different clusters for herbaceous, but the same

for hardwood. These results suggest that more homogenously sized cuttings, and the ability for all genotypes to photosynthesize throughout the entire process may have reduced the dependence on carbohydrate stores to develop an adventitious root system, resulting in lower environmental variance. While herbaceous propagation may be less suitable for direct applications to management or breeding, higher flexibility and lower environmental variance could be utilized for physiological or more basic science, like candidate gene studies.

1.5.3. Trait complexity shapes future studies and breeding

These genetic results provide novel QTL that indicate this parentage may be able to provide advantageous alleles for root architecture traits that facilitate soil exploration and water uptake. Transgressive segregation, phenotypes of progeny that are more extreme than the parental phenotypes, has been successfully leveraged in the past to make gains in plant breeding (Knapp et al., 2024; Rieseberg et al., 1999). Our traits showed variable transgressive segregation for all traits except for fibrousness in herbaceous cuttings, where 110R, one of the parents of the cross, was the most extreme phenotype (Figure 1.2). The individuals with more extreme phenotypes harbor alleles that could be useful for future breeding efforts and advance the drought resistance of rootstocks within this well-established, high performing ancestry while working on longer term projects, such as trait introgression from other genetic backgrounds.

Several previous successful family mapping studies for root traits in grapevine used crosses between *V. riparia* and *V. vinifera* (Alahakoon & Fennell, 2023; Bert et al., 2013; Tandonnet et al., 2018). *V. riparia* is not known for its drought resistance (Rahemi et al., 2022), which could limit the applicability of these markers for breeding rootstock drought resistance. The QTL identified in the current study are from progeny derived from *V. rupestris*, *V. berlandieri*, and *V.*

riparia, the most commonly used parents for rootstock breeding, which could make them more widely applicable for selecting grapevine accessions (Myles et al., 2009).

Though the studied population shows promise for breeding for root traits, there are some limitations to utilize the QTL identified in this study. This study compared two propagation methods, but the experiment was not replicated in a second year, so the QTL identified would need to be verified further before use in breeding programs. Just as rootstock selection affects vigor of the scion, previous research has shown scion selection significantly alters root diameter, density, fine root ratio, and biomass allocation of grafted rootstocks (Ferlito et al., 2020; Tandonnet et al., 2009). Interactions like these between scion and rootstock can result in unexpected outcomes, such as the drought resistant rootstock 1103P showing canopy collapse under drought due to excess vigor (Smart et al., 2012). Additionally, the direct effects of root traits from young plants (35 days old) on drought resistance and how the traits scale to the field is still largely unknown, especially for traits with low heritability, high plasticity, and complicated effects on physiology (Bernardo et al., 2025; Smart et al., 2006). Drought treatments in conjunction with QTL identification could be a valuable avenue to explore in the future to directly link QTL and root traits to physiological indicators of drought resistance.

While recent studies have been successful at mapping traits with smaller effect sizes ($< 10\%$), QTL mapping for root system traits has had mixed success (Mohtasebi, 2023; Schmitz, 2023; Smith, 2010; Thapa, 2022). In our study, low population size, collinearity of traits with plant size, high root system plasticity, and low heritability only allowed us to observe a small number of QTL with effect sizes greater than 15%. This leads us to believe that our results agree with previous findings that root system architecture of grapevine is a complex polygenic trait, with most QTL explaining less than 20% of variation (Alahakoon & Fennell, 2023). Marker assisted

selection, while useful for QTL with larger effect sizes, may not be as helpful for many small effect markers (Bhat et al., 2016). Future research on the genetics of RSA of grapevine should include higher throughput phenotyping methods to improve statistical power. Additionally, the root system of young plants may be more affected by differences in root-strike, so plants should be as well-developed as reasonably possible. Genomic selection models combined with higher throughput phenotyping methods may be a good route in the future to make selection for root traits a reality in grapevine breeding programs (Seyum et al., 2022).

1.6. Conclusion

This study identified quantitative trait loci and the effect of propagation method on the adventitious root system architecture in a diverse *Vitis* population derived from rootstocks 110R and 101-14 MGT. The two significant identified QTL occurred in traits with biological significance for drought resistance. Significant differences and interaction between genotype and propagation method shows that propagation method affects interpretation of results from scientific studies, and each propagation method has a scope of use. Genetic control of RSA is complex and multigenic, so future efforts for breeding should focus on genomic selection models and higher throughput phenotyping to improve power of QTL and GWAS studies.

1.7. Tables

Table 1.1. Measured traits, abbreviations, and units

Trait	Abbreviation	Units	Dataset
Total length	TL	mm	HW, HB
Total area	TA	mm ²	HW, HB
Total volume	TV	mm ³	HW, HB
Number of root tips	RT	unitless	HW, HB
Average angle	AA	degrees	HW, HB
Median diameter	MD	mm	HW, HB
Fibrousness	F	mm ⁻¹	HW, HB
Median diameter primaries	MDP	mm	HW, HB
Median diameter laterals	MDL	mm	HW, HB
Primary root length	PRL	mm	HW, HB
Lateral root length	LRL	mm	HW, HB
Lateral to primary ratio	LPR	unitless	HW, HB
Length of longest root	LR	mm	HW, HB
Pooled cutting mass	CM	g	HW
Pooled leaf mass	LM	g	HW
Pooled root mass	RM	g	HW
Rooting rate	RR	unitless	HW, HB

Dataset column shows what traits were measured for each propagation method; HW signifying hardwood propagation and HB signifying herbaceous propagation.

Table 1.2. Population and parental trait values

Trait	Population trait means		Parental Hardwood means		Parental Herbaceous means	
	Hardwood	Herbaceous	110R	101-14	110R	101-14
Total length	825.6 ±31.8	598.5 ±20.4	1169.7 ±147.5	1557.4 ±189	655 ±54.5	704.8 ±100.3
Total area	3116.9 ±111 *	2350.6 ±69.8 *	5011.5 ±598.5	5915.8 ±636.1	2756.7 ±209.1	2516.4 ±293.8
Total volume	1339.6 ±43.7 **	1010 ±27.5 **	2415.9 ±285.3	2477.5 ±247.8	1173.7 ±80.1	1028.7 ±88.6
Number of root tips	89.4 ±4 *	50.6 ±2.1 *	135.4 ±17.3	177.3 ±24.5	42.3 ±4.6	56.1 ±8.9
Average angle	49.7 ±0.2 **	47.2 ±0.3 **	51.8 ±1.2	51.3 ±0.7	43.4 ±0.64 ***	48.9 ±1.01 ***
Median diameter	1.08 ±0.002	1.05 ±0.004	1.21 ±0.07	1.06 ±0.04	1.18 ±0.024 ***	0.91 ±0.021 ***
Fibrousness	0.106 ±0.0013	0.084 ±0.0011	0.114 ±0.004	0.11 ±0.003	0.063 ±0.003 ***	0.078 ±0.004 ***
Median diameter primaries	1.422 ±0.02 *	1.295 ±0.01 *	1.62 ±0.04 ***	1.354 ±0.03 ***	1.306 ±0.03 ***	1.138 ±0.024 ***
Median diameter laterals	0.638 ±0.015	0.62 ±0.011	0.627 ±0.006	0.634 ±0.007	0.61 ±0.018 *	0.657 ±0.011 *
Primary root length	485.5 ±17.8	424.3 ±13	734.9 ±88.8	956.8 ±101.2	541 ±44.7	443.3 ±53.9
Lateral root length	340.1 ±15.3	174.2 ±8.9	434.8 ±63.1	600.6 ±93.3	114 ±15.8 **	261.4 ±50.4 **
Lateral over primary ratio	0.63 ±0.018 *	0.4 ±0.015 *	0.57 ±0.06	0.6 ±0.05	0.21 ±0.03 ***	0.56 ±0.05 ***
Length of the longest root	105.1 ±2.4	97.9 ±2.1	131.5 ±11.9	133.8 ±9.7	109 ±5.7	108 ±7.4

Significance determined by ANOVA for propagation method, and t test for parental differences (Significance: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$). Errors were calculated as standard error of the mean.

Table 1.3. Regression results between propagation methods on the set of common genotypes

Trait	r	R ²	p value
Lateral root length	-0.145	0.021	0.167
Length of the longest root	0.037	0.001	0.723
Average angle	0.131	0.017	0.212
Lateral over primary ratio	0.029	0.001	0.781
Total area	-0.132	0.017	0.207
Total length	-0.132	0.017	0.208
Rooting rate	-0.156	0.024	0.137
Primary root length	-0.087	0.008	0.407
Total volume	-0.122	0.015	0.242
Number of root tips	-0.159	0.025	0.128
Median diameter laterals	0.1	0.01	0.342
Fibrousness	0.019	0	0.858
Median diameter primaries	0.1	0.01	0.341
Median diameter	0.092	0.008	0.382

Table 1.4. Broad sense heritability of root traits

Trait	Broad sense heritability H^2	
	Hardwood	Herbaceous
Total length	0.31 (0.22-0.41)	0.29 (0.21-0.40)
Total area	0.32 (0.23-0.43)	0.30 (0.21-0.40)
Total mass	0.34 (0.24-0.44)	0.32 (0.23-0.42)
Root tips	0.31 (0.22-0.41)	0.33 (0.24-0.43)
Average angle	0.17 (0.10-0.25)	0.22 (0.15-0.32)
Median diameter	0.23 (0.15-0.32)	0.29 (0.20-0.39)
Fibrousness	0.14 (0.08-0.22)	0.20 (0.13-0.29)
Median diameter primaries	0.18 (0.11-0.27)	0.12 (0.06-0.20)
Median diameter laterals	0.08 (0.03-0.14)	0.18 (0.11-0.26)
Primary root length	0.33 (0.24-0.43)	0.28 (0.20-0.38)
Lateral root length	0.30 (0.21-0.4)	0.29 (0.21-0.39)
Lateral over primary	0.23 (0.15-0.32)	0.23 (0.15-0.32)
Longest root	0.26 (0.18-0.36)	0.23 (0.15-0.32)

Heritability presented with 95% confidence intervals.

Table 1.5. Mapped quantitative trait loci

Trait	Chrom.	Position (cM)	Physical position (Mb)	LOD	CI Low (Mb)	CI High (Mb)	% var. explained	Prop. method
Average angle	3	20	8.12	4.5	3.78	9.17	18.6	Herbaceous
Median diameter	4	2.7	2.1	5.48	0.54	2.87	22.1	Hardwood

1.8. Figures

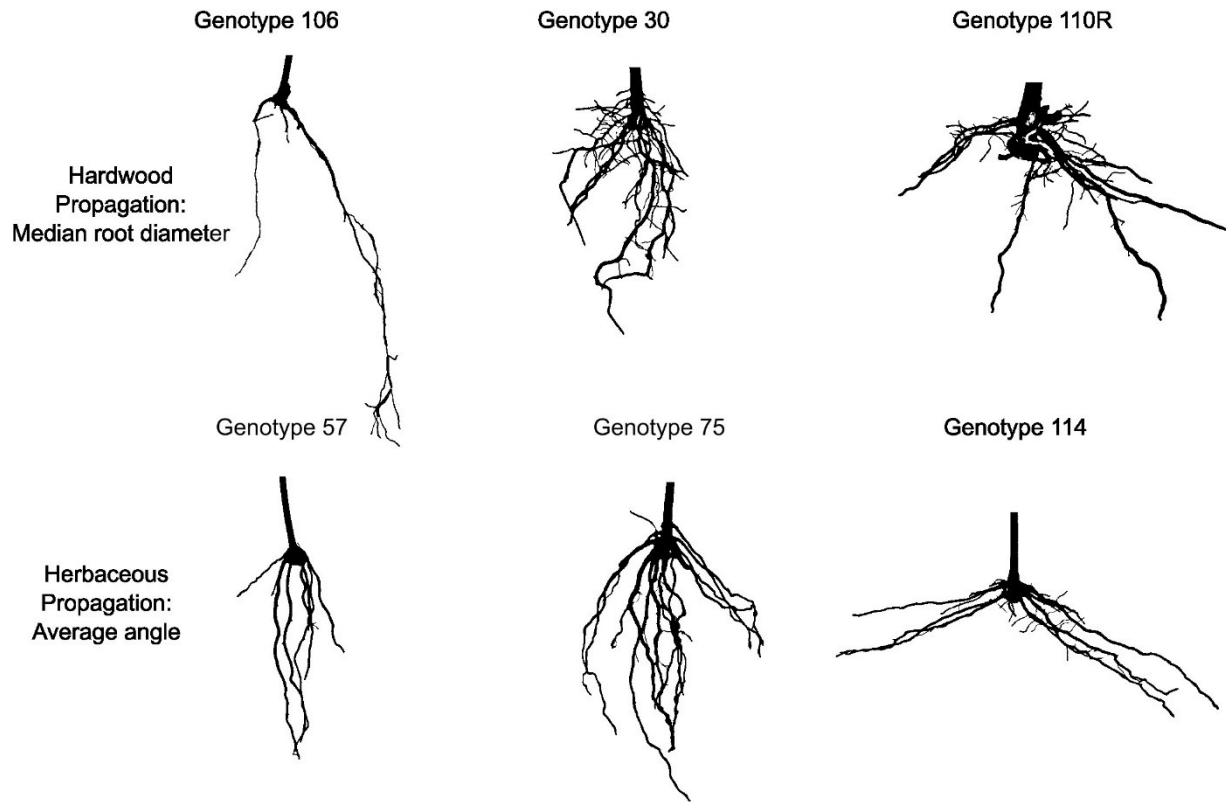


Figure 1.1. Segmented root system images

Segmented pictures of various root systems after 35 days of growth. Hardwood root systems on the top row represent an average phenotype (middle), and two extreme genotypes for median root diameter. Herbaceous root systems on the bottom row represent an average phenotype (middle) and two extreme phenotypes for average root initiation angle. A representative individual of the mean of each genotype was chosen for each image.

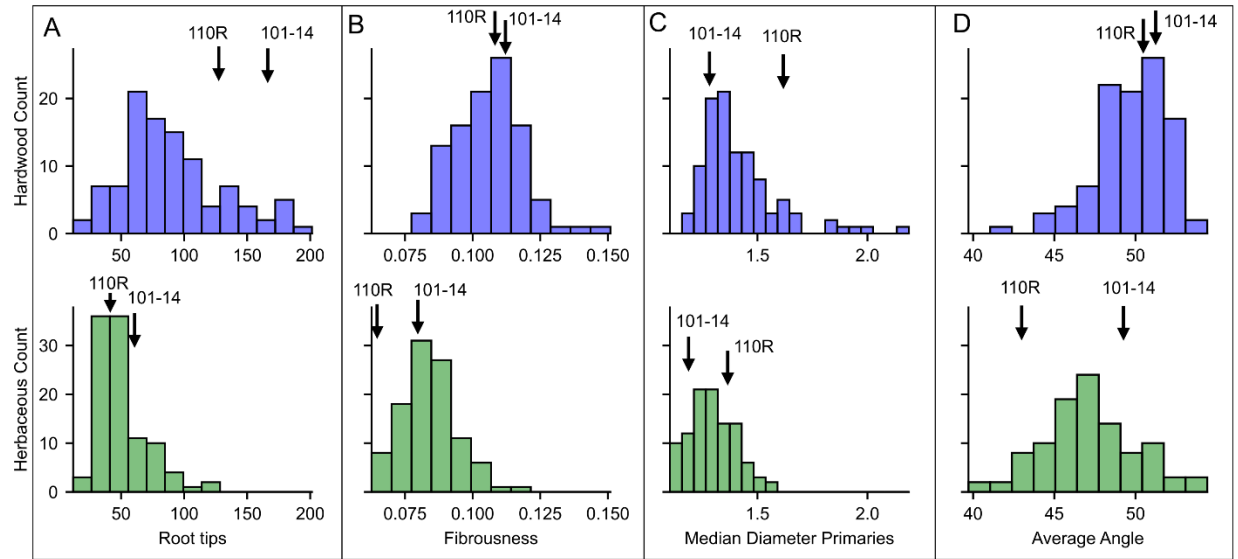


Figure 1.2. Population trait distributions

Whole population histogram comparisons between genotypic means for each propagation method. Histograms are shown for a subset of four important root traits. The height of the bars indicates the number of genotypes in each bin. Mean of each parent is indicated for each trait and propagation method. Panels correspond to the following traits: (A) Root tips (B) Fibrousness (C) Median diameter (D) Average angle.

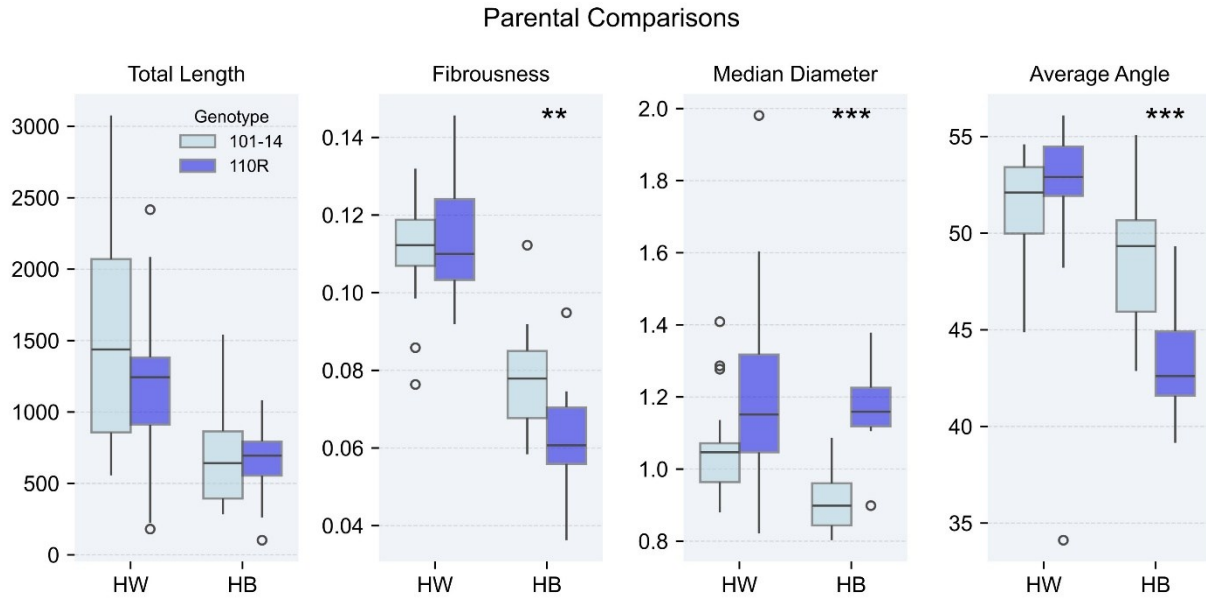


Figure 1.3. Parental trait distributions

Box plots comparing parental phenotypes for each propagation method for a subset of four important root traits. HW signifies hardwood propagation and HB signifies herbaceous propagation. Significance is indicated by asterisks, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (two-sided t-test).

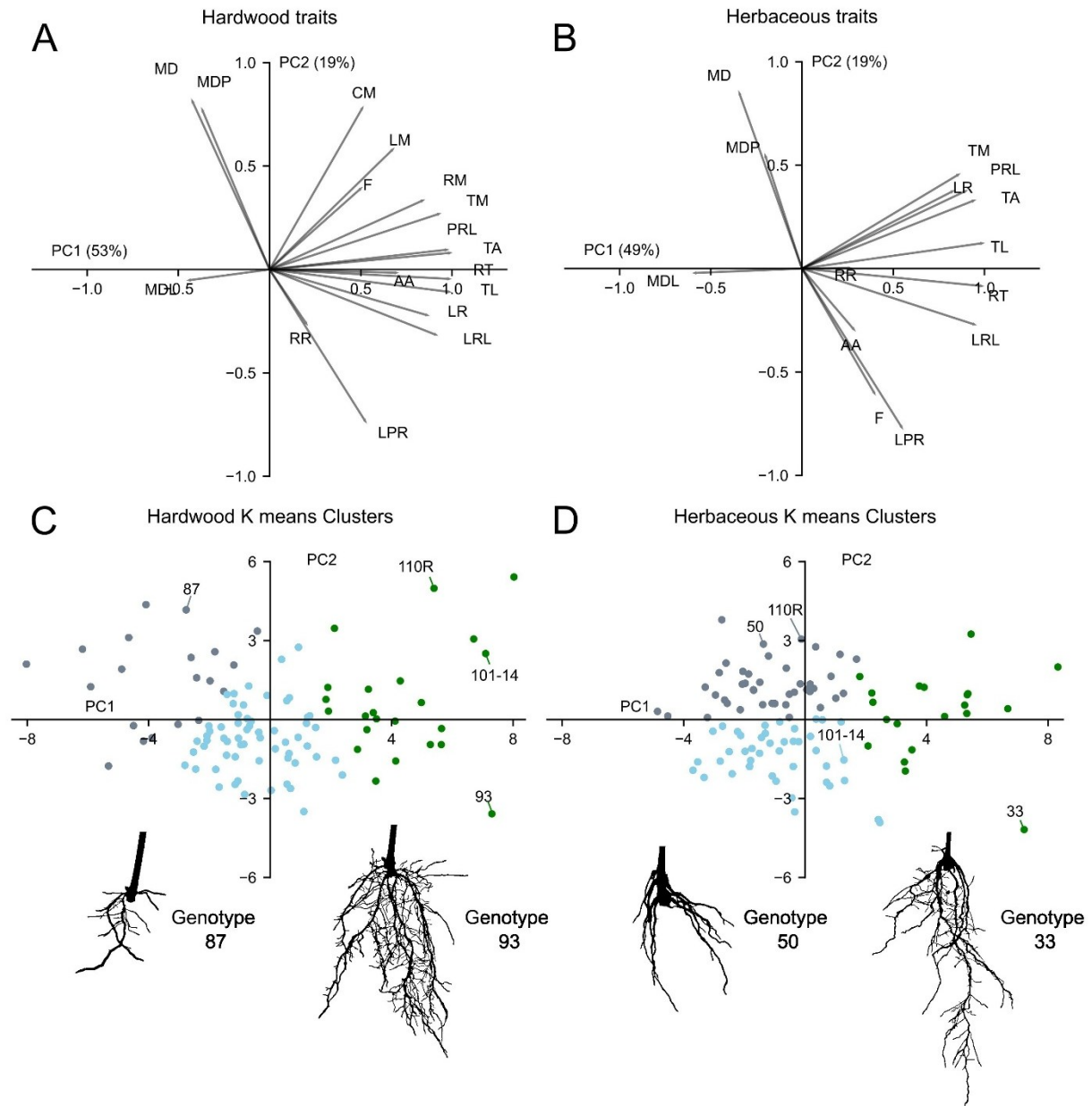


Figure 1.4. Principal component analysis

Principal component analysis (PCA) showing biplots and cluster analysis for both propagation types. (A, B) PCA biplot for hardwood and herbaceous traits with vectors showing loadings for the first two principal components. Trait abbreviations are: total length (TL), total area (TA), total volume (TV), number of root tips (RT), average angle (AA), median diameter (MD), fibrousness (F), median diameter primaries (MDP), median diameter laterals (MDL), primary root length (PRL), lateral root length (LRL), lateral to primary ratio (LPR), length of the longest root (LR), pooled cutting mass (CM), pooled leaf mass (LM), pooled root mass (RM), rooting rate (RR). (C, D) The K-means cluster analysis results of hardwood and herbaceous root systems. Representative binary images of root systems that differ across both PC axes are labeled on the cluster analysis to show contrasting phenotypes are shown below. Different colors represent different clusters.

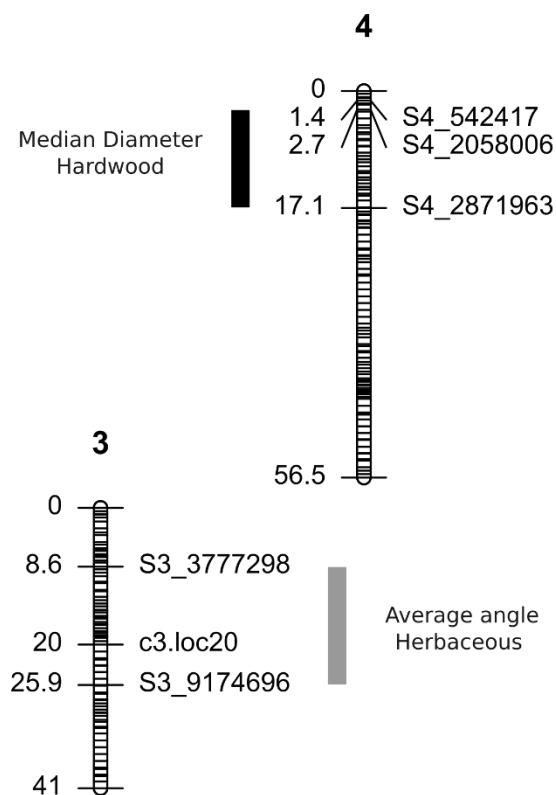


Figure 1.5. Mapped QTL

Mapped QTL at whole genome $p < 0.05$ significance. Peak and confidence interval markers for each QTL are marked on each linkage group. Bars beside each linkage group signify QTL confidence interval. Distances are in Centimorgans (cM).

1.9. References

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1.10. Supplemental materials

Table S 1.1. Hardwood trait correlations: *r* value

43	Trait	TL	TA	TV	RT	AA	MD	F	MDP	MDL	PRL	LRL	LPR	LR	CM	LM	RM
	Total length (TL)	-															
	Total area (TA)	0.97	-														
	Total volume (TV)	0.87	0.96	-													
	Root tips (RT)	0.99	0.96	0.88	-												
	Average angle (AA)	0.61	0.61	0.56	0.6	-											
	Median diameter (MD)	-0.45	-0.32	-0.18	-0.42	-0.29	-										
	Fibrousness (F)	0.42	0.44	0.41	0.5	0.42	0.08	-									
	Median diameter primaries (MDP)	-0.41	-0.3	-0.15	-0.36	-0.28	0.81	0.1	-								
	Median diameter laterals (MDL)	-0.38	-0.37	-0.35	-0.4	-0.31	0.11	-0.3	-0.03	-							
	Primary root length (PRL)	0.96	0.99	0.95	0.95	0.61	-0.3	0.44	-0.32	-0.34	-						
	Lateral root length (LRL)	0.95	0.86	0.72	0.94	0.56	-0.58	0.36	-0.47	-0.39	0.83	-					
	Lateral to primary ratio (LPR)	0.56	0.4	0.23	0.53	0.39	-0.83	0.07	-0.61	-0.31	0.34	0.74	-				
	Longest root (LR)	0.86	0.84	0.75	0.82	0.5	-0.48	0.2	-0.45	-0.33	0.82	0.83	0.6	-			
	Cutting mass (CM)	0.37	0.53	0.67	0.42	0.33	0.33	0.5	0.33	-0.23	0.53	0.18	-0.26	0.27	-		
	Leaf mass (LM)	0.54	0.67	0.77	0.6	0.41	0.07	0.54	0.12	-0.26	0.66	0.38	-0.03	0.4	0.85	-	
	Root mass (RM)	0.76	0.84	0.86	0.77	0.59	-0.09	0.41	-0.08	-0.29	0.83	0.61	0.16	0.67	0.65	0.72	-
	Rooting rate (RR)	0.22	0.18	0.14	0.2	0.17	-0.19	0.1	-0.25	-0.07	0.18	0.23	0.19	0.08	-0.13	0.02	0.01

The Pearson correlation coefficient is presented. Bold indicates a significant association ($p < 0.05$).

Table S 1.2. Herbaceous trait correlations: *r* value

Trait	TL	TA	TV	RT	AA	MD	F	MDP	MDL	PRL	LRL	LPR	LR
Total length (TL)	-												
Total area (TA)	0.97	-											
Total volume (TV)	0.88	0.97	-										
Root tips (RT)	0.94	0.88	0.81	-									
Average angle (AA)	0.19	0.13	0.08	0.25	-								
Median diameter (MD)	-0.28	-0.08	0.07	-0.37	-0.17	-							
Fibrousness (F)	0.24	0.14	0.13	0.52	0.26	-0.44	-						
Median diameter primaries (MDP)	-0.22	-0.07	0.09	-0.19	-0.1	0.74	0.02	-					
Median diameter laterals (MDL)	-0.47	-0.45	-0.42	-0.56	-0.37	-0.04	-0.4	-0.24	-				
Primary root length (PRL)	0.95	0.98	0.93	0.82	0.11	-0.07	0.02	-0.16	-0.37	-			
Lateral root length (LRL)	0.89	0.78	0.65	0.95	0.28	-0.52	0.52	-0.28	-0.52	0.71	-		
Lateral to primary ratio (LPR)	0.42	0.24	0.1	0.58	0.3	-0.78	0.69	-0.34	-0.4	0.14	0.75	-	
Longest root (LR)	0.84	0.85	0.78	0.72	0.12	-0.01	-0.03	-0.03	-0.48	0.85	0.67	0.2	-
Rooting rate (RR)	0.24	0.23	0.23	0.21	0.03	-0.14	0.08	-0.15	-0.07	0.25	0.17	0.03	0.13

The Pearson correlation coefficient is presented. Bold indicates a significant association ($p < 0.05$).

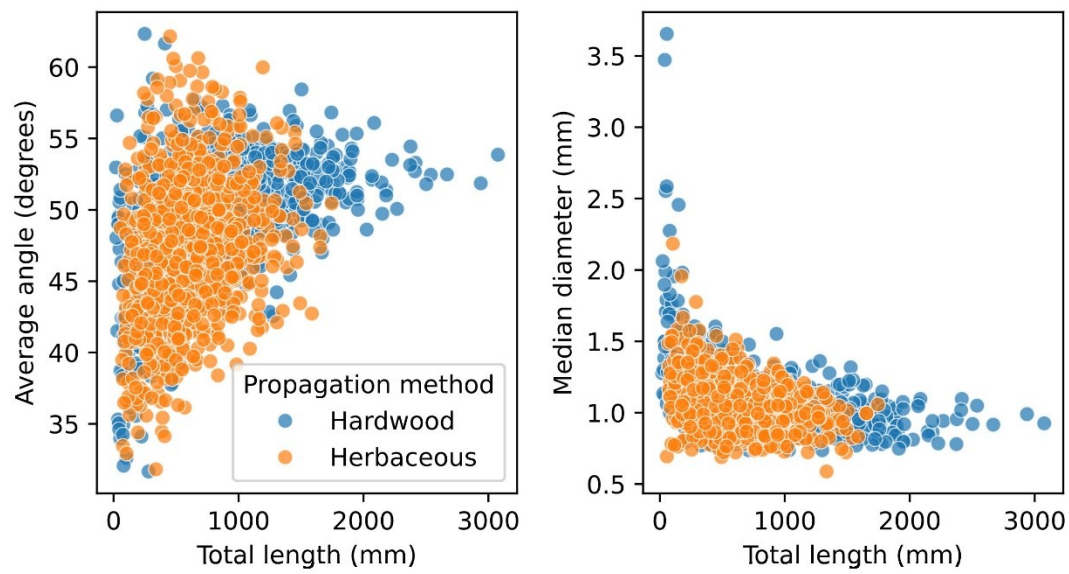


Figure S 1.1. Trait associations with root length

Scatterplots of the relationship between total length and the mapped QTL traits, average angle and median diameter, for individuals across both propagation methods.

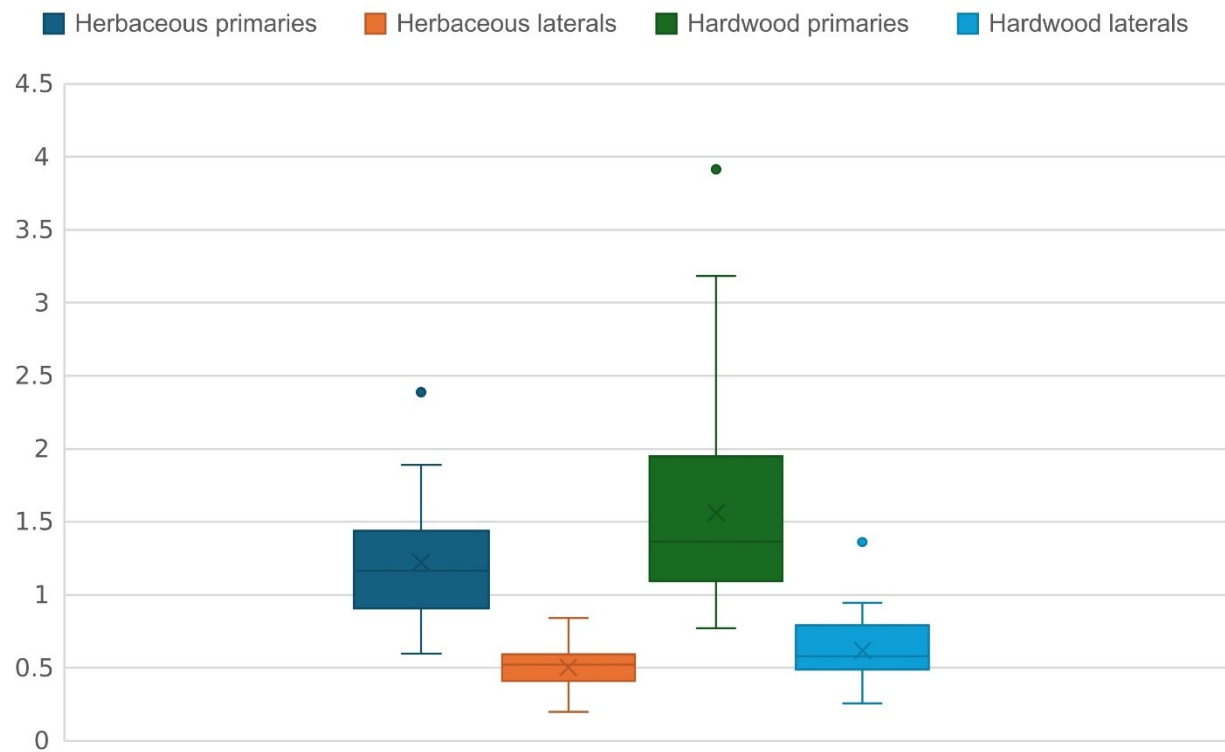


Figure S 1.2. Categorization of root order

Boxplots of widths of each root type, in mm. Rates of mis-categorization for each group are as follows: Herbaceous lateral: 0.059, Herbaceous adventitious: 0.114, hardwood lateral: 0.116, hardwood adventitious: 0.065.

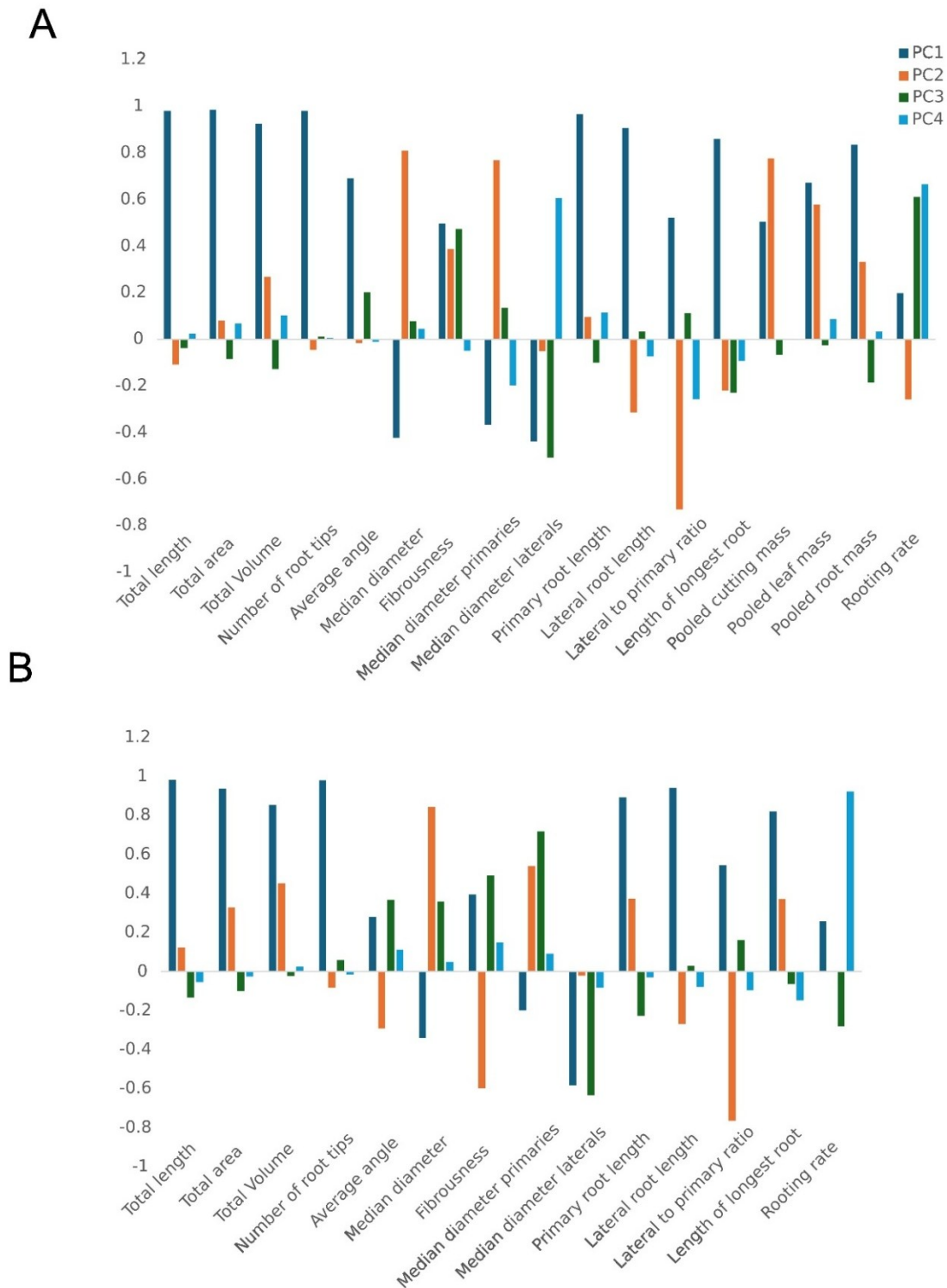


Figure S 1.3. PCA loadings

Principal component analysis loadings for principal components 1 – 4 for both propagation methods. (A) Hardwood principal component loadings (B) herbaceous principal component loadings.

Chapter 2: Root Growth and Architecture Traits Predict Plant and Agronomic Drought Resistance in Grapevine

2.1. Abstract

Many agricultural regions are facing increasing drought, and breeding more drought resistant rootstocks is a promising strategy to maintain production of woody perennial crops. These efforts have been limited by a lack of information about how root traits translate to plant drought performance. We used neutron radiography to measure root system traits for two groups of young grapevines to capture variation in root traits: seven commercial rootstocks with known drought resistance from prior field trials, and 14 genotypes from an F1 cross. We tested which traits were associated with drought resistance in the commercial rootstocks, and whether these traits predicted drought performance for the new genotypes and parents in a greenhouse drought experiment. In the commercial rootstocks, steeply angled roots, roots that tended to grow downward, and roots that grew preferentially in wetter soil were associated with drought resistance. However, drought performance in the F1 genotypes was unrelated to these traits and was instead associated with long and fast-growing 2nd order roots, which were generally absent in the commercial rootstocks. These results identify multiple traits that could be leveraged to breed more drought resistant rootstocks and demonstrate that root traits can be predictive of plant and agronomic drought resistance.

2.2. Introduction

Grapes are one of the world's most valuable horticultural crops, but increased severity and frequency of drought pose new challenges to agriculture in regions with mediterranean climates, reducing yield and quality of grapes (Arora, 2019; Cantu & Walker, 2019; Del Pozo et al., 2019; Hannah et al., 2013). In the past, drought resistant rootstocks have been successfully utilized to maintain yield and quality despite limited water (Chen et al., 2024; Ollat et al., 2019).

Development of new rootstock varieties has been used to improve tolerance to biotic and abiotic factors such as lime and phylloxera, but has not focused on resistance to drought (Ferris et al., 2012; Nesbitt, 1974). Developing new rootstock varieties specifically with drought resistance in mind, while a relatively new idea, holds promise (Bernardo et al., 2025; Ollat et al., 2019).

Rootstocks that consistently provide water throughout the season impart drought resistance to their scion, allowing them to avoid low water potentials (Serra et al., 2014; Zhang et al., 2016).

Root systems can achieve this through altering hydraulic architecture through fine root responses and root growth (Alsina et al., 2011; Barrios-Masias et al., 2015; Bauerle et al., 2008; Comas et al., 2005; Gambetta et al., 2013) or the architecture of the structural root system established by long lived woody roots (Bernardo et al., 2025; Fort et al., 2017). While the exact effect of root traits on the drought resistance of grapevine is still unknown and interactions between traits and environments complicates phenotypes, some traits are hypothesized to be especially advantageous under drought. From previous research in annual crops and traits observed in drought resistant rootstocks, deep root distributions (Fort et al., 2017; Gale & Grigal, 1987; Smart et al., 2006), steep roots or downward growing root angles (Bernardo et al., 2025; Fichtl et al., 2024; Fort et al., 2017), and thick roots (Materechera et al., 1992; Reingwirtz et al., 2021) have been identified as promising traits. Low branching frequency and a sparse root system have

also been identified as a possible advantageous phenotype (Reingwirtz et al., 2021; Wright & Westoby, 1999), though they have not been extensively studied, and there is never one root architecture phenotype that outperforms others in all situations (Alonso-Forn et al., 2025; Tron et al., 2015). While more root growth is generally seen as advantageous, the optimal timing and growth responses of roots to soil moisture are still not entirely known (Bauerle et al., 2008; Cuneo et al., 2021; Fort et al., 2017). Grapevine drought resistance is often attributed to its expansive rooting behavior, but measurement of total root system size in mature root systems in the field is extremely difficult (Archer & Saayman, 2018; Smart et al., 2006). In addition, environmental factors can tend to affect root system size more than genotypic effects (Saayman & Van Huyssteen, 1983; Southey & Archer, 1988).

While root traits are increasingly recognized as a means to improve rootstock drought resistance, phenotyping and selection of root traits remains notoriously difficult. Destructive harvesting of field plants, while tractable for annual crops, is far more difficult for perennial woody crops due to the size of the root system and time required for growth in the field (Wasaya et al., 2018; Zhu et al., 2011). To avoid costly phenotyping, recent efforts have increasingly focused on leveraging tools that can increase the throughput and efficacy of selection for root traits. Molecular tools such as genomic selection and marker assisted selection have been successfully implemented to improve tree crops and grapes (Seyum et al., 2022; Viana et al., 2016). Small plants are often used in these studies to investigate physiological mechanisms or map QTL, (Alahakoon & Fennell, 2023; Cuneo et al., 2021; Dudley et al., 2026; Fort et al., 2017; Thapa, 2022), however there have been few studies connecting small plant root traits to overall drought resistance in grapevine for novel genotypes.

To help close this gap, we tested how well root traits of young rootstocks are associated with their observed drought resistance using multiple methods. We measured root growth, soil moisture, and root architectural traits for young rootstocks in two groups: 1. Seven commercial rootstocks of varying drought resistance and 2. A novel F1 rootstock population developed from a cross between a drought resistant and a drought susceptible rootstock parent (110R $\times$ 101-14), to generate diverse combinations of root traits. Neutron radiography is a non-destructive imaging technique that can simultaneously visualize small root systems growing in soil and estimate soil moisture, making it an ideal tool to measure root growth and structure (Moradi et al., 2015; Oswald et al., 2008). We compiled theoretical drought resistance rankings based on the measured traits from neutron radiography using raw trait values and three methods that utilize multiple traits: principal component analysis (PCA), manual ranking based on multiple traits, and a structural functional plant model (SFPM) (Guadarrama-Escobar et al., 2024; Padgett-Johnson et al., 2003). PCA is a technique that can be used on many measured traits to reduce the dataset to a smaller number of dimensions (principal components) that can be used for clustering or ranking individuals (Abdi & Williams, 2010). One downside of PCA is that principal components are often difficult to interpret, so it could be complicated to determine how a PCA score will affect drought resistance. As an alternative, we also included a score that manually ranks individuals that compiles multiple traits based on how we expect the traits to contribute to drought resistance from the literature (Padgett-Johnson et al., 2003). Finally, root system SFPMs have shown success for a number of model species and allow for mechanistic simulation of observed drought resistance from multiple root system traits (Ndour et al., 2017; Schnepf et al., 2018). We used CPlantBox, a SFPM that has been utilized for functional hydraulic modeling of annual crops and structural modeling of grapevine (Fichtl et al., 2024; Zhou et al., 2020). CPlantBox uses

structural and hydraulic parameters as inputs and simulates spatially explicit, three-dimensional root system architecture, root system water uptake, and plant water potentials (Giraud et al., 2023).

After measuring root traits, we determined the drought resistance of the commercial rootstocks through published field trials and grower reports, and we tested the drought resistance of the novel rootstocks with an additional droughted greenhouse assay measuring drought physiology. Comparing our predictions from the raw trait values, PCA, manual ranking, and SFPM to observed drought resistance in both groups, we tested the following three hypotheses: 1) Drought resistant rootstocks have root traits that facilitate drought avoidance, including roots that grow at steeper angles, branch less frequently, attain higher root diameters, grow faster, and have stronger growth responses to moisture. 2) Plants with deeper roots and larger root systems will be better at avoiding drought in a greenhouse assay. 3) Root traits from small plants are correlated with gas exchange, rooting depth, and root mass in droughted plants in the greenhouse. 4) Approaches that integrate information from multiple traits (PCA, manual ranking, SFPM) are stronger predictors of gas exchange and root traits in novel rootstocks than individual traits.

2.3. Materials and methods

2.3.1. Plant materials and growing conditions: neutron radiography experiment

We used neutron radiography to measure root traits for two groups of plants. First, we measured seven commercial grapevine rootstocks with varying drought resistance and genetic backgrounds. Second, we measured 43 novel rootstock genotypes and both parents from an F1 population formed from a cross between a drought susceptible rootstock (101-14) and drought resistant rootstock (110R). The commercial rootstocks were measured in August 2024 ($N = 5 - 10$) and the 43 F1 genotypes and parents in May – July 2023 ($N = 3-7$). We collected softwood herbaceous cuttings 5 to 10 cm in length from greenhouse-grown mother vines, treated the bottom node with rooting hormone for 10 seconds (0.05 % indol-3-butyric acid, 0.025 % naphthalene acetic acid, Earth Science Products Corp., Wilsonville, OR), and placed the cuttings in moist perlite. The cuttings were then grown for 14-16 days in a greenhouse fog room, with automated frequent intermittent misting, to allow adventitious roots to form.

The cuttings were then transplanted into rectangular aluminum rhizotrons (30.5 x 31.5 x 2.5 cm) filled with 30 mesh quartz industrial sand (Basalite Concrete Products LLC., Rancho Cordova, CA), with care taken not to disturb root orientation. Aluminum and sand have low coefficients of attenuation, and sand holds relatively little water, allowing neutron imaging to achieve high contrast between roots and soil (Cuneo et al., 2021). Rhizotrons were filled by opening the side face and packing sand while lying flat to minimize sand layers that can distort images. Cuttings were moved to a greenhouse and allowed to acclimate under shade cloth for one to three days. Plants were then taken to the McClellan Nuclear Research Center (MNRC) for a five to seven-day acclimation period, and a nine-day imaging period. The plants were kept in a climate-controlled room at the MNRC under artificial lighting (14-hr days, $970 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ for

acclimation, $1100 \text{ } \mu\text{mol m}^{-2} \text{ s}^{-1}$ during imaging) in a randomized complete block design. Over the course of the three experiments, temperature and humidity directly adjacent to the plants ranged from 19.7 °C to 33 °C, and humidity ranged from 33.5% to 69.6%. While the plants acclimated in the greenhouse and at the MNRC, they were watered three times per week with water supplemented with macro and micro-nutrients. After the acclimation period, we watered the plants once, then withheld water and subjected plants to a nine-day dry down. The F1 genotypes were propagated and measured in three batches to make imaging times tractable. There was at least one replicate of 101-14 in all three experiments. There were no significant differences in root length between the first two experiments in 101-14, and though the third experiment only had one replicate survive, it was within the range of the values from the previous two experiments, so we continued with analysis.

2.3.2. Neutron radiography imaging

We performed neutron radiography imaging at the reactor at the McClellan Nuclear Research Center (Figure 2.1). All radiographs were obtained at an output of 750 to 1000 kilowatts. Images were captured using a NOG201013 neutron imaging camera (NeuronOptics Grenoble, Claix FR). Each imaging day, we also took three darkfield images, without a sample and with the neutron shutter closed, and three background images, without a sample and with the neutron shutter open, to correct for camera and neutron attenuation noise. We averaged the images to produce a composite background image and composite darkfield image for each day. The plants were imaged two days apart on the seventh and ninth day of the dry down, except for an unexpected facility shutdown during the first experiment that prompted plants to be measured five days apart, on the fifth and ninth days instead. Parent rootstocks that were measured with

both time periods showed similar growth rates across experiments, so we pooled these data in the analyses.

2.3.3. Image analysis of neutron radiographs

All image analysis was carried out in Python 3.9.21 and ImageJ (Schindelin et al., 2012). On each image, we first performed a flat field correction adapted from Oswald et al., (2008) and Moradi et al., (2015):

$$I' = k \cdot \frac{I_b - I_d}{I_r - I_d}$$

where I' is the flatfield corrected image, I_r is the raw image, I_b is the composite background image, I_d is the composite darkfield image, and k is a scaling factor to keep pixel values within the correct range, equal to the average exposure of the background image.

Because the contrast between roots and soil is largest when the soil is dry, we measured root structural traits on the radiograph from the peak of the drought. For the F1 population, we segmented neutron radiographs using a semi-automated pipeline in Python (Figure S 2.1) and manually overlaid a partially transparent version of the image from the last sampling date on the image from the previous sampling date in to visualize new growth and measure growth rate manually in ImageJ. For the commercial rootstocks, the images were noisier and segmentation was less accurate due to a longer exposure time during imaging, so we overlaid flatfield corrected images in a stack in ImageJ to manually measure growth rate.

We measured root orders separately and considered shoot borne adventitious roots to be 1st order, and subsequent lateral branches to be 2nd and 3rd order roots. For each radiograph, we used

ImageJ to manually measure a representative sample throughout the entire root system of two to 10 1st order roots and one to nine higher order roots, depending on the number of measurable roots in each image. We measured root growth, length, diameter, initiation angle, root tip angle, the distance between lateral roots, and the number of 1st order roots. We used these measurements to calculate root growth deviation (i.e., the ratio of initiation angle/root tip angle) and branching frequency (i.e., the number of lateral branches per unit root length).

We calculated a daily growth rate by measuring the length of root growth between the peak drought image and the image directly preceding it and dividing by the number of days between images. For the same roots, we also estimated soil moisture directly adjacent to the root from the pixel values in the flatfield adjusted images to calculate growth responses to moisture. To quantify differences in growth responses to heterogeneous moisture within individual root systems, we fit a mixed effect model to each commercial rootstock and F1 genotype. We used root growth rate as the independent variable, soil moisture adjacent to the root as a fixed effect, and individual plant as a random effect. To measure soil moisture for the growth responses, we measured a rectangular area without any other roots at least 5 mm across on the shortest side adjacent to where the new root growth took place. Here, we report mean pixel intensity for the measured area as a proxy for soil moisture. We tested this assumption with a ground truth experiment, where we filled rhizotrons with sand with a soil moisture varying from oven-dry to field capacity, imaged the rhizotrons, then measured pixel intensity and gravimetric water content from a marked area of each rhizotron. Gravimetric water content was calculated by sampling sand from the marked area and measuring the fresh weight and dry weight, after oven-drying for at least 48 hours at 105 °C. Pixel intensity and soil moisture were strongly correlated ($R^2 = 0.96$, $N = 22$), supporting this assumption (Figure S 2.2).

2.3.4. Droughted greenhouse experiment

Using the image data from the F1 population, we selected a subset of 14 genotypes that captured a wide range of variation in root traits. We then used these genotypes and the parent rootstocks (N = 16 genotypes) to conduct a second greenhouse experiment to measure plant performance under drought stress, following the methods from Berny Mier Y Teran et al. (2019). In March 2024, we propagated plants using the same method as for neutron radiography. Cuttings were grown in a fog room for 21 days, then transplanted into clear plastic cylindrical tubes filled with sand (diameter = 7.6 cm and height = 1.2 m), and placed in the greenhouse. The clear tubes allowed root depth to be monitored throughout the experiment. Opaque tarps were laid over each greenhouse table to exclude light from the rooting zone. Plants were watered every day for two weeks during establishment, then subjected to watering treatments.

Half the plants were randomly assigned to a well-watered and half to a drought treatment (N = 6 per genotype/treatment). Plants were arranged in six blocks in a randomized complete block design, with both treatments in every block. Well-watered plants received water every day, while droughted plants received water every Tuesday and Friday for seven weeks. To avoid producing layers of moisture at the top of the pot with dry sand underneath, we watered tubes to saturation and let excess water drain. Each Friday we irrigated with water supplemented with nitrogen (150 ppm), phosphorous (50 ppm), potassium (200 ppm), calcium (175 ppm), magnesium (55 ppm), sulfur (120 ppm), and iron, copper, boron, manganese, molybdenum and zinc (< 5 ppm). Over the course of the experiment, the daily temperature ranged from 15.4 °C to 32.5 °C, and humidity ranged from 34.1% to 87.9%. We uncovered the tubes and measured the depth of the deepest visible root weekly. Six weeks after starting the drought treatment, we measured stomatal conductance (g_s) at midday (between 1PM and 3PM) for all plants on the most recently

fully expanded leaf with a porometer (LI-600, LICOR Environmental, Lincoln, NE). Sixty-three days after transplanting, we destructively harvested the plants by dumping the soil and roots onto a metal mesh and washing the sand off the roots with gentle running water. To measure biomass, we cut the roots and leaves at the root crown and bud and dried them in an oven at 70 °C for at least 48 hours. To account for differences in leaf area and estimate a proxy for total transpiration, we multiplied the midday g_s by the above ground biomass at destructive harvest to obtain adjusted g_s ($\text{mol g m}^{-2} \text{ s}^{-1}$).

2.3.5. Ranking of expected drought resistance

To define ground truth drought resistance groups for the commercial rootstocks and ranking for the F1 population, we used published research and the results from the droughted greenhouse experiment, respectively. For the commercial rootstocks, we reviewed published research from experimental physiology field trials, pot studies, and observational multi-year field trials. We started with classifications from nursery guides and textbooks, which are often determined from observational studies of yield and vigor under drought conditions, however, when possible, we amended the rankings to give more weight to experimental field trials or experiments measuring drought stress physiology (Table 2.1). Ultimately, we categorized the commercial rootstocks as resistant (Ramsey, 110R, 1103P), moderate or mixed (SO4 and Freedom), and susceptible (101-14 and 1616C). For the F1 population, we used the adjusted g_s to rank ground truth drought performance. We assumed under drought that higher adjusted g_s is associated with higher drought performance.

Then, we tested whether integrating variation in multiple traits would improve predictions for whole-plant performance or drought resistance compared to single traits in three ways. For each multiple trait approach, we analyzed the two groups (commercials and F1 population) separately.

We also calculated PCA scores and manually ranked drought resistance scores from trait values for the first two root orders separately.

First, we used principal components analysis (PCA) to produce a ranking system integrating variation in all the root growth and structural traits. We included root growth rate, thickness, root tip angle, root growth deviation, branching frequency, and growth responses to moisture in the PCA. For commercial rootstocks, we used PC1 and PC2 to perform K-means cluster analysis with three clusters to group alike rootstocks together. We did not perform cluster analysis for the 2nd order roots in the commercial rootstocks due to lack of statistical separation between genotypes, and that the branch frequency and growth responses to moisture data were not available. For the F1 genotypes for both root orders, we standardized all traits to a mean of 0 and standard deviation of 1 and calculated PC1 scores for each individual, which were averaged to produce mean PC1 scores for each genotype. Commercial rootstocks separated more clearly and accurately according to drought resistance across PC1, and PC2 was more difficult to interpret without clear separation according to drought resistance, so we excluded PC2 from ranking the F1 population.

Second, we adapted the approach from Padgett-Johnson (2003) to develop a drought resistance score based on the five traits we expected to be the most adaptive, based on previous findings for trait differences between drought resistant and susceptible grape rootstocks: root growth rate, thickness, root tip angle, branching frequency, and growth responses to moisture. The PCA analysis is a naïve analysis of all traits that reduces dimensions based on the amount of variance explained, while manual scoring allowed us to focus on the traits we expect to be most predictive, and weight the trait values that are in the direction we expect to be advantageous. For each trait, we used individual trait values to calculate a percentile for each plant, then averaged

each commercial rootstock or F1 genotype and divided the percentile by 100 to produce a score ranging from 0 to 1. We ordered trait values such that a higher percentile was associated with traits we expect to be the most drought resistant. Thus, the fastest root growth, thickest roots, steepest-angled roots, least root branching, and strongest positive growth response to moisture corresponded to higher percentile values for their respective traits (Fort et al., 2017; Reingwirtz et al., 2021; Reyes, 2021). For growth responses to soil moisture, we used the calculated slope from the mixed effect model to rank genotypes. We summed the percentiles for the five traits to produce an overall score for each variety.

2.3.6. Structural functional plant model

For our third approach, we tested using a structural-functional plant model to integrate variation in multiple root traits and predict root system architecture and water uptake. We used traits measured from neutron radiographs to parameterize the root structural parameters for the CPlantBox model (Giraud et al., 2023; Schnepf et al., 2018). CPlantBox takes several root trait parameters as inputs to output a stochastic root system architecture that can account for different pot shapes and growth times. Using root and soil hydraulic properties, coupled with the generated root architecture, CPlantBox dynamically calculates water potentials and water flow in the soil-plant continuum.

We parameterized root hydraulics by measuring radial hydraulic conductivity for fine roots under well-watered conditions for a subset of four genotypes, using the hydrostatic method adapted from Barrios-Masias et al. (2015) and Miyamoto et al. (2001) (Figure S 2.3). Hydraulic conductivity was not significantly different between genotypes (Type I ANOVA, $p > 0.05$), so we parameterized all genotypes with the mean value from the measured genotypes. We used published data for grapevine to parameterize the decline in radial conductivity with root age

(Gambetta et al., 2013). We parameterized soil hydraulics using published water relations parameters for sand (Supplemental Table S 2.5).

For each genotype, we simulated root growth for 49 days in rooting tubes with the same dimensions as in the greenhouse experiment ($N = 100$ simulations/genotype) and recorded maximum rooting depth. To predict gas exchange, we simulated a three day dry down on the full-grown root systems, equal to the dry down in the tubes. Due to computational limitations, we simulated root hydraulics for a reduced sample size ($N = 10$ simulations/genotype). We recorded predawn and midday water potential at the root collar and the water flow rate at the root collar at midday at peak drought, analogous to whole plant transpiration rate.

2.3.7. Statistical analysis

All statistical analysis was carried out in Python 3.9.21 and R 4.2.3. For all analyses, unless otherwise specified, we analyzed the commercial rootstocks and the 14 F1 genotypes with both parents separately. Prior to analysis, we confirmed the assumptions for ANOVA were met by using the Anderson-Darling test to test the residuals for normality and Levene's test to test for homogeneity of variances. The assumptions were met for nearly all traits, and any deviations were small, so we proceeded with the analysis.

First, for the commercial rootstocks and all 43 F1 genotypes and parents, we tested each rootstock and genotype for significant root growth responses to soil moisture (growth response significantly different than zero) and deviation in root angle for each root order. P values for significant growth responses to moisture were taken from the mixed effect model output and were corrected with the false discovery rate method (FDR) (Benjamini & Hochberg, 1995). Root growth deviation was tested for differences from 0 for each genotype with a two-sided t-test. P values were again corrected for multiple tests FDR for both groups. Second, for commercial

rootstocks and all 43 F1 genotypes/parents, we used type III ANOVAs to test each root trait for differences between genotypes or rootstocks, root orders, and their interaction. Third, we used linear regression to test for collinearity between traits, for rootstock groups (commercial and F1) and root type independently. Fourth, to ensure the drought treatment effectively droughted the plants in the greenhouse experiment we performed type II ANOVA for adjusted g_s with treatment and genotype as main effects to check for reduction in adjusted g_s in the drought treatment. We then analyzed the drought treatment alone with a Type I ANOVA to test for differences between genotypes under water stress. Within the drought treatment, we also performed regression between adjusted g_s and the root depth and mass of each individual plant within the drought treatment to check if total rooting depth or mass were good proxies for drought resistance.

Finally, we conducted two analyses to compare the capacity for individual traits and integrated trait metrics to predict drought performance and root traits. We grouped commercial rootstocks into groups that were statistically significant from each other, using Tukey HSD pairwise comparisons for the individual traits and outputs from the percentile score and SFPM, and using PCA unsupervised K-means clustering with the first two principal components. We evaluated the success of each metric by comparing the number of mis-grouped commercial rootstocks from expected drought resistance groupings. To evaluate the F1 population, we applied a linear model to the performance variables from the greenhouse experiment under drought (adjusted g_s , maximum root depth, and root mass) to 1) each trait measured from the neutron radiographs, 2) the PC1 axis score, 3) the percentile drought resistance score incorporating *a priori* knowledge about drought adaptation, and 4) the root depth and root water uptake predictions from CPlantBox across the 14 F1 genotypes and 2 parents. For each linear model we calculated the

Pearson r value, R^2 , and the Akaike Information Criterion corrected (AICc) to evaluate the performance of each model.

2.4 Results

2.4.1. Root traits differ by root order and genotype

For all traits in both sets of plants, we observed significant differences between rootstocks (Type III ANOVA, $p < 0.05$, Table 2.2). For all traits in both groups of rootstocks, we either observed significant differences between root orders, or significant interactions between genotype and root order (Table 2.3). Interactions between root order and genotype varied in direction and magnitude, indicating that the trait differences between root orders are highly dependent on genotype. There were scattered significant associations between traits, with the commercial rootstocks containing more significant associations. The most notable associations occurred between root length and growth rate, root tip angle and growth deviation, and root length and thickness in 2nd order roots in the F1 population (Table S 2.1).

2.4.2. Root traits vary between rootstocks of known drought resistance

Using a PCA to group commercial rootstocks matched our expectations from the literature for some traits, but not others. The first two principal components explained 55 and 23% of trait variation, respectively. K-means clustering analysis identified three clusters. One cluster contained all individuals of Ramsey and 110R, which are both classified in the literature as drought resistant (Table 2.1). A second cluster grouped together 1616C, Freedom, SO4, and, unexpectedly, 1103P. 1616C and SO4 are considered low to moderate in drought resistance, while reports for Freedom are mixed, but 1103P is classified as highly drought resistant (Table 2.1). Surprisingly, 101-14 was clustered almost entirely independently from the other rootstocks, in the third cluster. Most individuals of each rootstock grouped together, with some minor inconsistencies. One individual for 1103P and 1616C and two individuals in SO4 were grouped in different clusters from the rest, though the overall grouping was still clear (Table 2.4, Figure

2.2). The first and second cluster mostly separated along PC1, with Ramsey and 110R at one extreme and Freedom at the other. The third cluster, of 101-14, mostly separated from the others along PC2.

We used the PCA loadings and ANOVAs to investigate trait differences between the rootstocks from different groups. PC1 had the largest contributions ($\geq 45\%$) from root diameter, growth deviation, and root tip angle with more minor contributions from growth and growth responses to moisture (35 to 40%), while PC2 was dominated by root growth rate and branching frequency ($> 50\%$ contribution) (Figure 2.2). Ramsey and 110R, the drought resistant rootstocks that clustered together, showed significant differences in 1st order root traits that were consistent with our hypotheses. The 1st order roots for these rootstocks were thicker, more steeply angled, and less branched than the other rootstocks (Tukey HSD tests, $p < 0.05$) (Table 2.2, Figures 2.3 and 2.4). 110R and Ramsey also had stronger downward growth deviations and growth responses to moisture. Both rootstocks showed significant preferential growth in the 1st order roots in wetter soil (mixed effects model, $p < 0.05$), while for the other rootstocks, the slope of the relationship between root growth rate and soil moisture was not significantly different from zero (Figure 2.5). In general, roots generally grew roughly in their direction of branching, but with more deviation in the negative direction than the positive direction (i.e., on average, the roots bent so that the tips were angled more steeply downward than the base) (Table 2.2). 110R and Ramsey were the only rootstocks that showed significant downward growth deviation (t-test, $p < 0.05$).

Unexpectedly, Freedom was an extreme phenotype for almost all measured traits across both root orders, and clustered on the other extreme of PC1. Freedom had many 1st order roots, which were also thin, shallow, less branched, and slow growing (Table 2.2, Figure 2.3). Freedom was also the only rootstock with a significantly positive growth deviation (i.e., with roots bending so

that the tips angled upwards towards the surface) (t-test, $p < 0.05$). In the commercial population, 2nd order roots did not show much statistical separation between rootstocks.

While these results generally agreed with our hypothesis, some rootstocks did not match the phenotypes that we expected. 101-14, a drought susceptible rootstock, separated from all of the other rootstocks along PC2 because it exhibited the most branched roots and, contrary to our predictions for drought resistance, fastest root growth rates (Table 2.2). 101-14 was also situated near Ramsey and 110R along PC1 and exhibited some similar traits, including thick roots, also contrary to our hypotheses. Likewise, 1103P, which is considered drought resistant in the literature, had thickness, root length, and growth deviation more similar to 1616C and SO4 than the other drought resistant rootstocks and did not cluster with Ramsey and 110R (Figure 2.1, Table 2.4). In contrast with the 1st order roots, we observed few significant differences between rootstocks in the second order root traits, likely because there were fewer observable second order roots in the neutron radiographs for some genotypes, creating large confidence intervals.

2.4.3. Predicting rootstock drought resistance classifications

The percentile ranking method was most successful in correctly grouping rootstocks by observed drought resistance in the field, indicating that this was the most accurate method to integrate the root trait information to capture field performance (Table 2.4). The percentile method based on 1st order roots identified Ramsey and 110R as most drought resistant group, followed by 1103P, then by overlapping groups containing 101-14, 1616C, SO4, and Freedom, which was identified as the most drought susceptible. This method misplaced SO4, which is generally considered low to moderately drought resistant, but generally succeeded at distinguishing drought resistant from susceptible rootstocks. Percentile ranking the second order root traits was less successful,

producing a smaller range in rankings and less separation between rootstocks, and more shuffling between drought resistant and susceptible rootstocks.

The percentile method was also more accurate than ranking drought resistance from predictions for root system water uptake at the peak of the dry-down from the structural-functional plant model (SFPM, CPlantBox). The model correctly ranked 110R and Ramsey as highly drought resistant, but miscategorized SO4 as highly drought resistant and 1103P as drought susceptible. Overall, the percentile method miscategorized fewer rootstocks than the SFPM or the PCA analysis (Table 2.4).

2.4.4. Second order root length and growth correlate with greenhouse rooting depth and transpiration under drought

The greenhouse experiment with a subset of the F1 population showed that drought reduced adjusted g_s and there were significant differences between genotypes within the drought treatment for all traits. First, we confirmed that the drought treatment was sufficient to impact vine physiology by testing that adjusted g_s was significantly lower in the droughted than the well-watered plants (Type II ANOVA, $p < 0.001$, Figure 2.6). We then confirmed that the genotypes varied in drought performance, finding significant genotypic differences in adjusted g_s , rooting depth, and biomass within the drought treatment ($p < 0.05$). When we regressed adjusted g_s to rooting depth and root mass across individuals in the drought treatment, root depth and adjusted g_s were very weakly but significantly associated ($p < 0.01$, $R^2 = 0.08$), however root mass was not associated with adjusted g_s ($p > 0.05$, $R^2 = 0.03$). This suggests differences in root depth played a small but significant role in maintaining adjusted g_s ($p < 0.01$, $R^2 = 0.08$), but differences in root mass likely did not.

After regressing each predictor variable against the validation traits, we used AICc (Aikake Information Criteria corrected for small sample sizes) to identify the most strongly supported models for each drought performance variable (Table 2.5). The best-supported models showed that contrary to our predictions, the integrated trait indices were not stronger predictors of adjusted g_s and rooting depth compared to individual traits. The most strongly supported models predicted adjusted g_s from the length of second order roots ($R^2 = 0.51$), plant rooting depth from the length of second order roots ($R^2 = 0.50$), adjusted g_s from the growth deviation of the 1st order roots ($R^2 = 0.32$), and root biomass from the length, growth rate, and PC1 score of the second order roots ($R^2 = 0.16 - 0.26$) (Figure 2.8).

Interestingly, in this subset of the F1 population, the second-order root traits were more predictive of drought performance than the 1st order roots, contrary to the commercial rootstocks. Using the percentile score for the 1st order roots, which was the most successful metric for predicting drought resistance categories in the commercial rootstocks, as a predictor only weakly captured variation in plant rooting depth ($r^2 = 0.29$) and was unrelated to adjusted g_s ($r^2 = 0.09$) and root biomass ($r^2 = 0.08$) (Table 2.5).

2.5. Discussion

In general, we observed that the drought resistant commercial rootstocks contained roots that were more steeply angled, tended to orient downwards, and had stronger growth responses to moisture. Other traits did not show clear patterns between rootstocks, and their contribution to drought resistance is less clear. Alternatively, in the F1 population we found that root mass, depth, and adjusted g_s were all overwhelmingly determined by length and growth rate of the 2nd order roots. Though root traits generally did successfully predict drought resistance grouping in the commercial rootstocks and the drought performance ranking in the F1 population, the most successful methods differed between groups. This is likely due to new combinations of traits in the novel F1 population and different methods of drought resistance determination.

2.5.1. Root tip angle, thickness, and growth responses to moisture are likely drought resistant traits

For root tip angle, root growth deviation, and growth responses to moisture, the observed phenotype matched what we expected. We saw the rootstocks considered more drought resistant generally had steeper 1st order roots, which has been shown to cause increased drought tolerance in other agronomic crops (Guo et al., 2024). We saw more negative growth deviation was related to steeper root tip angles (Table S 2.1), and significantly negative growth deviation occurred in drought resistant rootstocks 110R and Ramsey, which has been observed in previous studies (Fichtl et al., 2024; Fort et al., 2017; Reingwirtz et al., 2021). These results agree with the PCA results, especially along PC1. Root tip angle, root deviation, and root thickness were the traits that contributed to PC1, which separated out Ramsey and 110R from SO4, 1616C, and Freedom. This data strengthens the argument that steeper and thicker roots can enhance drought resistance,

although there is still a significant knowledge gap of how root angles and thickness affect root architectures and distributions in different field conditions (Smart et al., 2006).

110R and Ramsey both showed the strongest growth responses to moisture compared to the other rootstocks. New root growth is the main mechanism in which grapevines increase their root hydraulic conductivity (Gambetta et al., 2013), and previous research has shown that preferential root growth in deeper soil and recovery of root growth in wet soil after drought are traits associated with drought resistance (Alsina et al., 2011; Bauerle et al., 2008; Cuneo et al., 2021). This further supports our findings that preferential root growth with moisture is likely a drought resistant trait. From previous root growth experiments in the field and previous neutron radiography work with lupine, it's also likely that all rootstocks contain some level of root responses to moisture (Comas et al., 2005; Dara et al., 2015). Because we did not apply heterogenous watering treatments to the pots, it is possible we didn't have a large enough range of soil moisture values for each plant to be able to consistently detect weaker responses. To further investigate this phenomenon, future experiments could apply watering treatments to different areas of the root zone similar to Dara et al. (2015).

The root traits also showed some unexpected patterns. First, all of the categorization methods grouped 1103P with the drought susceptible rootstocks (Figure 2.2, Table 2.4). Unlike 110R and Ramsey, 1103P did not have especially thick roots, strong downward growth deviation, or growth responses to moisture in the 1st order roots (Table 2.2). Second, 101-14 separated from all of the other rootstocks along PC2, due to its exceptionally fast growth rates and extensive branching in the 1st order roots (Figure 2.3). Interestingly, although 101-14's root angle, thickness, and growth rates aligned more closely with Ramsey and 110R than the other susceptible rootstocks, it had the lowest growth responses to moisture (Figure 2.5, Table 2.2).

This finding may be indicative of a poor carbon investment and emphasizes the importance of prioritizing root growth in areas of high moisture.

Misplacement of 1103P and 101-14 could also be due to the presence of functional root traits that we did not measure that may impart drought resistance. In the literature, the two rootstocks have been shown to differ in their root growth both temporally and spatially (Alsina et al., 2011; Bauerle et al., 2008), transcriptomic and stomatal response to drought (Bianchi et al., 2023), and to have differences in root pressure-volume relationships (Bartlett et al., 2022). Other traits, though there are not direct comparisons between 1103P and 101-14 in the literature, have also been shown to differ between grapevine rootstocks. These traits, such as embolism resistance (Knipfer et al., 2015), suberization and lacuna formation (Barrios-Masias et al., 2015; Cuneo et al., 2021), and aquaporin expression (Gambetta et al., 2013; Perrone et al., 2012) can all lead to differences in hydraulic conductivity and water uptake during drought (Delval et al., 2024).

Finally, all of the categorization methods classified Freedom as the most drought susceptible, even though the literature is mixed. Freedom had the most extreme phenotype for nearly every trait, including having the shallowest, thinnest, slowest-growing, and most unbranched roots (Table 2.2). Some older sources classified freedom as drought resistant (Coombe et al., 1988; Rahemi et al., 2022), likely due to reports of high vigor and a set of dry land field trials in south Australia (Cirami et al., 1994; Cirami & McCarthy, 1988; Hardie & Cirami, 1988), though other more recent sources have classified it as susceptible to drought (Carroll, 2006; McCarthy et al., 1997; Perry & Sabbatini, 2015). While our results agree with Carroll (2006), McCarthy et al., (1997), and Perry & Sabbatini (2015), and suggest that Freedom likely does not have advantageous root structure for drought resistance, there is clear interaction between genotype and environment. In the 1997 study by McCarthy et al. (1997), they also reported poor

performance from 110R and 1103P, rootstocks with demonstrated drought resistance, which could have been due to a shallow hardpan that prevented root penetration. The results from the current study and previous findings highlight that different root architectures could perform better or worse in certain environments. For example, in shallow and well-draining soils with intermittent irrigation or rainfall, similar to the environment present in the study by McCarthy et al. (1997), fibrous and dense root systems may outperform plants with deep root systems (Tron et al., 2015). This could explain how Freedom's drought resistance matched that of 110R and 1103P in the drier year and far surpassed 110R and 1103P in the irrigated year. This could also explain its high vigor in irrigated conditions, despite evidence that it is not drought resistant and would likely perform poorly in dry farmed conditions.

2.5.2. Successful methods of prediction differed between commercial and F1 rootstocks

Screening root architecture traits to predict drought performance showed some promise. 1st order root traits successfully predicted drought resistance for the commercial rootstocks, especially when the information from multiple traits was integrated into a percentile ranking score (Table 2.4). Alternatively in the greenhouse experiment, the integrated trait metrics, including the PCA scores, percentile ranking scores, and SPFM predictions for root system architecture and water uptake, were not more predictive than the most predictive individual traits. The most strongly supported models (i.e., the models with AICc scores within 2 units of the minimum) predicted adjusted g_s from the length of second order roots ($r^2 = 0.51$), plant rooting depth from the total length of second order roots ($r^2 = 0.50$), and root system biomass from the length, growth rate, and PC1 score of the second order roots ($r^2 = 0.16 - 0.26$) (Table 2.5). Surprisingly, the second-order root traits were more predictive of rooting traits and drought physiology than 1st order root traits, contrary to our findings for the commercial rootstocks (Table 2.2). These results indicate

that long 2nd order roots resulted in both higher drought performance and deeper, larger root systems, although we did not observe any consistent effect of root mass on drought performance in this study.

The presence of long 2nd order roots in the F1 population that were generally absent in the commercial rootstocks could also explain the difference between the commercial rootstocks and F1 population. When compared to the F1 population, commercial rootstocks had relatively short lateral roots. The F1 parents, 110R and 101-14, had 2nd order roots comparable to or longer than the rest of the commercial rootstocks, but many F1 progeny had longer 2nd order roots than both parents. Transgressive segregation in the progeny, when phenotypes of the progeny are more extreme than either parent, may have resulted in long 2nd order roots that maintained adjusted g_s better in drought conditions than both the parents with shorter 2nd order roots.

These findings further support the necessity of phenotyping root traits on an order and morphology basis (Guo et al., 2024; Mapfumo et al., 1994; McCormack et al., 2015). Though we did not evaluate function or survivability within root orders in the F1 population, sometimes even within a single plant, there were thick and fast-growing lateral roots and thinner shorter roots. Similar observations of pioneer roots (unusually fast growing daughter roots) in Poplar, Maple, and Walnut showed variation within root orders could affect survivability and long-term root architecture (Bagniewska-Zadworna et al., 2014; Zadworny & Eissenstat, 2011), which suggests variation in morphology within a root order may also be an important trait to monitor in the future to fully optimize root system architecture.

2.5.3. Selection for drought resistance based on root traits in small plants is promising

In total, our results suggest that phenotyping roots in young plants may be a feasible strategy for early selection for drought resistance, with some caveats. Root architecture disregards hydraulic

functional traits, which play a large role in drought resistance (Gambetta et al., 2013; Reingwirtz et al., 2021). Also, although we identified root order is an important determination, we do not yet know the strength of contribution to drought resistance for traits within or between root orders. Commercial rootstock prediction of drought resistance was determined by multiple traits within 1st order roots, while adjusted g_s and root traits the subset of the F1 population was overwhelmingly determined by length and growth rate of 2nd order roots.

Additionally, the literature basis for drought resistance of root traits (as used in the high performing percentile method) comes from studies that utilize the same rootstocks that appear in this study. This method could be analogous to resubstitution bias, which is attempting to validate a model with the same dataset used to train it (Kerr et al., 2015). In the F1 population, although it was tested with different training and validation datasets, the effect of 2nd order root length on drought response (adjusted g_s) could be overestimated because of the tube setup of the experiments. Differences in rooting depth in the greenhouse pots allowed for more soil exploration in some genotypes than others, which has not been observed in the field (Smart et al., 2006). This could have caused overestimated differences in adjusted g_s than would exist in the field. The tubes may also have forced roots to grow downward when they may not have otherwise, further exacerbating this effect.

To truly know the contributions that root traits play in drought resistance, it is necessary to evaluate the root traits and drought performance of many novel accessions in the field, without prior knowledge of their drought resistance. Fast growing and long lateral roots in the F1 population are promising traits, but the true magnitude of their effect needs to be validated before they could be capitalized upon in breeding efforts.

2.6. Conclusion

We found that steep root angles, downward deviation of root growth, and root growth responses to moisture occurred in drought resistant commercial rootstocks and are likely traits of importance for conferring drought resistance. We observed long and fast-growing 2nd order roots were the most predictive of drought performance in the novel F1 population, suggesting a potentially advantageous trait. This, combined with differences in traits between and within root orders strengthens the argument that root phenotyping should be based on order when possible. We showed that to some extent, measurements of root traits on small plants may be used for early selection for grapevine rootstocks. Despite seeing parallels between small plant root traits and drought performance in both rootstock groups, using prediction methods that integrated multiple root traits did not improve selection for novel rootstocks. Additionally, while prediction showed some success, average root growth rate, root thickness, and branching frequency did not show clear patterns with drought resistance in the commercial rootstocks and support the hypothesis of multiple root system ideotypes for different environments or management.

2.7. Tables

Table 2.1. Expected drought resistance of seven commercial rootstocks

Rootstock	Common name	Ancestry	Expected drought resistance
Ramsey (Salt Creek)	Ramsey	<i>V. x champinii</i>	Very high (a) ¹⁻¹⁰
110 Richter	110R	<i>V. berlandieri</i> , <i>V. rupestris</i>	High - very high (a) ¹⁻¹⁴
1103 Paulson	1103P	<i>V. berlandieri</i> , <i>V. rupestris</i>	High - very high (a) ^{1-6,8-14}
Selektion Oppenheim 4	SO4	<i>V. berlandieri</i> , <i>V. riparia</i>	moderate - low (b) ^{1,4-6,8-11,13,15}
Freedom	Freedom	<i>V. x champinii</i> , <i>V. solonis</i> , <i>V. riparia</i> , <i>V. labrusca</i> , <i>V. vinifera</i>	mixed (b) ^{1-4,6,8,9,12,14,15}
101-14 Millardet de Grasset	101-14	<i>V. riparia</i> , <i>V. rupestris</i>	low (c) ^{1,4-11,13,15}
1616 Couderc	1616C	<i>V. riparia</i> , <i>V. longii</i>	low (c) ^{1,4,6,8-10}

Letters in the expected drought resistance columns indicate expected drought resistance groupings: (a) resistant, (b) moderate or mixed resistance, (c) susceptible. Expected drought resistance is determined from the following references. Sources are a mix of literature reviews, field experiments, pot experiments, nursery guides, field observations of yield and vigor under droughted conditions, and anecdotal accounts.

1. (Cirami et al., 1994)
2. (Cirami & Mccarthy, 1988)
3. (Mccarthy et al., 1997)
4. (Coombe et al., 1988)
5. (N. Dry, 2015)
6. (P. R. Dry & Coombe, 2005)
7. (Fort et al., 2017)
8. (Rahemi et al., 2022)
9. (Verslype et al., 2023)
10. (Wolpert et al., 2002)
12. (Carroll, 2006)
13. (Pritchard, n.d.)
14. (Williams, 2010)
15. (Perry & Sabbatini, 2015)

Table 2.2. Mean values of measured traits for commercial rootstocks

Root order	Genotype	Growth rate (mm/day)	Root diameter (mm)	Tip angle (degrees)	Root length (mm)	Growth deviation (degrees)	Branch frequency (mm ⁻¹)	Number adventitious roots	Growth with moisture slope
1st	Ramsey	4.9 a	1.8 d	160.9 c	100.8 ab	-32.3 a***	0.026 abc	11 a	30.4 ab*
	110R	7.7 bc	1.87 d	156.7 c	148.2 c	-21.5 ab***	0.037 bc	11 a	40.8 b**
	1103p	4.9 ab	1.21 b	151.1 c	104 ab	-3.7 bc	0.004 a	19 b	12.8 ab
	SO4	3 a	1.42 bc	125.5 b	87.9 a	-0.1 bc	0.056 cd	10 a	7.0 a
	101-14	8 c	1.77 cd	149.5 c	134.1 bc	-9.3 b	0.082 d	10 a	0.24 a
	1616C	3.6 a	1.17 b	130 b	104.6 ab	-9.5 b	0.041 bc	16 ab	17.9 ab
	Freedom	2.6 a	0.71 a	110 a	89.2 a	19.2 c*	0.006 ab	31 c	5.4 a
2nd	Ramsey	1.9 a	0.58 a	116.3 ab	30.2 a	-6.2 a			
	110R	4.2 b	0.71 ab	103.6 ab	58 b	-2.8 a			
	1103p	3.9 ab	0.38 ab	100.5 ab	51 ab	-2.3 a			
	SO4	2.8 ab	0.72 ab	140.5 b	26.2 a	-6.4 a			
	101-14	4 b	0.81 b	106.9 ab	59.7 b	-4.56 a			
	1616C	2 a	0.63 ab	112.4 ab	34.8 ab	-5.7 a			
	Freedom	1.5 a	0.55 a	86.2 a	36.4 ab	3.1 a			

For each genotype for growth deviation and growth with moisture we tested each value against the null hypothesis: Trait value = 0. Asterisks show significant differences from zero (One sample t-test, Mixed effects model; * = $p < 0.05$, ** = $p < 0.01$, *** = $p < 0.001$). Letters show significance grouping from Tukey HSD pairwise comparisons for each trait within root orders.

Table 2.3. Type III ANOVA results for both rootstock groups

Group	Trait	Root order	Rootstock	Interaction
Commercial	Root length	p < 0.001	p < 0.001	0.45
	Growth rate	p < 0.001	p < 0.001	0.02
	Diameter	p < 0.001	p < 0.001	p < 0.001
	Root tip angles	p < 0.001	p < 0.001	p < 0.001
	Growth deviation	0.56	p < 0.001	0.04
	Branch frequency		p < 0.001	
F1	Root length	p < 0.001	p < 0.001	0.728
	Growth rate	0.844	p < 0.001	0.881
	Diameter	p < 0.001	p < 0.001	p < 0.001
	Root tip angles	0.003	0.014	0.027
	Growth deviation	0.625	p < 0.001	0.038
	Branch frequency	0.046	p < 0.001	0.011

Bold indicates significance at $p < 0.05$.

Table 2.4. Commercial Rootstock Prediction Methods

Ranking method	Rootstock	Mean/cluster	Grouping	Expected Resistance
Percentile ranking (1st order)	Freedom	2.305	a	mixed
	SO4	2.547	ab	low - medium
	1616C	2.883	bc	low
	101-14	3.036	c	low
	1103P	3.612	d	high - very high
	Ramsey	4.025	e	very high
	110R	4.296	e	high - very high
Percentile ranking (2nd order)	Freedom	1.800	a	mixed
	1103P	2.220	abc	high - very high
	101-14	2.334	ab	low
	1616C	2.436	b	low
	Ramsey	2.567	bc	very high
	SO4	2.637	bc	low - medium
	110R	3.074	c	high - very high
SFPM ranking (root water uptake)	Freedom	0.240	a	mixed
	1103P	0.323	a	high - very high
	1616C	0.370	ab	low
	101-14	0.540	bc	low
	110R	0.581	c	high - very high
	SO4	0.611	c	low - medium
	Ramsey	0.623	c	very high
PCA cluster analysis (1st order)	Freedom	1	a	mixed
	1616C	1	a	low
	SO4	1	a	low-medium
	1103P	1	a	high-very high
	101-14	2	b	low
	110R	3	c	high-very high
	Ramsey	3	c	very high

Rankings and groupings of commercial rootstocks from the different methods of prediction. Groups were determined from a Tukey HSD test and PCA unsupervised K-means clustering for the PCA analysis.

Table 2.5. Correlations between prediction and validation data

Validation metric	Prediction method	Pearson r	R ²	AICc
Plant depth	Root length (2nd)	0.7	0.496	103.03
	PC1 (2nd)	0.63	0.392	106.05
	Growth rate (2nd)	0.6	0.363	106.79
	Percentile score (1st)	0.54	0.289	108.54
	Root length (1st)	0.51	0.26	109.19
Adjusted g_s	Root length (2nd)	0.71	0.511	-32.42
	Growth rate (2nd)	0.56	0.313	-26.98
	Growth rate (1st)	0.54	0.289	-26.43
	Modeled depth	0.52	0.267	-25.94
	Root length (1st)	0.49	0.242	-25.40
Root mass	Growth rate (2nd)	0.51	0.26	27.51
	Root length (2nd)	0.46	0.21	28.47
	PC1 (2nd)	0.40	0.16	29.39
	Branch frequency (1st)	-0.34	0.11	30.30
	Growth with moisture (2nd)	-0.29	0.09	30.81

2.8. Figures

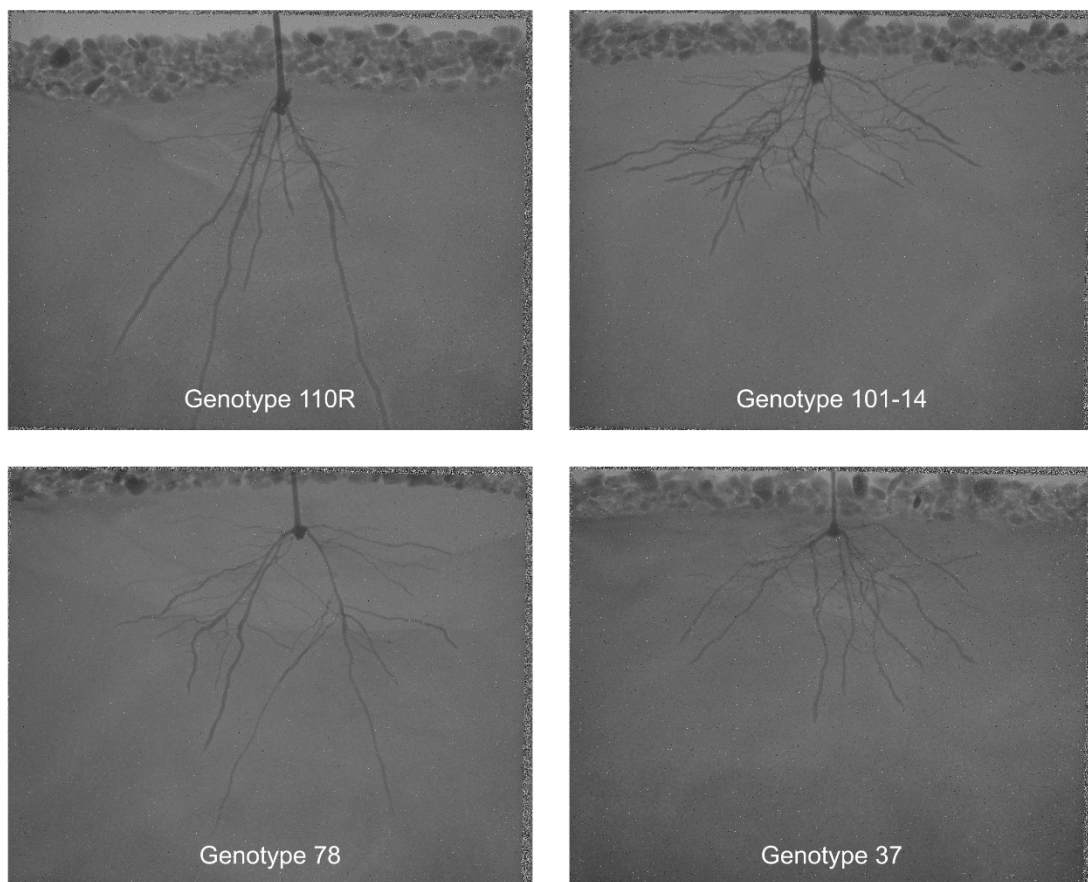


Figure 2.1. Neutron Radiographs

Example neutron radiographs showing contrasting phenotypes.

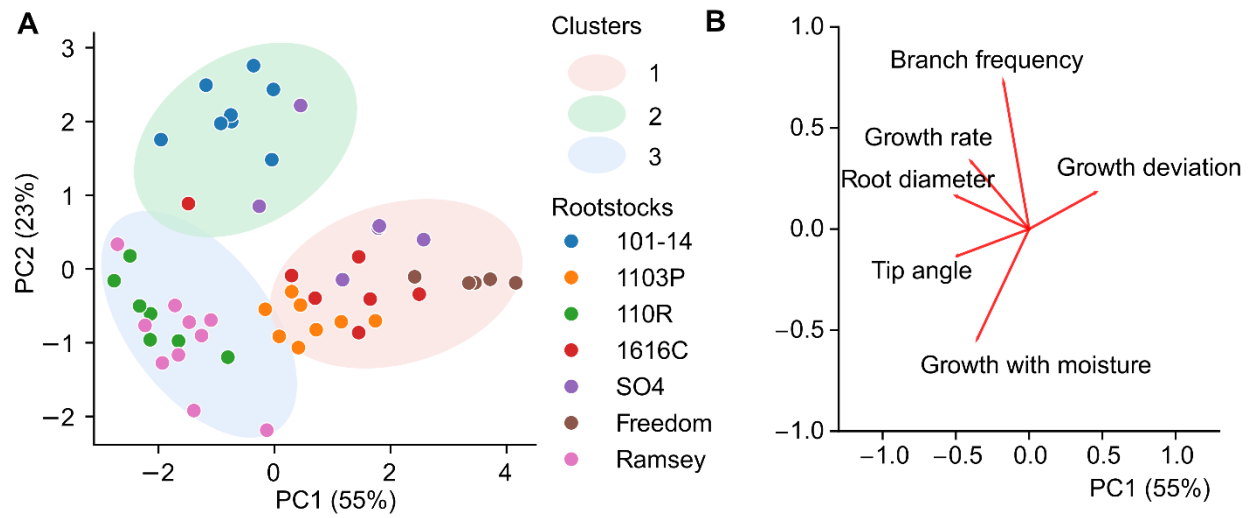


Figure 2.2. Principal component analysis

PCA analysis for the seven commercial rootstocks. A: Cluster analysis of commercial rootstocks. B: PCA loading biplot for the cluster analysis.

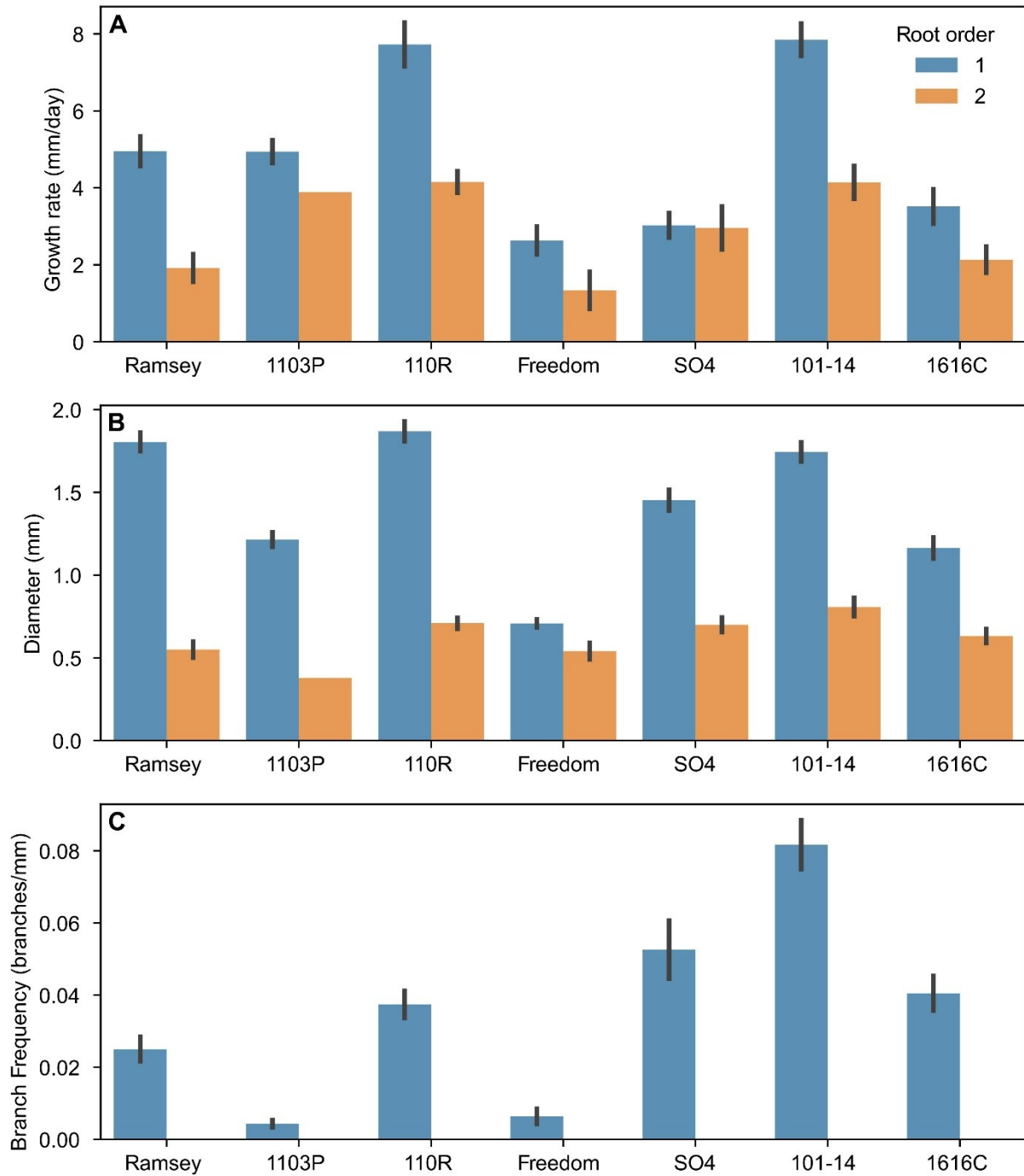


Figure 2.3. Commercial rootstock traits

Mean root traits for the seven commercial rootstocks. Root order 1 are adventitious roots and root order 2 are lateral roots. A: Measured growth rate for roots by root order for all rootstocks. B: Root radius for each root order for all rootstocks. C: Estimated branching frequency for adventitious roots for all rootstocks.

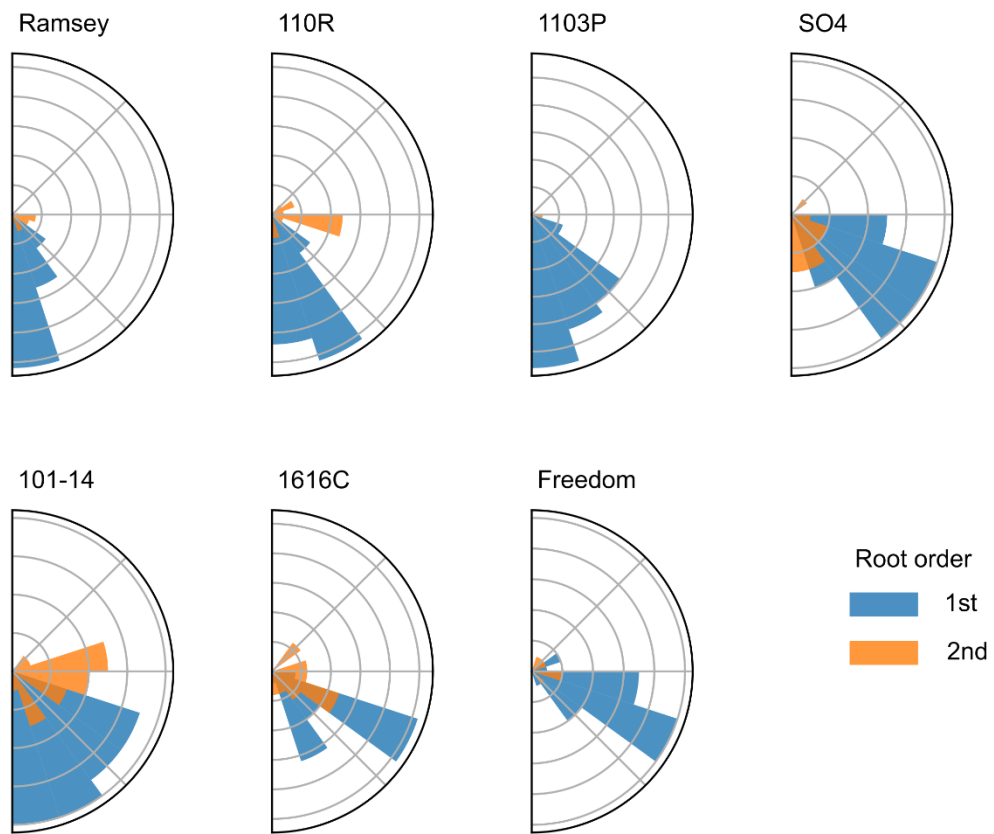


Figure 2.4. Root angles

Root tip growth angles of each commercial rootstock measured for both root orders.

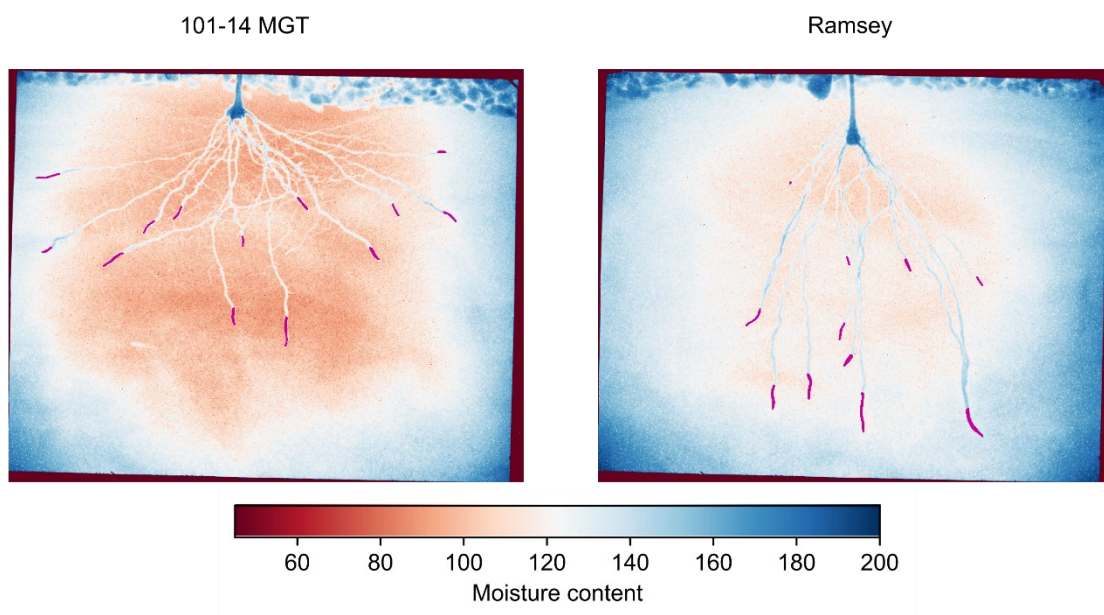


Figure 2.5. Growth responses to moisture

Neutron radiographs of root systems with moisture content and 1st order root tip growth shown. Blue indicates areas with more water and red indicates areas with less water (unitless). Pink root tips indicate root growth of 1st order roots over two days. (Left) Rootstock 101-14, drought susceptible rootstock showing no root growth response to moisture. (Right) Ramsey, drought resistant rootstock showing growth responses to moisture.

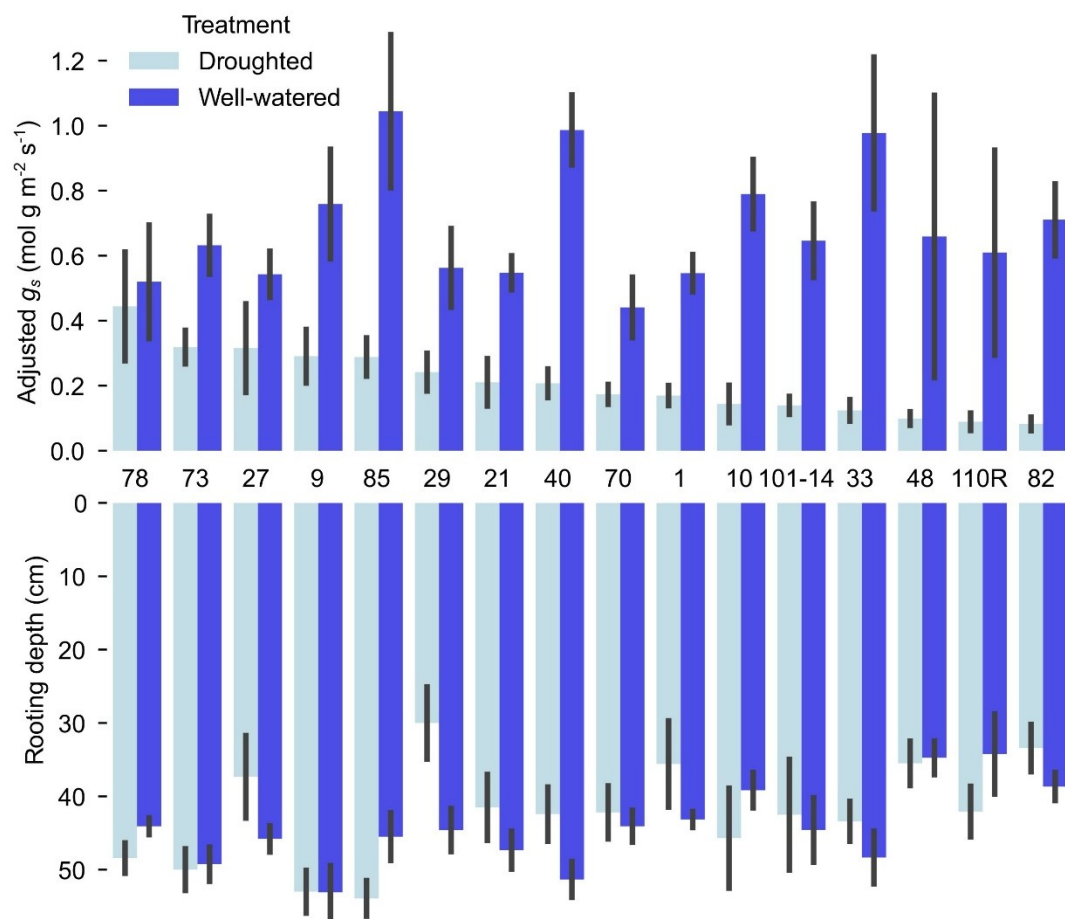


Figure 2.6. Greenhouse assay

Adjusted g_s and rooting depth at day 49 for each genotype for both well-watered and drought treatments in the greenhouse experiment.

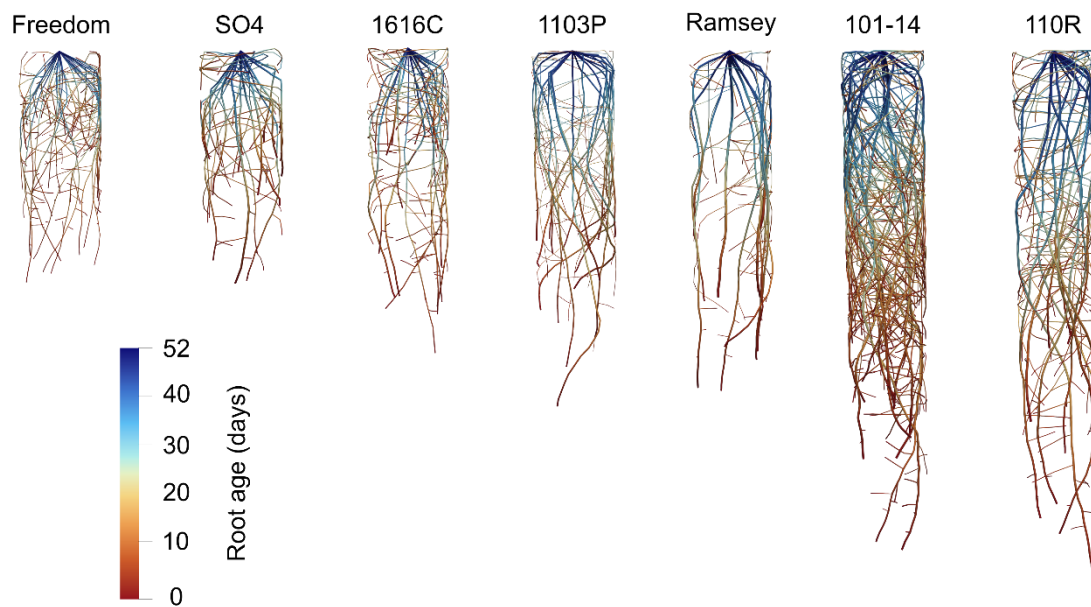


Figure 2.7. SFPM simulations

Example root structures produced from CPlantBox after 49 days of simulated growth for seven commercial rootstocks.

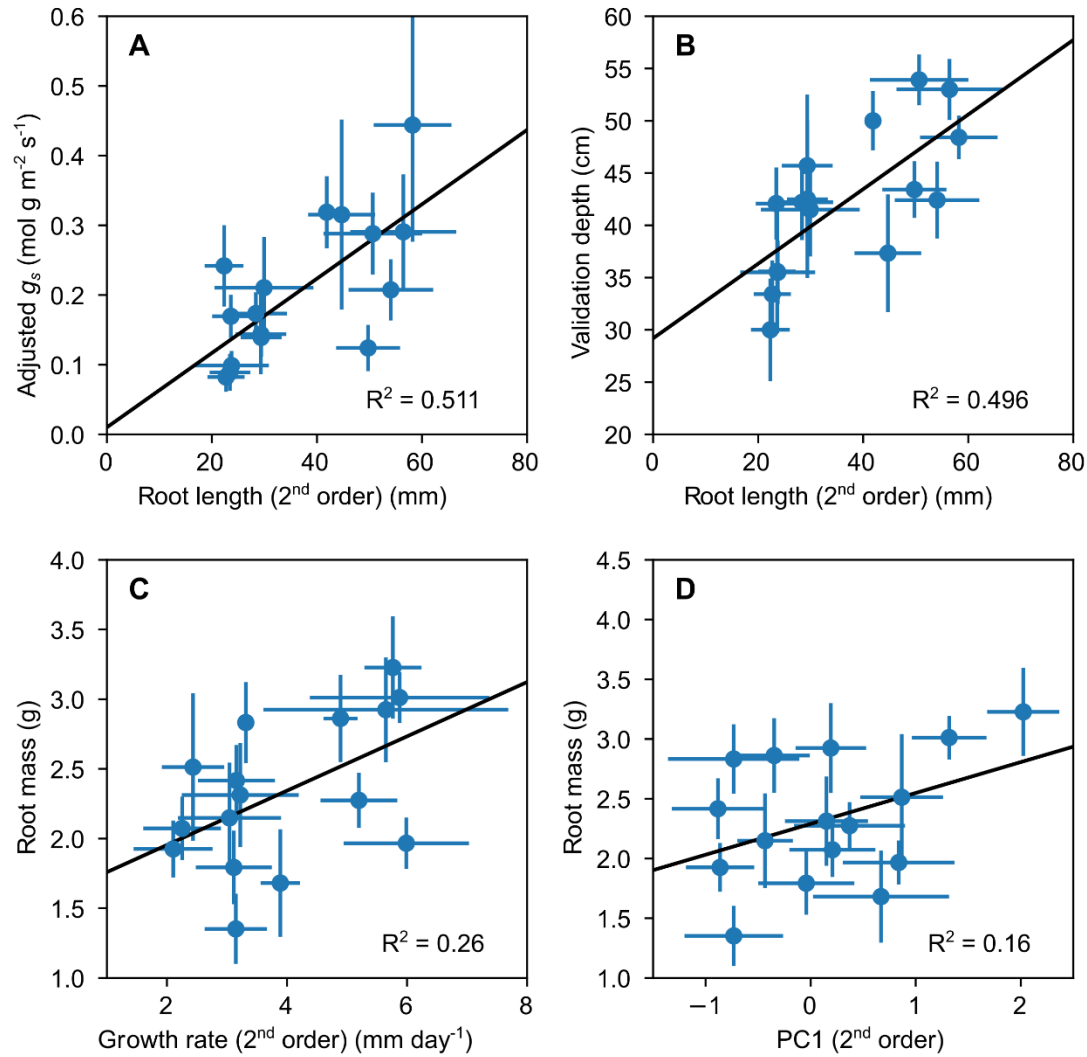


Figure 2.8. Regressions between *F1* genotypes and greenhouse traits

Linear regressions between trait predictors from neutron radiography and validation phenotypes for each genotype as measured in the greenhouse experiment after 49 days. Each point represents the mean of each genotype for the observed and predicted values. Error bars indicate the standard error of the mean. The black line indicates the line fit from the linear model. A: The regression between root length of 2nd order roots and the adjusted g_s measured in the greenhouse experiment. B: The regression between root length of 2nd order roots and the actual depth measured in the greenhouse experiment. C: The regression between the growth rate of 2nd order roots and the actual root mass measured in the greenhouse experiment. D: The regression between PC1 for 2nd order roots and the actual root mass measured in the greenhouse.

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2.10. Supplemental materials

Table S 2.1. Pairwise correlations of traits for both group and root orders

Group	Root order	Traits	Root length	Growth rate	Diameter	Num adv. Roots	Tip growth direction	Growth deviation
F1	1st	Growth rate	0.81	-				
		Diameter	0.31	0.26	-			
		Num adv. Roots	-0.03	-0.10	-0.23	-		
		Tip growth direction	0.20	0.13	0.20	0.00	-	
		Growth deviation	-0.05	-0.05	-0.05	0.12	-0.58	-
		Branch frequency	-0.30	-0.19	-0.05	-0.33	-0.04	0.00
	2nd	Growth rate	0.63	-				
		Diameter	0.51	0.24	-			
		Tip growth direction	0.15	-0.04	0.28		-	
		Growth deviation	-0.15	-0.12	-0.06		-0.38	-
		Branch frequency	0.19	-0.51	0.55		-0.09	0.40
Commercial	1st	Total length	-					
		Growth rate	0.67	-				
		Diameter	0.55	0.66	-			
		Num adv. roots	-0.26	-0.37	-0.73	-		
		Tip growth direction	0.44	0.56	0.73	-0.50	-	
		Growth deviation	-0.29	-0.37	-0.61	0.48	-0.71	-
		Branch frequency	0.17	0.36	0.42	-0.58	0.10	-0.16
	2nd	Total length	-					
		Growth rate	0.57	-				
		Diameter	0.43	0.37	-			
		Tip growth direction	-0.25	-0.03	0.22		-	
		Growth deviation	-0.11	-0.20	-0.08		-0.21	

The commercial rootstocks had higher collinearity than the F1 population, likely due to fewer genotypes and higher number of individual replications creating population structure. For both commercial rootstocks and F1 population rootstocks, some traits were significantly correlated, but r values were low, below 0.4 for the F1 population and below 0.7 for the commercial rootstocks. Growth rate and individual root length (F1 $r = 0.81$, commercial $r = 0.67$), as well as root tip growth direction and growth deviation (F1 $r = -0.58$, commercial $r = -0.71$) were more closely related than the other traits. Collinearity was low for lateral root traits, except for the significant positive correlation between growth rate and root radius (F1 $r = 0.51$, commercial $r = 0.43$). We omitted root length and growth deviation from the manual percentile ranking because of the high collinearity with growth rate and root angle. The other traits showed low collinearity for the F1 genotypes, so we included the rest.

Table S 2.2. Traits for correlation

Neutron radiography traits	Validation traits
Angle of root tip (1st)	Plant depth
Angle of root tip (2nd)	Adjusted g_s
Branch frequency (1st)	Root mass
Diameter (1st)	
Diameter (2nd)	
Growth deviation (1st)	
Growth deviation (2nd)	
Growth rate (1st)	
Growth rate (2nd)	
Growth with moisture (1st)	
Growth with moisture (2nd)	
Modeled depth	
Modeled root water uptake	
PC1 (1st)	
PC1 (2nd)	
Percentile score (1st)	
Percentile score (2nd)	
Root length (1st)	
Root length (2nd)	

We tested correlations between individual neutron radiography root traits and greenhouse experiment root traits. We tested 19 neutron radiography traits against three validation traits, to produce 56 tests.

Table S 2.3. Correlations between prediction and validation data: bottom performing models

Validation metric	Prediction method	Pearson r	R ²	AICc
Plant depth	Growth deviation (2nd)	-0.5	0.252	109.36
	Modeled depth	0.49	0.243	109.55
	Growth rate (1st)	0.46	0.21	110.22
	Growth with moisture (2nd)	-0.42	0.174	110.93
	Diameter (2nd)	0.4	0.16	111.21
	Branch frequency (1st)	-0.35	0.123	111.9
	Modeled root water uptake	-0.33	0.112	112.09
	PC1 (1st)	0.33	0.107	112.19
	Growth deviation (1st)	-0.27	0.071	112.82
	Diameter (1st)	0.27	0.071	112.82
	Growth with moisture (1st)	0.22	0.048	113.21
	Percentile score (2nd)	0.13	0.017	113.73
	Angle of root tip (2nd)	0.07	0.004	113.93
	Angle of root tip (1st)	0	0	114.00
Adjusted g_s	PC1 (2nd)	0.49	0.24	-25.36
	Growth deviation (1st)	-0.45	0.2	-24.54
	Percentile score (2nd)	0.39	0.15	-23.57
	Angle of root tip (2nd)	0.38	0.142	-23.42
	Percentile score (1st)	0.37	0.134	-23.28
	Modeled root water uptake	-0.31	0.096	-22.60
	PC1 (1st)	0.3	0.092	-22.53
	Diameter (2nd)	0.27	0.075	-22.23
	Branch frequency (1st)	-0.26	0.068	-22.11
	Growth deviation (2nd)	-0.17	0.03	-21.45
	Growth with moisture (1st)	0.11	0.011	-21.16
	Angle of root tip (1st)	0.03	0.001	-20.99
	Diameter (1st)	0.02	0.001	-20.98
	Growth with moisture (2nd)	-0.01	0	-20.98
Root mass	Percentile score (1st)	0.28	0.08	30.97
	Diameter (2nd)	0.27	0.07	31.03
	Angle of root tip (1st)	-0.23	0.05	31.39
	Growth deviation (1st)	0.22	0.05	31.48
	Growth deviation (2nd)	-0.20	0.04	31.6
	Diameter (1st)	0.19	0.04	31.64
	Root length (1st)	0.15	0.02	31.89
	Percentile score (2nd)	0.12	0.02	32.00
	Growth rate (1st)	0.08	0.01	32.15
	Modeled root water uptake	0.06	0	32.18
	Angle of root tip (2nd)	0.06	0	32.19
	Growth with moisture (1st)	0.04	0	32.22
	PC1 (1st)	-0.04	0	32.23

Table S 2.4. Genotype means for measured traits from neutron radiography

Root order	Genotype	Root length (mm)	Growth rate (mm day ⁻¹)	Diameter (mm)	Tip angle (degrees)	Growth deviation (degrees)	Branch frequency (mm ⁻¹)	Number adventitious roots
1	1	72	3.49	1.93	140.2	0.8	0.078	4.3
	9	98.2	3.91	2.37	155.6	-21.2	0.036	5
	10	113.3	5.32	1.67	150.1	-13.6	0.04	8.3
	21	85.9	2.65	2.02	144.7	-2.3	0.079	8.3
	27	89.6	3.91	1.54	141.8	-18.9	0.094	6
	29	73.5	2.03	1.68	152.2	-15	0.054	6.7
	33	96	6.37	1.66	149.5	-19.2	0.091	5
	40	135.3	7.25	1.67	150.5	-2.4	0.025	7
	48	70	2.78	1.71	159.6	-6.8	0.06	8.3
	70	78.1	3.7	1.88	132.9	-6	0.047	6
	73	87.9	3.67	1.63	127.6	5.2	0.023	9.3
	78	139.6	11.39	1.88	160.3	-30.1	0.031	5
	82	84.9	2.19	1.81	136.8	2	0.057	6.3
	85	100.2	4.82	1.74	142.6	-11.2	0.072	5.7
	101-14	75.2	2.46	1.62	140.3	-7.9	0.068	6.9
	110R	93.5	4.37	2.15	155.3	-10.7	0.051	6.3
2	1	23.6	3.22	1.12	106.4	-4.3		
	9	56.4	5.77	1.53	116.5	-9.4		
	10	29.3	3.04	1.05	91.4	-5.2		
	21	29.9	2.25	1.12	111	-4.2		
	27	44.7	3.89	1.12	115.4	-5.3		
	29	22.3	3.15	0.72	110.5	0.3		
	33	49.7	5.2	0.93	110.9	-2.1		
	40	54.1	5.65	0.81	110.8	-7.1		
	48	23.7	3.16	0.86	99.4	1.6		
	70	28.4	4.89	0.82	98.8	-5.2		
	73	41.9	3.31	0.79	108.2	6.3		
	78	58.2	5.99	1.04	111.7	-9.1		
	82	22.7	2.1	0.86	108.5	4.8		
	85	50.6	5.88	0.97	107.1	-25.3		
	101-14	29.4	2.43	1.17	122.7	-6.3		
	110R	23.4	3.12	0.86	99.4	-19.9		

Mean trait values for the 14 F1 genotypes and parents used in the greenhouse experiment.

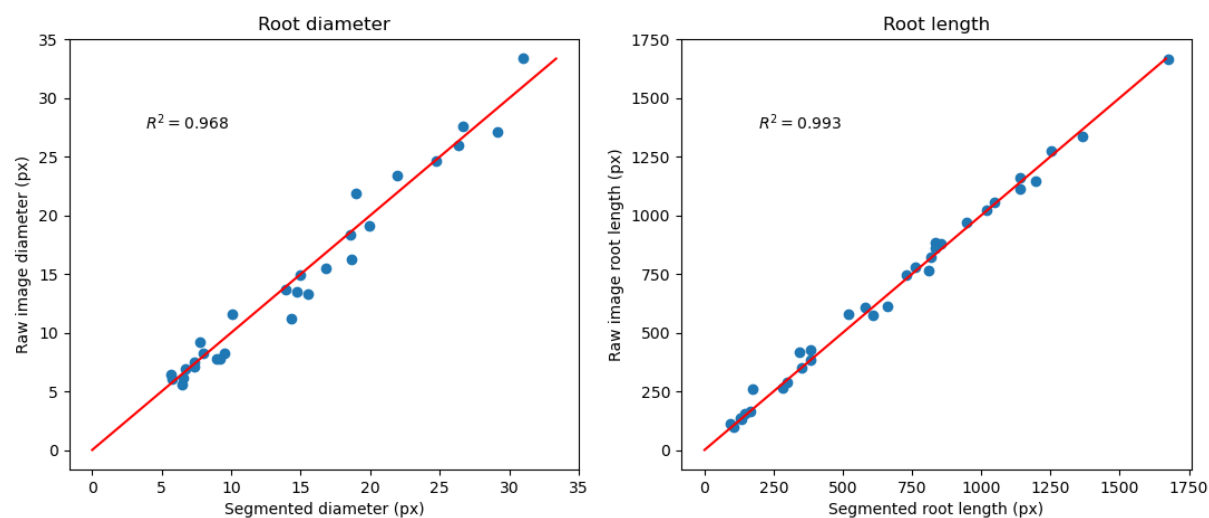


Figure S 2.1. Ground truth comparison for image segmentation

Ground truth comparison to segmented images for two traits, root diameter and root length. The red line is the expected values of diameter and root length with slope 1. R^2 was calculated for each dataset against expected validation values from raw images.

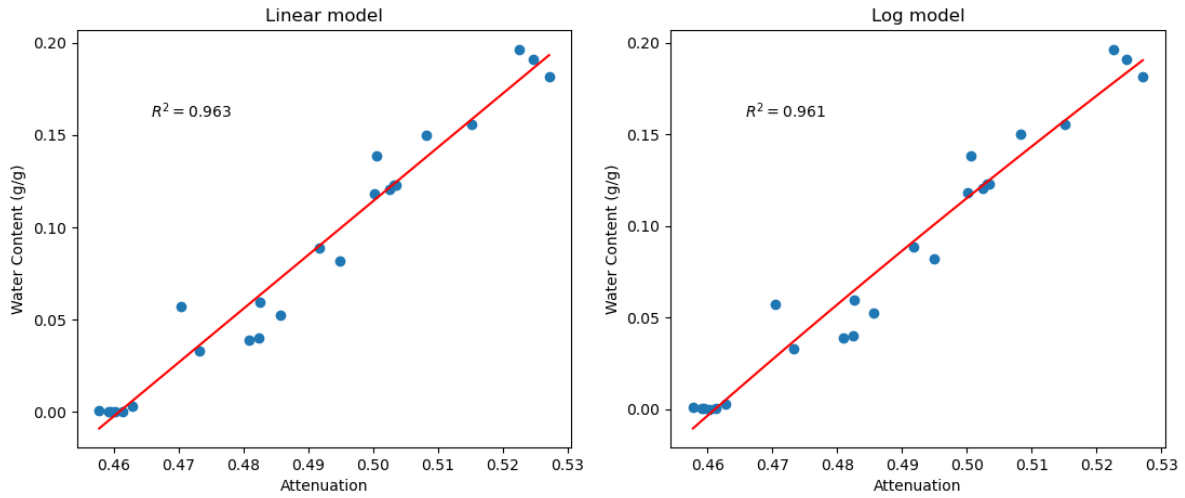


Figure S 2.2. Neutron radiography moisture ground truth

Comparison of two moisture attenuation models. The red lines denote fitted models. R^2 is presented for each respective model. A logistic and linear model were tested against validation soil moisture data. Neutron attenuation normally follows an adaptation of the Beer – Lambert law (Moradi et al., 2015). Oswald et al. (2008) demonstrated a model to estimate soil moisture based on the Beer – Lambert law, but due to differences in neutron flux and imaging setup between calibration and phenotyping experiments, we could not fit a logistic model to the commercial rootstock data. We found that our observed soil moisture values occurred in a limited range, and a linear model performed equally as well as a logistic model on our calibration data, so we assumed pixel values were proportional to soil moisture (linear model $R^2 = 0.96$, logistic model $R^2 = 0.96$).

Table S 2.5. CPlantBox simulation parameters

Parameter	Description	Units	Type	Variable by genotype	Additional information
Root type	Root order		Structural	No	
MaxB	Number of shoot borne adventitious roots		Structural	Yes	
r	Growth rate	cm day ⁻¹	Structural	Yes	
La	Root apical zone	cm	Structural	Yes	
Lb	Root basal zone	cm	Structural	Yes	
ln	Distance between lateral roots	cm	Structural	Yes	
a	Root radius	cm	Structural	Yes	
Theta	Angle between root and parent root	radians	Structural	Yes	
TropismT	Type of tropism		Structural	No	
TropismN	Number of trials of root tropism		Structural	Yes	
TropismS	Mean value of expected change of root tropism	cm ⁻¹	Structural	No	
kx	Axial root conductivity	cm ³ s ⁻¹ kPa ⁻¹	Functional	No	
kr	Radial root conductivity	cm ³ s ⁻¹ kPa ⁻¹	Functional	No	Figure S3
	Reduction of radial root conductivity based on age	days	Functional	No	
Age dependent	age	days	Functional	No	
PlantContainer	Pot dimensions	cm	Simulation	No	
rs age	Initial plant age	days	Simulation	No	
sim time	Simulation time	days	Simulation	No	
soil	Soil type		Simulation	No	Table S6
	Initial soil matric potential	cm	Simulation	No	
initial sm	Potential	cm	Simulation	No	
PET	evapotranspiration	cm ³	Simulation	No	

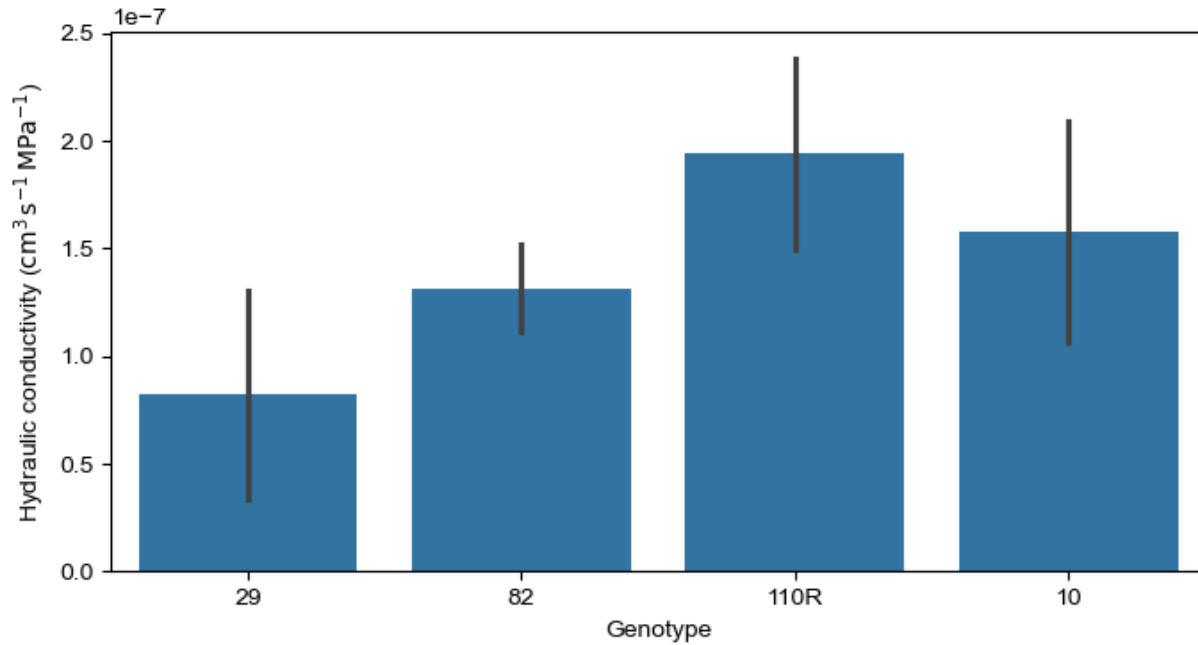


Figure S 2.3. Hydraulic conductivity of fine roots

Measured fine root hydraulic conductivity of each genotype (NS, ANOVA type I). Error bars indicate the standard error of the mean. We conducted an experiment in November of 2023 to estimate radial hydraulic conductivity. We performed a neutron radiography experiment the same as previously, but with three replicants of four genotypes of varying root traits. Pots were prepared and grown the same as previously, but over the course of the experiment we watered the pots every Monday, Wednesday, and Friday. At the end of the experiment we destructively harvested the root systems to measure well-watered hydraulic conductivity.

We measured two roots from each plant for fine root hydraulic conductivity (Lp_r). We cut roots of length 8 cm underwater and kept them submerged until measurement. Lp_r was measured with the hydrostatic method using a pressure chamber (Miyamoto et al., 2001). With the root submerged in a water bath inside a pressure chamber (PMS model 1505D), we pressurized the chamber to four increasing values (0.05, 0.10, 0.15, and 0.20). At each pressure the root was left for 300 seconds with a microcentrifuge tube with a tissue wipe to collect any water exuded, and then we weighed the tube. We estimated the surface area of the root by measuring the longitudinal section area with a LI-3000C leaf area meter (LI-COR Environmental, Lincoln, NE) and squaring the result. We did not find any significant differences between genotypes (Fig S.7, ANOVA type I, $p > 0.04$). We took the average of each genotype, then took the average of all genotypes to estimate a radial conductivity value.

Root conductivity was parameterized in CPlantBox according to the “age dependent” parameter designated by the model in the xylemfluxpython module (root_conductivities.py). Radial conductivity from 0-5 days of age was set equal to an experimentally determined value $1.41 \times 10^{-7} \text{ cm}^3 \text{ s}^{-1} \text{ MPa}^{-1}$. Conductivities beyond 5 days were set to 10 fold less than the mean value based on observations from Gambetta et al., (2013). Conductivities for roots 2 to 5 days old were set to decrease linearly from the tip conductivity value to the reduced value at secondary growth. We set the axial conductivity values to be 100 times higher than the radial conductivity values (Steudle & Peterson, 1998).

Table S 2.6. Parameters for soil type

Parameter	Soil type	
	Sand	Loam
Q_r	0.01	0.08
Q_s	0.26	0.43
α	0.14	0.04
n	6.6	1.6
K_s	712	50

Parameterization of soil type and PET. For the F1 population, we ran the model with the parameters below for sand to be directly comparable to the validation experiment (Table S.4). We obtained values from Hilberts et al. (2005) and Guarracino (2007), and set the soil matric potential at field capacity to -7.4 kPa (Krueger & Ochsner, 2024). Due to the model severely underestimating some genotypes' depths, we set the potential evapotranspiration to 4 mL day⁻¹ to prevent the plants from reaching the predetermined wilting point (water potential = 15000 cm). We parameterized commercial rootstocks to loam, since drought resistance estimates overwhelmingly come from field trials with natural soil.

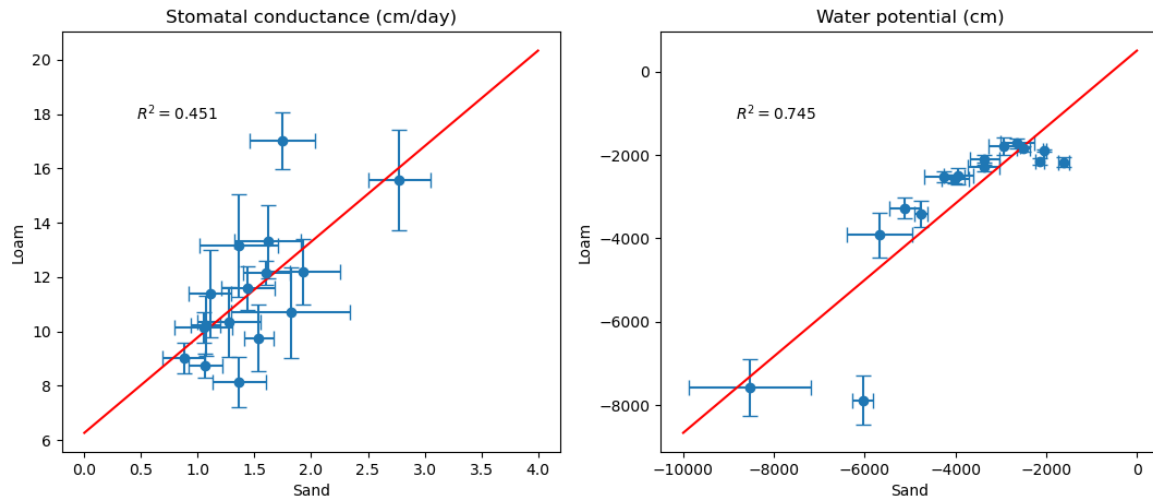


Figure S 2.4. Regressions between sand and loam parameterizations for the F1 population.

Error bars indicate standard error of the mean. The red line is a fitted linear model to the data. To test if soil type caused large differences in results, we also ran simulations for the F1 population with loam as soil type, which was standard in the model. The results showed good collinearity (Fig S.7), so we determined altering the soil parameters from the standard parameters in the model likely did not have a serious effect on the output of the model, or cause differences between the commercial and F1 simulation.

The ranges of simulated rooting depth from the SFPM largely overlapped with the observed values, with observed values ranging from 12.5 to 64.5 cm, and the modeled values ranging from 10.5 to 75.8 cm. The model somewhat underestimated rooting depth for most genotypes, except for two genotypes that were overestimated.

Chapter 3: Linking Root System Architecture to Aboveground Drought Responses in Grape Rootstocks with Contrasting Drought Resistance

3.1. Abstract

Drought resistant grapevine rootstocks can be leveraged to maintain quality and yield under dry conditions, but the traits that confer drought resistance in rootstocks are poorly understood. We tested whether root traits that would promote deeper soil exploration would enable 110R to reduce vine water stress despite soil drying, including maintaining higher gas exchange rates and water potentials. We compared field-grown vines of a drought resistant (110R) and drought susceptible (101-14) rootstock grown at the same site and grafted to the same scion (Chardonnay). We measured aboveground physiology and soil matric potentials for three growing seasons at a hot (Winkler zone IV) experimental vineyard in Davis, California with a deep ($\geq 3.8\text{m}$) soil profile, under dry-farmed or deficit irrigation conditions, then measured root traits from soil cores and vine excavations. Drought resistant 110R had significantly thicker, more steeply angled, and sparser (less branched) roots than 101-14 ($p < 0.001$). 110R also had a smaller overall root length, but a deeper root distribution ($p < 0.001$). Vines grafted to 110R also maintained significantly higher mean gas exchange rates and predawn water potentials in a severely droughted growing season and had less sensitive responses in gas exchange and water potential to soil drying ($p < 0.05$). These findings contribute to evidence of a link between root architecture traits and aboveground drought responses in the field for grape rootstocks. We expect identifying trait differences to inform breeding efforts to enhance rootstock drought resistance.

3.2. Introduction

Severe droughts are commonplace in growing regions with Mediterranean-like climatic conditions, which challenges grape production in these regions (Del Pozo et al., 2019; Van Leeuwen & Darriet, 2016). Drought resistant rootstocks could be a tool to maintain grape yield and quality under these conditions (Ollat et al., 2016). One way grapevine rootstocks confer drought resistance and maintain physiological function in the scion is by exploring the soil effectively for water, avoiding low vine water potentials (Archer & Saayman, 2018; Bauerle et al., 2008; Serra et al., 2014; Smart et al., 2012). Previous research has indicated that this may be a more important drought resistance trait for grapevines than tolerance for low water potentials (Alsina et al., 2011; Charrier et al., 2018). Despite this finding, the root system traits and ideotypes that improve drought resistance in the field are not well understood, due to the challenges of measuring root traits in mature field-grown vines (Bernardo et al., 2025; Wolpert et al., 2002). Historically, grapevine rootstock drought resistance has been determined from anecdotal and observational evidence from growers and scientists, vigor and yield, or field studies (Rahemi et al., 2022; Serra et al., 2014), which complicates selection and breeding of rootstocks based on root system traits.

Though there is no one accepted drought resistant ideotype for grape rootstocks previous work has identified root system traits that could contribute to drought resistance (Bernardo et al., 2025). Deeper and steeper (more downwardly angled) roots, as well as faster root growth recovery after drought have been identified as potentially advantageous traits for drought resistance (Cuneo et al., 2021; Fort et al., 2017). Studies in small potted plants have shown that the drought resistant rootstock 110 Richter (110R) has thicker roots and a sparser and steeper

root system than the drought susceptible rootstock 101-14 Millardet et de Grasset (101-14) (Alonso-Forn et al., 2025; Fort et al., 2017; Reingwirtz et al., 2021), though it is unknown to what extent these differences are maintained in mature, field-grown vines. Finally, although there is no clear relationship between rootstock and total rooting depth (Smart et al., 2006), field studies have identified differences in root distributions with depth between grape rootstocks (Morano & Kliewer, 1994; Perry et al., 1983; Swanepoel & Southey, 1989). Because of this, vertical root distribution with depth has been proposed as an alternative to total rooting depth as a drought resistance trait (Gale & Grigal, 1987; Perry et al., 1983; Smart et al., 2006).

While deep rooting behavior of rootstocks is a sought-after trait for drought resistance, there are also several difficulties associated with measuring grapevine root systems. Grapevines are relatively deeply rooting with roots observed as deep as six meters and their maximum lateral spread estimated as wide as 10 meters (Richards, 1983; Seguin, 1972; Smart et al., 2006), which makes it difficult to measure their entire root system in the field. Root system size and root system architecture are also highly dependent on soil type (Smart et al., 2006; Southey & Archer, 1988) and management decisions, such as irrigation and vine spacing, which makes it difficult to compare these traits across rootstocks from different sites (Archer et al., 1988; Soar & Loveys, 2007; Van Zyl, 1988).

Deep root systems have been linked to drought resistance ecologically and agronomically in trees (Gale & Grigal, 1987; Schenk & Jackson, 2005; Song et al., 2020), but to our knowledge, no research has directly linked root distributions to drought resistance in grapevines. Previous research has investigated root distributions between different grapevines without measuring physiology (Perry et al., 1983; Smart et al., 2006; Swanepoel & Southey, 1989), measured above ground physiology only (Ezzahouani & Williams, 1995; Stevens et al., 2010), linked root growth

and hydraulic redistribution to physiology (Alsina et al., 2011; Romero et al., 2025), and in two cases compared root growth with depth between rootstocks of varying drought resistance (Alsina et al., 2011; Bauerle et al., 2008). Bauerle et al. (2008) and Alsina et al. (2011) showed that under drought stress, rootstock 1103P had higher levels of root growth at depth (> 60 cm), which increased its hydraulic conductivity and gas exchange compared to drought susceptible 101-14. To our knowledge, this is the closest link between root distributions and drought physiology of field grown grapevines in the literature.

We dug deeper into this phenomenon by studying the relationship between deep rooting and drought resistance by measuring water relations and root system traits for two rootstocks of different drought resistance, 110R and 101-14, over three growing seasons to experimentally link drought resistance with root traits in the field. We studied the grafted rootstocks under drought conditions in an experimental field trial, measuring their physiological responses and root traits. We also installed soil moisture sensors at individual vines to estimate physiological responses to drought on an individual plant basis across the vineyard. We tested the following hypotheses: (i) 110R will have a deeper, sparser root distribution, with thicker and more steeply angled roots; (ii) Under drought conditions, 110R will maintain higher stomatal conductance, photosynthesis, and water potentials; and (iii) 110R will have less sensitive responses in gas exchange and vine water potentials to drying soil.

3.3. Materials and methods

3.3.1 Growing conditions and experimental design

All measurements were taken between May of 2021 and January of 2026 at a vineyard on the University of California, Davis campus (lat: 38.53, long: -121.79). The site has a Mediterranean climate with hot dry summers and cool rainy winters. The site is in a hot growing region with a 20-year average for growing degree days of 2046 °C (Winkler zone IV), and maximum daily temperatures in the summer ranging from 27 to 42 °C (National Weather Service, n.d.) . The vineyard is 22 meters above sea level and flat (a slope of 0 degrees), on deep alluvial soils with the water table at roughly 15 meters below the soil surface (Department of Water Resources, n.d.). Soils consist of Yolo silt loam and Reiff very fine sandy loam (*SoilWeb*, n.d.). The rows have an east to west orientation, and the vine by row spacing is 2.43 meters by 5.49 meters. The wide row spacing allows the rows to have independent irrigation treatments. The vineyard was managed as a spur pruned bilateral cordon system with vertical shoot position trellising system in 2021 and was transitioned to a cane pruned California Sprawl system in the winter of 2021-2022. The vineyard was planted in 2011, and each row contains consecutive groups of five replicant vines of seven different rootstocks, all grafted to Chardonnay. The order of each rootstock group varies randomly between rows. Here, we focused on two rootstocks (110R and 101-14) to capture extremes in drought resistance. As shown in Figure 3.1, in 2021 and 2022, we selected two vines per rootstock to measure in three locations of the vineyard, resulting in six vines per rootstock. In 2024, we selected three vines per rootstock in the same three locations and added an additional location to the east side of the vineyard, resulting in 12 vines per rootstock. Due to root herbivory in some of the vines prior to 2024, we shifted the vine selection slightly for one of

the existing locations to exclude those vines that had been affected, maintaining at least one vine in common with the previous years. We used vines from the middle of each five-vine group. The measured blocks containing rootstocks of each type were approximately 25-50 meters apart. Textural analysis of the soils in each block showed the southwest corner of the vineyard was classified as silt loam, the northwest corner of the vineyard was loam, and the east side of the vineyard contained a mix of loam and silt loam.

The vineyard was dry farmed until 2021, when continued severe drought prompted installation of drip irrigation to prevent vine death. Each vine had two 2.08 liter per minute drip emitters installed, one on either side of the trunk with an approximately 30 cm drip radius. In 2021, the vineyard was dry farmed except for irrigation on August 9th, 17th and September 9th to prevent vine death. In 2022, the vineyard was irrigated with drip irrigation to 60% of estimated loss from evapotranspiration. There were also five days of rainfall in 2022. The first three on 11 July, 16 July and 14 September totaled less than 0.3 mm each, and the final two days of rain at the end of the growing season (18 and 19 September) resulted in 73.4 mm. In 2024 there was no irrigation or rainfall for the growing season. The vines were trunk suckered in the spring but not hedged during the measurement period to avoid large changes in leaf area. The vineyard floor has a natural cover crop of wild grasses that are disced each spring.

3.3.2. Vine physiology measurements

During the summers of 2021, 2022, and 2024, we measured vine gas exchange and water potential one or twice per week from veraison to harvest. We measured predawn leaf water potentials (Ψ_{PD}) from 04:00-06:00 and midday leaf water potentials (Ψ_{MD}) from 11:00am-13:00pm on the same day on two (2021 and 2022) or one leaf per vine (2024). We sampled mature, sunlit leaves 6-10 nodes below the shoot tip by sealing the leaves in double Whirl-Pak bags (Whirl-Pak Filtration Group, Madison WI) and measured water potential in the lab with a pressure chamber (PMS Instrument Co., Model 1505D). We considered Ψ_{PD} above -0.4 MPa to be mild stress, Ψ_{PD} between -0.4 MPa and -0.8 MPa to be moderate drought stress, and Ψ_{PD} below -0.8 MPa to be severe drought stress, determined from the literature (Deloire et al., 2020; Deloire & Heyns, 2011; Ojeda et al., 2002; Williams & Araujo, 2002). Using the same criteria for leaf selection, we measured gas exchange (g_s) and photosynthesis (A) with a LI-COR 6800 portable photosynthesis system. We used a reference CO₂ concentration of 400 $\mu\text{mol mol}^{-1}$, fan speed of 10,000 rpm, light level of 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$, and ambient temperature and humidity.

3.3.3. Soil moisture and climate monitoring

In 2021, we installed soil matric potential sensors (Campbell Scientific 253-L) at 30 cm from the trunk on the south side of the vines at a depth of 1.2 meters in 2021 ($N = 12$). Soil matric potential (Ψ_{smp}) was measured every minute, and the average was recorded every hour with a datalogger (Campbell Scientific CR1000X). We used the measurements from 06:00 on the same sampling dates as the vine physiology measurements to test relationships with physiological variables during 2021 and 2022. Numerous probes were damaged after the 2022 growing season, so we excluded the Ψ_{smp} data for 2024 from the physiology analyses. We compiled climate data

from each year from the Davis station of the California Irrigation Management Information System (Hart et al., 2009).

3.3.4. Canopy and yield measurements

In 2021, we harvested the fruit once it reached a target Brix (~24 degrees) and measured the yield for each vine ($N = 12$). We did not attempt to measure yield in 2024 due to severe herbivory on the clusters for many vines. In 2021, we measured vigor with pruning weights, by measuring the mass of the excised shoots for each vine in the winter after the growing season ($N = 12$). In 2024, we measured vigor as total canopy leaf biomass. After harvest in September, we randomly selected one side of each vine and removed all leaves from every shoot, oven-dried them at 70 °C for at least 48 hours and multiplied the dry weight by two to estimate the total leaf mass per vine ($N = 24$).

3.3.5. Root distribution measurements

We measured root distribution with depth for a subset of vines ($N=16$, 8 per genotype) in September of 2024 with a 7822DT drill rig (Geoprobe Systems. Salina, KS). We collected soil cores 7.7 cm in diameter and 2.43 m deep at three distances from the base of the vine (0.5, 1.0 and 1.5 meters) extending straight out into the space between rows ($N = 3$ cores per vine). Root distributions were measured by dividing each core into eight 30 cm depth intervals and passing the sample through a 1mm fine mesh sieve with water. Roots remaining in the sieve were washed and scanned with an EPSON 12000XL scanner using photo mode to acquire root images. After image acquisition, the roots were dried in an oven at 70 degrees C for at least 48 hours, and the dry biomass was measured for each root core. Images were processed with a custom semi-automated python script (Dudley et al., In press) to measure the number of roots,

root length, mean diameter, branching frequency (branches per unit length), and specific root length (SRL), measured as the ratio of root length to root mass. To test for the presence of roots deeper than 2.43 meters, we used a hand auger (AMS inc, American Falls, ID) to core two additional holes to four meters depth, one for each rootstock, and measured the presence or absence of roots approximately every 15 cm.

3.3.6. Root system excavation

In March of 2025, we excavated around the base of the root system for a subset of experimental vines ($N = 6$, $N = 3$ per genotype) and measured root traits. We first excavated a two by two by two meter trench 60 cm away from the north side of each vine. Then, using an Air Spade 2000 (Guardair Corporation, Chicopee MA), we excavated a hole around the base of the vine approximately 75 cm deep and 120 cm in diameter by blasting soil away from the root system. For a representative subset of roots in each root system, we measured the total number of root orders visible for each vine, and, for each root order, we measured maximum root diameter, branching frequency (number of branches divided by root length), branching angles in relation to parent roots, and vertical root angle direction (where zero degrees is horizontal, negative is down, and positive is up). We measured root traits for all adventitious roots (1st order), then for each adventitious root we measured a random subset of one to nine daughter roots of all orders, always measuring at least one root per order. In total, we measured 153 total roots for 110R, and 202 total roots for 101-14.

3.3.7. Statistical analysis

All statistical analyses were carried out in Python 3.9.21. To examine differences in physiology between years, for each vine within each year, we took the average of all physiology traits from veraison (15 July) to approximately one month after harvest (8 September) and performed Tukey

pairwise comparisons to determine significance between years. For the rest of the analyses, the three growing seasons were analyzed separately due to slight differences in management and measured vines. To determine differences in physiology between rootstocks for each year, we fitted mixed effects models. Each measured physiological trait was the response variable; rootstock, date, and block were fit as categorical fixed effects; each individual vine was fit as a random effect with a fixed slope and random intercept. Several models failed to converge, so to simplify the model we repeated those models excluding the block term. To further examine differences between rootstocks for specific dates, we used two-sided, two-sample t-tests as post-hoc tests for each trait and date and corrected the resulting p-values using the SciPy multiple tests false discovery rate (FDR) correction within each trait. To test relationships between physiology and soil moisture, we used a mixed effects model with each physiological trait as the response variable, rootstock, Ψ_{smp} , and block as categorical fixed effects, and individual vine as a random effect. Models that failed to converge were run again without the block term.

To compare root distributions we compared two metrics: We fitted the exponential parameter β to the root system distributions as done in in Gale and Grigal (1987) and Smart et al. (2006) using the equation:

$$\gamma = 1 - \beta^d$$

where γ is the cumulative root fraction at each depth, d is the measured depth, and β is a fitted parameter. We then fit the best value of β for each root system to determine differences in rooting distributions. Due to relatively poor fits from the model, we also estimated the depth at which 50% of the cumulative root length occurred (D50) with linear imputation when it occurred between measured depths. For each vine, we pooled all three sampling distances because there was not a clear pattern of reduced root density extending into the interrow (Figure S 3.1). We

compared values of D50, β , estimated leaf area, and yield between genotypes with a two-sided, two-sample t-test. For the other traits from the root cores, we used a type II ANOVA with depth and rootstock as predictors. To compare root traits from whole root excavation, first we averaged root traits within each root order and individual vine, resulting in one value per order, per vine. For each measured trait, we ran a type III ANOVA with root order, genotype, and root order:genotype interaction as predictors.

3.4 Results

3.4.1. 2021 was a more severe drought year

The weather and growing conditions, as well as the soil matric potential differed between measured years (Table 3.1, Figure 3.2). Drought, as measured with matric potential and precipitation, was the most severe in 2021, followed by 2024, then 2022 (Table 3.1). Soil matric potentials showed different trajectories over the growing season, but at the end of the growing season, after harvest but before irrigation and rain events (31st August), there were significant differences in Ψ_{smp} between years (Type I ANOVA, $p < 0.05$) (Figure 3.2). Ψ_{smp} means and standard errors at 1.2 m depth were -111.9 ± 3.9 kPa in 2021, -69.7 ± 7.3 kPa in 2022, and -92.7 ± 17.6 kPa in 2024. These differences were mostly due to water inputs, since there were large differences in precipitation between growing seasons, but only minor differences in growing degree days, reference evapotranspiration, and soil temperature. Precipitation was considerably lower in the 2021 water year (1st October 2020-30th September 2021) (133.5 mm) than in the water years for 2022 (475.7 mm) or 2024 (408.1 mm) (Table 3.1).

3.4.2. Rootstock performance differs under severe drought

Consistent with Ψ_{smp} , Ψ_{PD} and Ψ_{MD} also indicated that 2021 was the driest year over the course of the measurement period (July 15th to September 8th) (Table 3.2). The mean Ψ_{MD} in 2021 (-1.62 ± 0.015 MPa) was significantly drier than in 2022 (-1.56 ± 0.02 MPa) and 2024 (-1.55 ± 0.013 MPa). All three years showed significantly different mean Ψ_{PD} from each other. 2021 showed the lowest Ψ_{PD} (-0.82 ± 0.014 MPa), followed by 2022 (-0.65 ± 0.011 MPa), and 2024 (-0.39 ± 0.005 MPa) (Tukey pairwise comparisons, $p < 0.05$). g_s and A were significantly lower in 2024,

potentially due to differences in management and canopy size between the years. 2021 and 2022 did not differ significantly in their g_s , but A was significantly greater in 2022 than 2021.

Rootstock type significantly affected vine A , g_s , Ψ_{PD} , and Ψ_{MD} , but only in the driest growing season (Figures 3.3 and 3.4, Table 3.3). In 2021, A , g_s , and Ψ_{PD} were significantly higher in vines grafted to 110R than 101-14, while Ψ_{MD} was higher in vines grafted to 101-14 (mixed effects model, $p < 0.05$). From the posthoc t-tests measuring rootstock differences in physiology for each individual date, A and g_s showed the most consistent differences over the growing season, with significantly higher values in 110R for five out of 14 measurement days, compared to two out of 14 days for Ψ_{PD} ($p < 0.05$). In 2021 there were no significant differences between rootstocks in Ψ_{MD} ($p > 0.05$) for any single dates. In 2022 and 2024 there were no significant differences between rootstocks in A , g_s , Ψ_{PD} , or Ψ_{MD} for any single dates ($p > 0.05$).

3.4.3. 101-14 is more sensitive to soil drying

Rootstocks showed different levels of physiological responses to drying soil in 2021, where 101-14 showed stronger responses than 110R (Figure 3.5, Table 3.4). For both rootstocks, A and g_s declined with Ψ_{smp} and there was a significant interactive effect of Ψ_{smp} and rootstock. 110R had significantly lower g_s at full soil hydration ($\Psi_{smp} = 0$ kPa), but the slope of the decline in g_s with soil drying was significantly shallower. 110R had higher Ψ_{MD} at full soil hydration that slightly declined with Ψ_{smp} , while midday Ψ_{MD} slightly increased with soil drying for 101-14. In 2022, there were no significant differences between rootstocks or interactions between rootstock and Ψ_{smp} for any of the physiology variables.

3.4.4. 110R has a deeper, steeper, and sparser root system

110R had a deeper root distribution than 101-14 as measured with soil coring ($N = 16$), and both the D50 and β metrics agreed (Figure 3.6, Table 3.5). 110R had a D50 value of 132.9 ± 2.9 cm,

while 101-14 had a D50 value of 115.8 ± 4.3 indicating a deeper value that 50% of the root length occurred (t-test, $p < 0.001$). 110R also had a higher β value (0.9937 ± 0.0002) than 101-14 (0.9929 ± 0.0002), indicating a proportionately deeper root system (Gale & Grigal, 1987) (t-test, $p < 0.001$).

Root cores showed that 110R also had a sparser root system. Both the total root length and total number of roots were lower for 110R for the same excavated soil volume (Table 3.5) (ANOVA, $p < 0.001$). Differences in root length and number were more pronounced in the top 1.25 meters of the root distribution, with no significance observed for deeper soil (Figure 3.6) (posthoc t-tests, $p < 0.05$). 110R also had a lower branching frequency than 101-14 (ANOVA, $p < 0.001$). Posthoc tests did not show significant differences for any one depth, but the branching frequency was significantly different over all depths pooled. 110R had thicker roots and higher SRL, however these differences were not significant (Table 3.5). Hand coring to four meters depth showed roots to a depth of 3.8 meters, and multiple roots below 2.4 meters for both rootstocks, though we were not able to determine rootstock differences or measure distributions at these depths.

Root system excavations showed that both root order and rootstock had a significant effect on root diameter, branch angle from the parent root, and vertical branch angle ($N = 6$) ($p < 0.05$) (Figure 3.7). 110R had more vertical roots that branched at a larger angle from the parent root for all root orders (Figure 3.8). There was a significant interaction between root order and rootstock for root diameter. 110R had thicker roots for the first five root orders, while roots were thicker in 101-14 for the sixth root order. Distance between branch points was not significantly different between root orders or rootstocks.

3.4.5. Rootstocks showed similar yield and vigor

In 2021 we observed 110R had higher yield (5.42 ± 0.52 kg/vine) and pruning weight (542.6 ± 79.0 g/vine), compared to 101-14's yield (4.06 ± 0.74 kg/vine) and pruning weight (307.9 ± 66.7 g/vine), though these values were not significantly different (t test, $P > 0.05$). In 2024, estimated leaf mass was very similar for 110R (0.99 ± 0.10 kg/vine) and 101-14 (1.04 ± 0.08 kg/vine).

3.5. Discussion

Overall, we found that the rootstock 110R had a deeper, sparser, steeper, and less branched root system with thicker roots than 101-14 in mature, field-grown vines grafted to the same scion. The physiological differences between rootstocks were consistent with these differences in root architecture, with vines grafted to 110R maintaining higher gas exchange rates and water potentials during severe drought conditions. Under severe drought, 110R also had less sensitive responses in gas exchange and water potential to drying soil.

3.5.1. In the field, 110R outperforms 101-14 under severe, but not moderate, drought

Under severe drought stress in 2021 ($\Psi_{PD} < -0.80$ MPa), 110R maintained higher A , g_s , and Ψ_{PD} throughout the measurement period, which lasted approximately from veraison (mid-July) to after harvest (mid-September) (Figures 3.3 and 3.4). In addition, the mixed effects models investigating physiological responses to soil moisture show that both A and g_s reduced much more strongly with drying soil for 101-14 than for 110R (Table 3.4, Figure 3.5). These findings are consistent with previous research investigating root hydraulic traits. Drought resistant rootstocks have shown advantageous functional root traits such as preferential growth in wet soil (Alsina et al., 2011; Bauerle et al., 2008; Padgett-Johnson, 1999; Smart et al., 2006; Soar & Loveys, 2007), timing of active root growth and hydraulic conductance throughout the season (Alsina et al., 2011; Bauerle et al., 2008), and fine root anatomy and physiology (Barrientos-Sanhueza et al., 2024; Cuneo et al., 2021; Gambetta et al., 2013), which could allow for maintained physiological function under drought and dampen extreme responses to drought stress.

Our results agree with drought classifications from the literature that rank 110R as more drought resistant than 101-14, largely based on nursery guides, pot studies, and field classifications

based on yield and vigor (Carbonneau, 1985; Dry, 2015; Pritchard, n.d.; Rahemi et al., 2022; Verslype et al., 2023). These findings are also consistent with numerous pot studies demonstrating that 110R maintains greater root hydraulic function under drought (Bartlett et al., 2022; Cuneo et al., 2021) and maintains greater stomatal conductance under drought (Carbonneau, 1985).

Though we observed differences between the rootstocks under severe drought in 2021, we did not observe differences in more moderate drought years of 2022 and 2024, and previous field studies have shown mixed results. From site to site, physiology can be partially driven by the underlying soil characteristics (Ehosioko et al., 2026), making comparisons between field studies difficult. Previous field studies directly comparing drought physiology of the two rootstocks in the field showed conflicting evidence; For 110R, Stevens et al. (2010) found lower water potentials and gas exchange compared to 101-14 under drought, Theocharis et al. (2024) showed higher water potentials and gas exchange, while Ezzahouani & Williams (1995) showed no differences. Our results suggest that the previous studies may not have subjected the plants to sufficient drought stress to see consistent differences in rootstock performance. The midday leaf water potentials in these studies were all above -1.40 MPa, and Theocharis et al. (2024) reported predawn water potentials above -0.53 MPa. We only saw meaningful separation in the physiology of the rootstocks when the plants had leaf water potentials below -1.40 MPa and had very low predawn water potential values (below -0.80 MPa).

Variability due to field and soil conditions are also apparent in the observed physiological responses to drying soil. While the effect of soil matric potential on Ψ_{MD} itself was not significant, significant interaction resulted in a regression for 101-14 in the opposite direction than expected. 101-14 had a negative slope compared to 110R's slight positive slope, indicating

a more negative Ψ_{MD} in wetter soil (Figure 3.4). Though unexpected, this outcome could be due to several reasons. Stomatal closure in 101-14 in response to low soil moisture could have reduced vine transpiration so strongly that Ψ_{MD} increased, resulting in a positive slope as well as higher Ψ_{MD} later in the drought (Tombesi et al., 2015) (Figures 3.4 and 3.5). Alternatively, differing root system responses to soil moisture and irrigation could explain the unexpected outcome. 101-14 could have failed to recover its root growth and hydraulic conductivity quickly in response to irrigation when compared to 110R, causing Ψ_{MD} in 101-14 to continue to decline despite increasing Ψ_{smp} after irrigation (Cuneo et al., 2021; Smart et al., 2012). To address outliers with isolated high Ψ_{smp} after the third irrigation event in 2021 (Figure 3.5), we repeated the mixed effects model with all data points after 9 September removed, and the results remained comparable (Table S 3.1), so we kept the results from the full model.

3.5.2. Field grown 110R has more drought resistant root traits than 101-14

This study only focused on two rootstocks, so while it is not possible to determine which root system traits are most strongly correlated with drought resistance, the results are consistent with previous findings that suggest sparser, deeper, and thicker root systems may perform better under drought conditions (Fort et al., 2017; Reingwirtz et al., 2021). In our study, 110R also had a significantly higher value for both D50 and β than 101-14, indicating a higher portion of roots in deeper soil layers, strengthening the argument that rootstock affects root distribution with depth (Perry et al., 1983; Swanepoel & Southey, 1989). Higher proportions of roots in deeper soil layers could explain the less severe responses to soil drying and better maintenance of A and g_s of 110R during drought. 110R also had a sparser root system than 101-14, with lower branch frequencies and lower root length per unit volume of soil. Differences in root length density between the rootstocks could also partially explain why the rootstocks diverged in 2021, the

driest year. 101-14 had higher measured root length at all depths, which could have resulted in a root uptake tragedy of the commons, where high root density caused quicker depletion of water (Cabal et al., 2024; Gersani et al., 2001; Maina et al., 2002). In the dry year especially, depletion of water early in the growing season could have caused reduced physiology later compared to 110R, which had lower root density.

In dry soil, grapevine roots halt growth, which reduces hydraulic conductivity of the root system (Alsina et al., 2011; Cuneo et al., 2016, 2018; Zwieniecki et al., 2002). Thus, having a higher proportion of roots in deeper soil layers could allow 110R to reduce losses in hydraulic conductivity when compared to 101-14, and ensure continued water uptake even as the upper layers of soil dry. Shallower root systems, such as for 101-14, would have reduced A and g_s as well as more severe responses to drying soil when compared to the deep root system of 110R. Deep rooting distributions may be especially important in dry farmed or rarely irrigated vineyards in mediterranean climates, like the one in the study, where the top layers of the soil are not frequently re-wetted by irrigation or rain.

From the root system excavation, we also observed thicker roots, steeper root angles, and wider branch angles for 110R. Interestingly, differences we observed in the field regarding root angle, branch frequency, root distribution, and root density have been observed previously in studies involving greenhouse grown plants (Alonso-Forn et al., 2025; Fort et al., 2017; Reingwirtz et al., 2021). We also observed the same differences in small plants in previous experiments (4 weeks old) of root angle, thickness, and branch frequency (Dudley et al., In press) which indicates that these traits likely scale from small plants to the field. To fully understand how consistently root traits scale from small plants to the field and the effect root traits play on drought resistance,

future work will need to study a wider array of different rootstocks with a variety of root traits and drought resistance strategies.

3.5.3. Management practices could have contributed to deep, expansive root systems

Management choices, combined with functional root traits, could have affected the observed drought resistance of the vines. Coarse textured deep soils, limited irrigation, and reduced root uptake competition due to wide vine spacing may have caused the root systems to be larger, sparser, and deeper than more traditionally farmed vineyards (Archer et al., 1988; Archer & Saayman, 2018; Archer & Strauss, 1990; Padgett-Johnson, 1999; Southey, 1992; Williams & Smith, 1991). Expansive root systems, without much competition allowed even the drought susceptible rootstock to be mostly dry farmed throughout the duration of the study. As a result of these management practices, we could not measure the entire vertical extent of the root system. When we hand-cored to four meters depth, we found roots for both rootstocks below 2.43 meters, and the deepest root we found was at a depth of 3.8 meters. Since the most active roots during veraison would likely be the deepest in a dry-farmed vineyard, missing information on the distributions of those deepest roots is a major limitation, and likely a reason for the poor fit of the exponential root distribution model. Moving forward, non-invasive measurement of root systems can provide information on rooting depth, biomass, and activity without destructive sampling, and could help close this gap while providing more complete data than traditional methods of root system measurement (Ehosioka et al., 2020, 2026).

3.6. Conclusion

Under mostly dry farmed conditions in the Central Valley of California, the rootstock 110R outperformed 101-14 under severe drought stress, but showed similar performance under moderate drought stress. 110R had less sensitive responses of gas exchange and leaf water potential to drying soil than 101-14. 110R had a sparser root system with thicker roots, lower branch frequency, more steeply angled roots, and wider branch angles, which matches previously published research. 110R also had a proportionately deeper root distribution, with 50% of its root length occurring deeper in the soil profile than 101-14. A deep total rooting depth of 3.8 meters allowed for mostly dry farming both rootstocks and illustrates the importance of management when developing vineyards for drought resistance. The deep rooting distributions also highlight phenotyping difficulties and the importance of non-invasive techniques in future studies.

3.7. Tables

Table 3.1. Weather and growing conditions for experimental years

Year	GDD	Eto (mm)	Soil Temperature (°C)	Precipitation (mm)
20 year avg	2046.3 ± 41.7	1173.7 ± 12	18.7 ± 0.2	424.5 ± 41
2021	2268.9	1240.5	19.3	133.5
2022	2383.7	1228.8	18.7	475.7
2024	2102.3	1100.3	19.9	408.1

Data and standard errors are shown from the California Irrigation Management Information System (CIMIS) (Hart et al., 2009). Cumulative growing degree days (GDD) and mean reference evapotranspiration (ET_o) and soil temperature were calculated from April 1st to October 31st for each year. Total precipitation was calculated for each water year (i.e., from October 1st of the prior year to September 30).

Table 3.2. Physiology means for each year

Year	A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	Ψ_{MD} (Mpa)	Ψ_{PD} (Mpa)
2021	12.73 ± 0.791 c	0.18 ± 0.016 b	-1.62 ± 0.015 a	-0.82 ± 0.014 a
2022	13.22 ± 0.798 b	0.17 ± 0.014 b	-1.56 ± 0.020 b	-0.65 ± 0.011 b
2024	6.13 ± 0.098 a	0.12 ± 0.003 a	-1.55 ± 0.013 b	-0.39 ± 0.005 c

Means and standard errors for each measured trait from comparable measurement periods (i.e., July 15th to September 8th). Letters indicate significant differences between years determined from Tukey pairwise comparisons ($p < 0.05$). Each year is analyzed separately.

Table 3.3. Linear mixed effect model outputs

		A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	Ψ_{MD} (Mpa)	Ψ_{PD} (Mpa)
2021	Intercept [ref: 101-14]	15.41	0.21	-1.46	-0.64
	Genotype [110R]	3.27**	0.07**	-0.09**	0.08***
2022	Intercept [ref: 101-14]	14.77	0.2	-1.55	-0.6
	Genotype [110R]	0.31	-0.01	-0.04	-0.02
2024	Intercept [ref: 101-14]	6.75	0.14	-1.12	-0.24
	Genotype [110R]	0.034	-0.001	-0.013	-0.006

The outputs of the mixed linear models comparing mean physiology variables for each growing season. Each year is analyzed separately. The genotype term is represented as the difference of 110R compared to the reference genotype, 101-14. Asterisks indicate significance: * < 0.05, ** < 0.01, *** < 0.001.

Table 3.4. Soil moisture mixed effect model outputs for each growing season

		A ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	g_s ($\text{mol m}^{-2} \text{s}^{-1}$)	Ψ_{MD} (Mpa)	Ψ_{PD} (Mpa)
2021	Intercept [ref: 101-14]	21.27	0.29	-2.02	-0.89
	Genotype [110R]	-8.34**	-0.0802	0.51	-0.105
	Ψ_{smp} (kPa) [ref: 101-14]	0.12***	0.0016***	-0.0047*	-0.0003
	Genotype: Ψ_{smp} [110R]	-0.11***	-0.0014**	0.0062*	-0.0015
2022	Intercept [ref: 101-14]	13.73	0.201	-1.16	-0.54
	Genotype [110R]	-1.92	0.079	0.13	0.07
	Ψ_{smp} (kPa) [ref: 101-14]	-0.00163	-0.00034	0.00525**	0.00066
	Genotype: Ψ_{smp} [110R]	-0.029	0.0012	0.00096	0.00101

The outputs of the mixed linear models comparing physiological responses to soil matric potential for two years of the trial. The intercept and Ψ_{smp} terms represent the model output for the reference genotype, 101-14, while the genotype and interaction term represent the difference between 110R and the reference genotype for the intercept and slope, respectively. Asterisks indicate significance: * < 0.05, ** < 0.01, *** < 0.001.

Table 3.5. Root traits for each studied rootstock

Trait	Rootstock	
	101-14	110R
Length (mm)	906.8 ($\pm$ 76.5)	611.6 ($\pm$ 41.4)
Thickness (mm)	0.8295 ($\pm$ 0.010)	0.8473 ($\pm$ 0.021)
Branch freq. (mm ⁻¹)	0.0368 ($\pm$ 0.0014)	0.0307 ($\pm$ 0.0019)
Dry mass (g)	0.49 ($\pm$ 0.09)	0.34 ($\pm$ 0.05)
SRL (mm/g)	4649.9 ($\pm$ 393.3)	5299.7 ($\pm$ 405.8)
D50 (cm)	115.8 ($\pm$ 4.3)	132.9 ($\pm$ 2.9)
β (unitless)	0.9929 ($\pm$ 0.0002)	0.9937 ($\pm$ 0.0002)

Root traits measured from root cores. β is the exponential fitting parameter for root system distributions, and D50 is the depth at which 50% of the cumulative root length occurred in the soil profile. Statistics are the mean values across all depths, and errors are standard error. Bold indicates a significant difference between the genotypes ($p < 0.001$, Type II ANOVA, two-sample two-sided t-test).

3.8. Figures

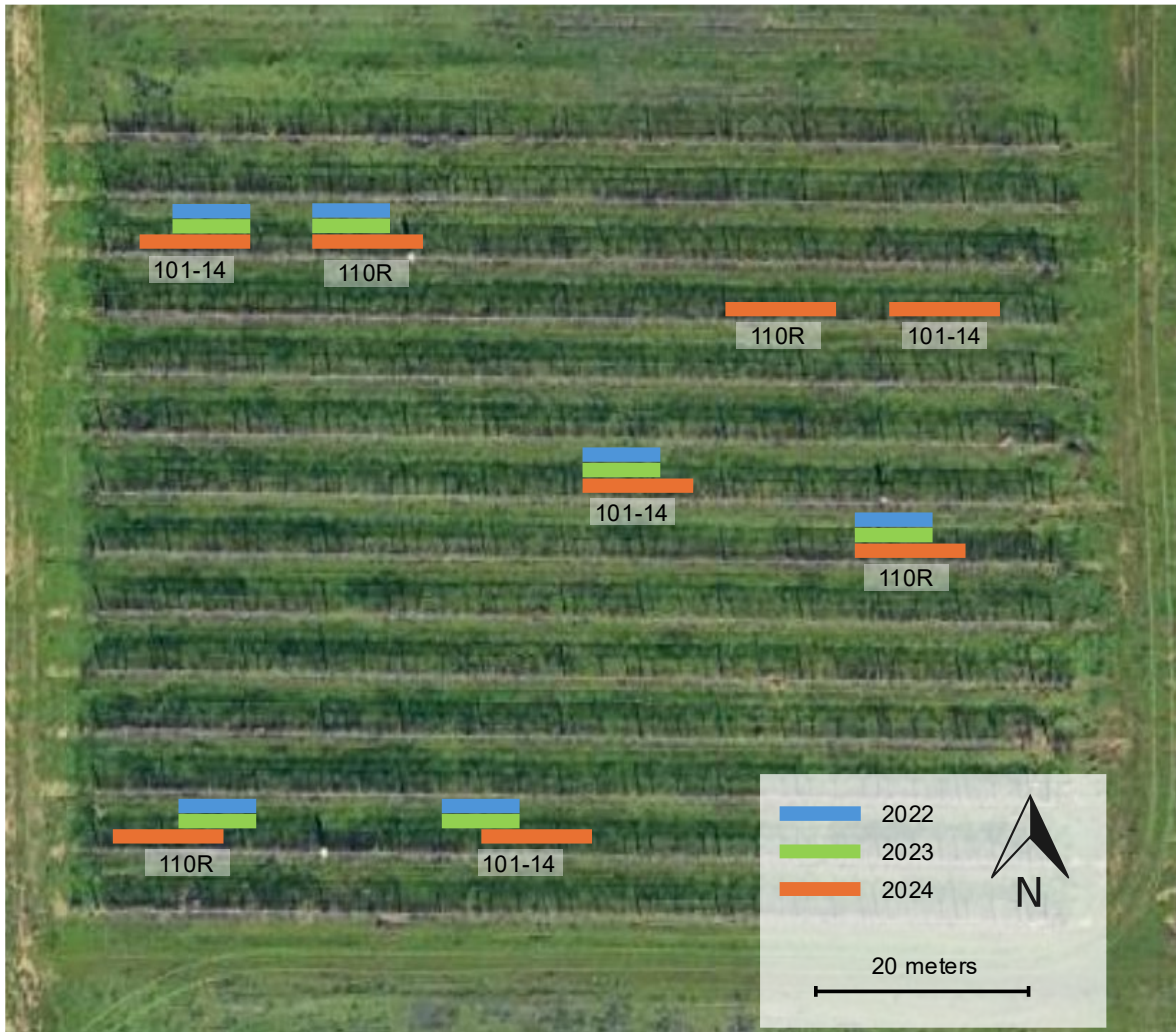


Figure 3.1. Vineyard layout

Satellite image of the measured vineyard. Bar color indicates the year each vine was measured, and bar length indicates how many vines and their location in the row (short bar is two vines, long bar is three vines). Four measured blocks are shown, one in the northwest corner, one in the southwest corner, and two on the east side of the vineyard.

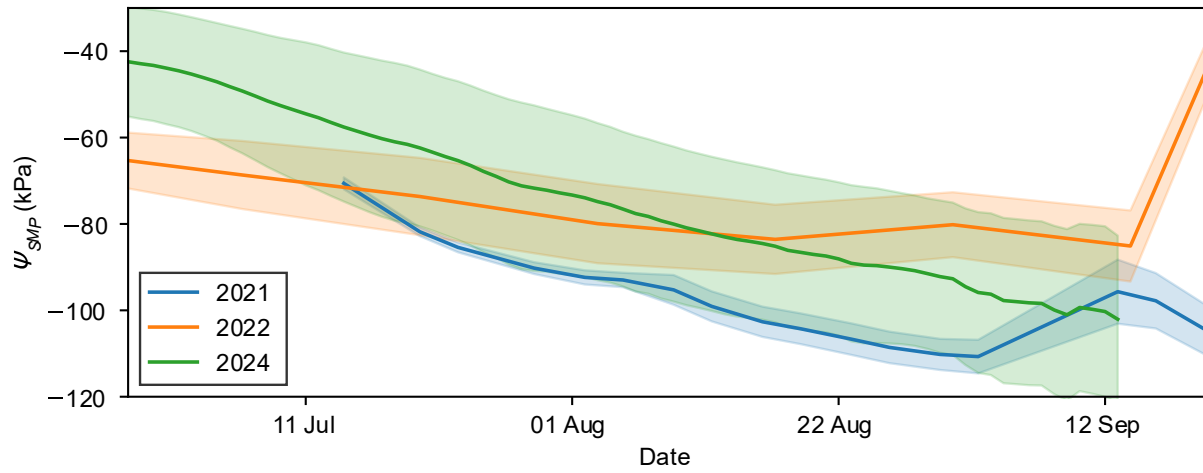


Figure 3.2. Soil matric potential

Ψ_{smp} at 1.2 meters depth at various locations throughout the vineyard for each measured year measured with soil matric potential probes. Error bars indicate standard error.

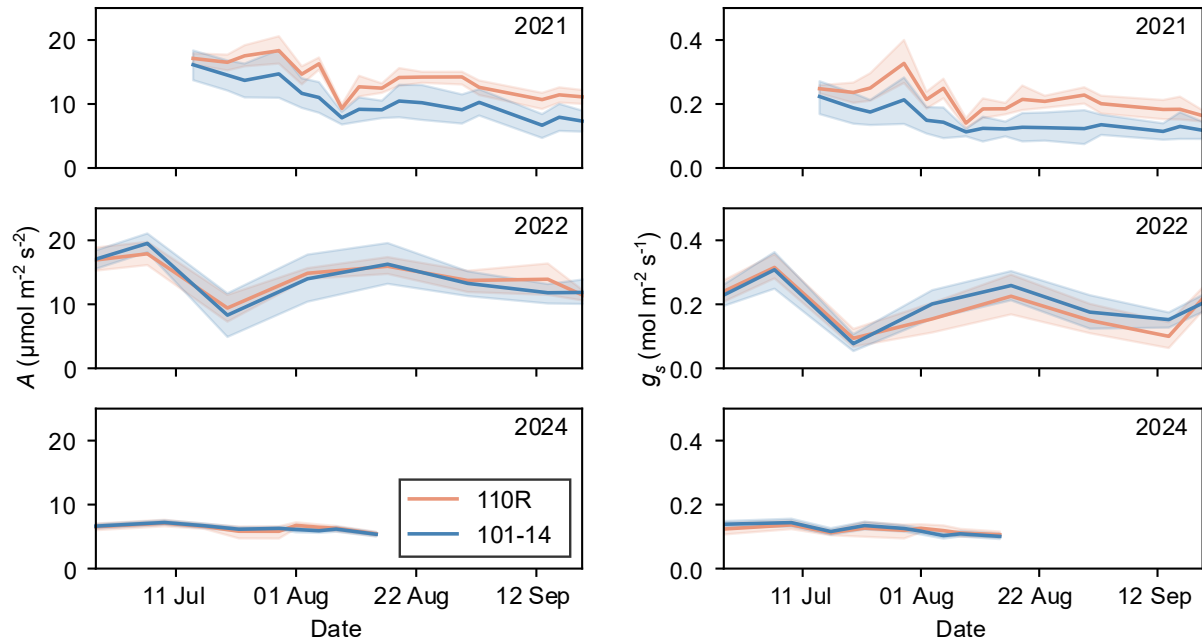


Figure 3.3. Vine gas exchange

Measured photosynthesis (A) and stomatal conductance (g_s) at midday over the study period for all years is shown for both rootstocks under dry conditions. Error bars indicate 95% confidence interval for each date.

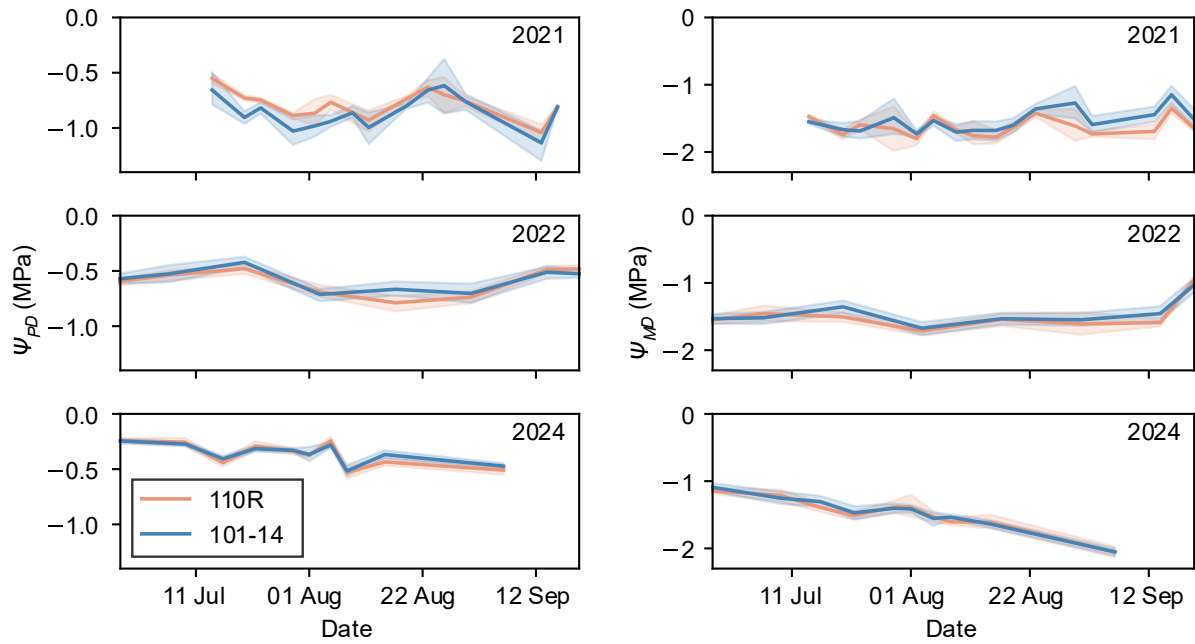


Figure 3.4. Vine water potentials

Measured predawn water potential (Ψ_{PD}) and midday water potential (Ψ_{MD}) over the study period for all years is shown for both rootstocks under dry conditions. Error bars indicate 95% confidence interval for each date.

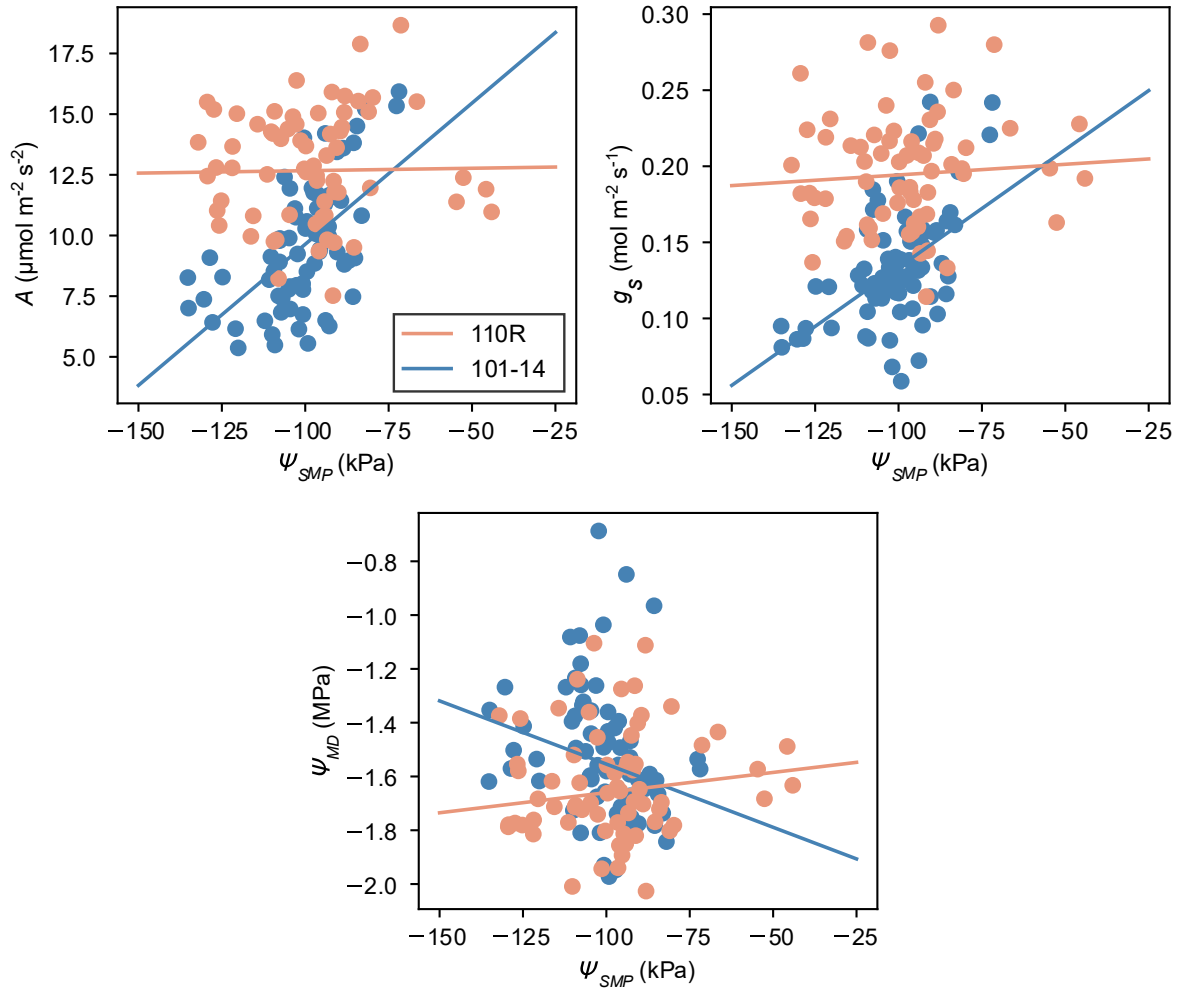


Figure 3.5. Physiological response to soil matric potential

Visualization of the mixed effect model output for the physiological responses to soil moisture for each rootstock. Lines are fit from the slope and intercept from the mixed effect models. Data points are corrected for their random effects; the random effect (individual plant variation in intercept) was subtracted from each data point to produce scatterplots.

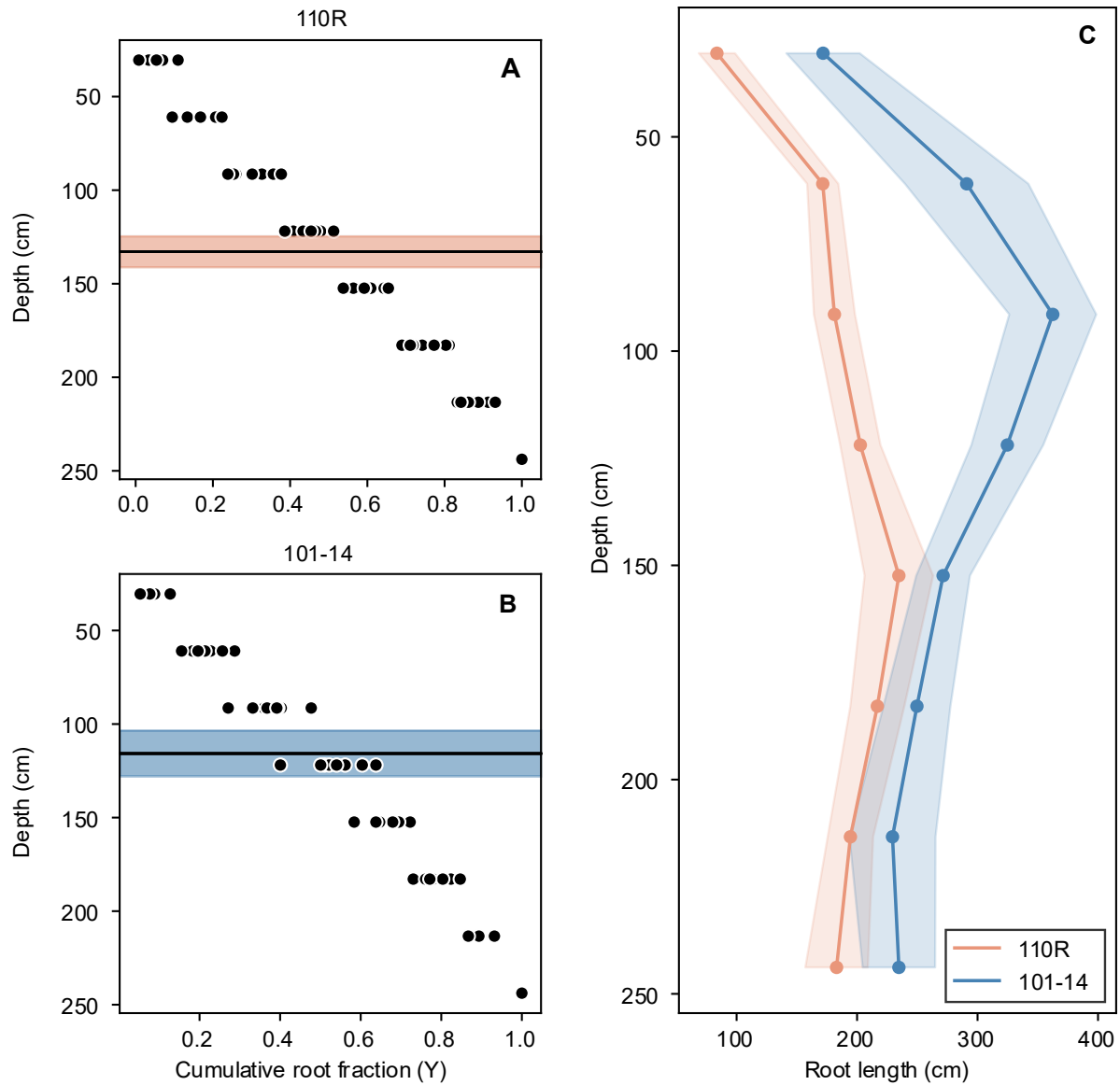


Figure 3.6. Vine root distributions

Root length distributions with depth as measured from soil coring to 2.43 meters. Horizontal lines in plots A and B indicate D50 and the standard deviation ($N = 16$). C shows the total root length measured at each 30 cm interval, and colored areas show standard errors.

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Figure 3.7. Excavated root systems

Example root systems from both genotypes after excavation from the field. Soil pits are approximately one meter deep.

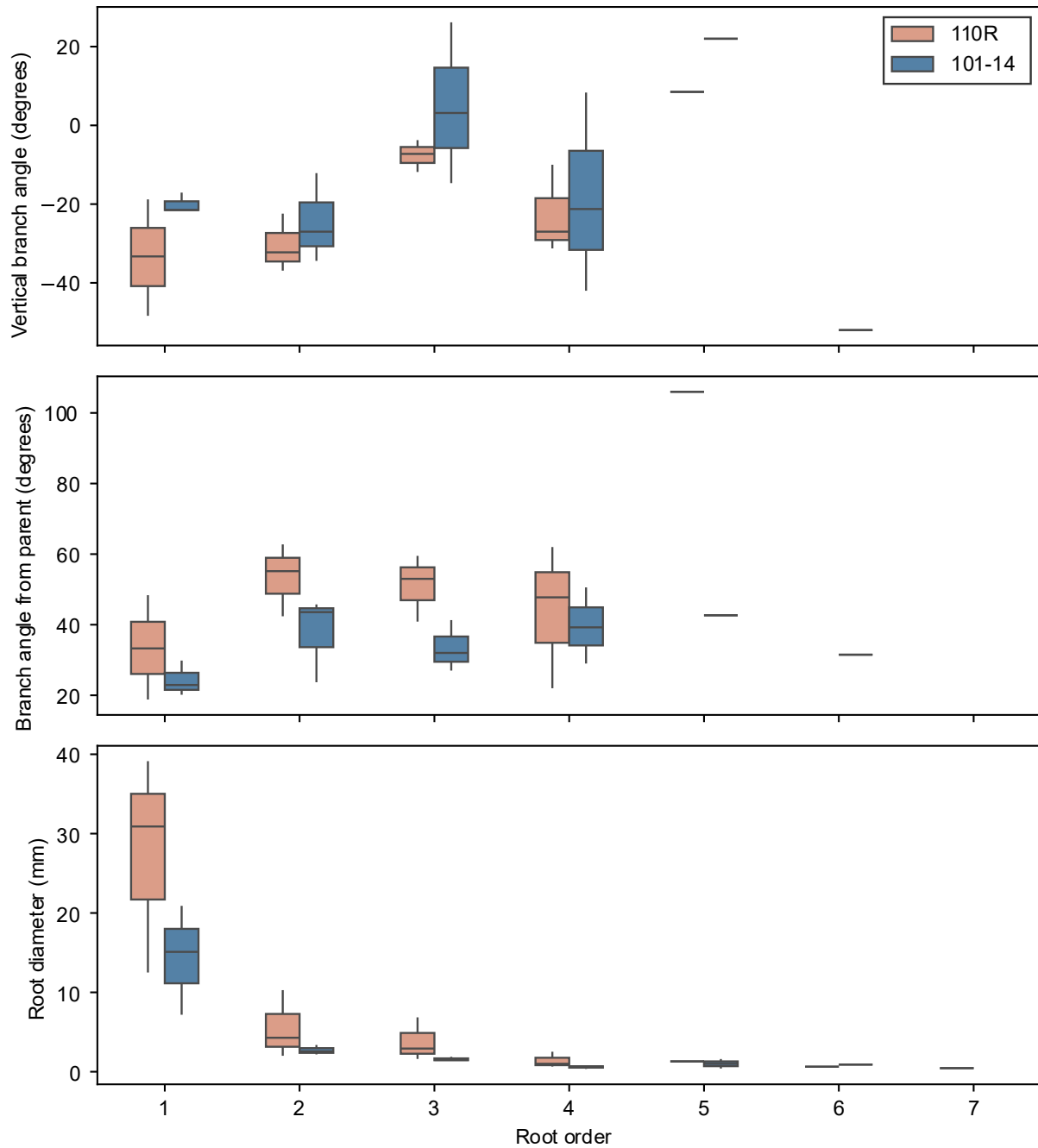


Figure 3.8. Vine traits by root order

Root traits measured from multiple root orders from excavated vines ($N = 6$) for three traits for both rootstocks. Vertical branch angle, branch angles from the parent root, and root diameter are shown.

3.9. References

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3.10. Supplemental materials

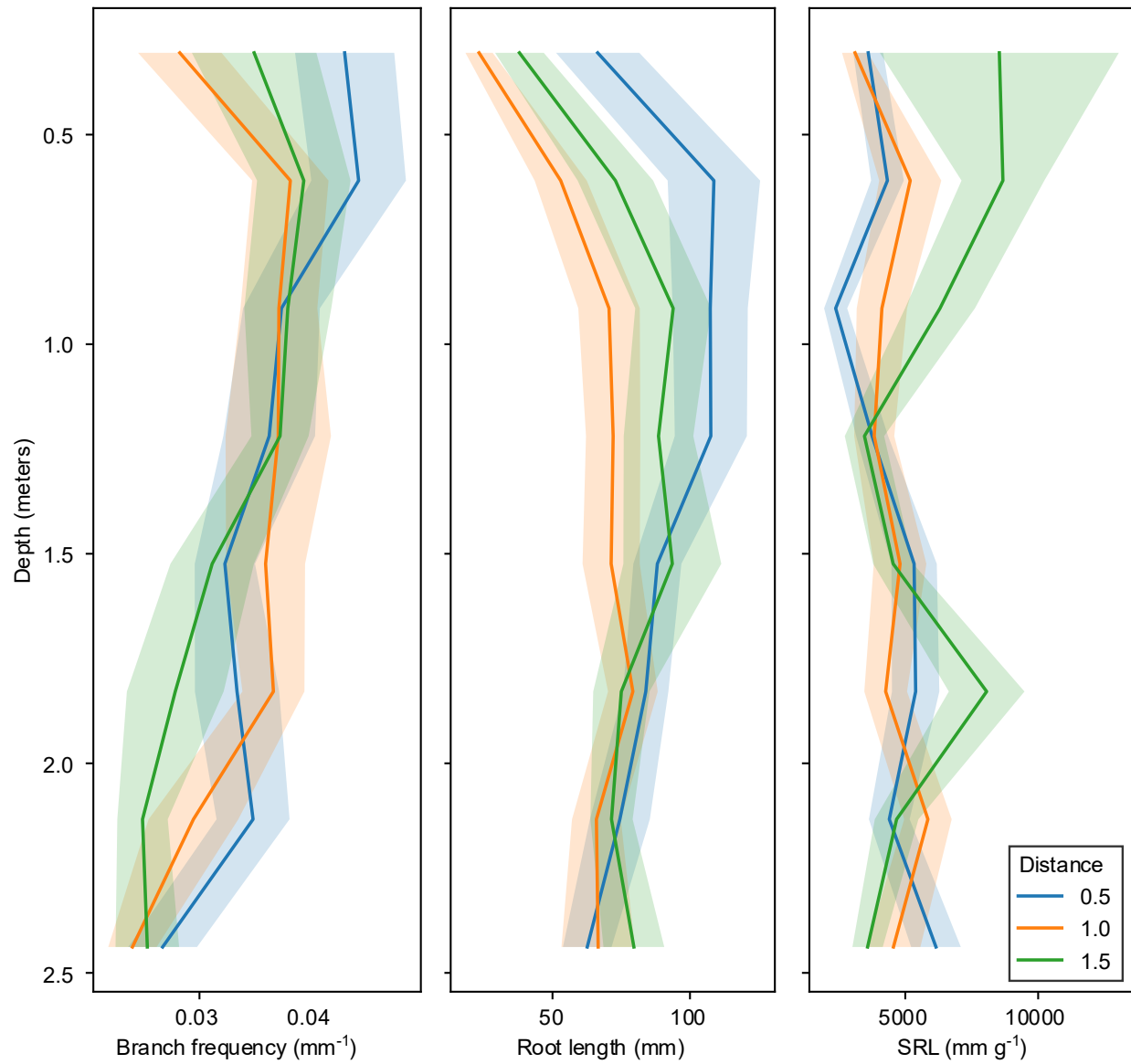


Figure S 3.1. Root distributions by sampling distance

Root distributions of each sampling distance for three traits. Specific root length (SRL) is root dry mass divided by root length.

Table S 3.1. Soil moisture mixed effect model outputs for 2021 with dates after 9 September removed

		A	g_s	Ψ_{MD}	Ψ_{PD}
2021	Intercept	25.02***	0.37***	-2.05***	-0.86***
	Genotype	-8.60**	-0.18	0.52	0.05
	Ψ_{SMP} (kPa)	0.1473***	0.0023***	-0.0049*	-0.0005
	Genotype: Ψ_{SMP}	-0.1178***	-0.0024**	0.0060*	0.0001

The outputs of the mixed linear models comparing physiological responses to soil matric potential for two years of the trial. Asterisks indicate significance: * < 0.05, ** < 0.01, *** < 0.001.