

Adaptation and Early Establishment of Olive Trees (*Olea europaea* L.) under the Humid Subtropical Climate of the Southeastern United States

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Abstract. Olive cultivation in the southeastern United States is challenging due to the warm and humid subtropical climate of the region. This study examined the impact of cultivar selection, transplant strategy, and environmental conditions on the adaptability and early establishment of olive trees in Florida. Two research orchards were established at two different locations (Citra and Jay), featuring three cultivars (Arbequina, Arbosana, and Koroneiki) transplanted at two sizes (large and small). Shoot and root growth were monitored over 2 years (Jul 2017 to May 2019) to assess transplant stress, tree establishment, and environmental adaptation. Small transplants exhibited higher growth rates than large ones, with differences in tree height and trunk cross-sectional area diminishing over time. Large transplants experienced greater transplant stress, as evidenced by a significant reduction in number of primary branches. Severe winter temperatures at Jay caused damage to ‘Arbosana’ and ‘Koroneiki’ trees, leading to high mortality rates and poor post-winter recovery growth. In contrast, ‘Arbequina’ demonstrated greater tolerance to both transplant stress and cold damage, maintaining stable vegetative growth. Root growth was primarily influenced by environmental factors rather than cultivar selection or transplant size. Total root length declined from 4 to 9 months after transplant (MAP), but it remained stable from 9 to 20 MAP. Most roots were concentrated within 20- to 40-cm soil depth, likely due to irrigation. These findings highlight the complex interactions among cultivar, transplant size, and environmental conditions. In addition, our results suggest ‘Arbequina’ has potential for commercial cultivation in the region. Further research is needed to assess long-term effects on productivity and resilience.

The olive tree (*Olea europaea* L.) originated in the Mediterranean Basin, which is characterized by a semiarid climate (Rallo et al. 2018), with dry and hot summers and wet and mild winters (Giorgi and Lionello 2008). In addition to the Mediterranean region, olives are also grown commercially in other areas with similar climate, such as California, central Chile, South Africa’s Cape Province, and southeastern Australia. However, the establishment or productivity of olive trees under the warm and humid subtropical climate

of the southeastern United States has been barely explored. Over the past decade, the impact of citrus greening disease on Florida’s citrus industry has sparked growing interest in olive production among local growers.

Olive trees are highly sensitive to climatic conditions and seasonal changes (Ben Abdallah et al. 2017), resulting in distinct growth patterns (Benlloch-González et al. 2024). When grown under warm climates, olive trees have two growth cycles (Wiesman and Lavee 1994): a first growth flush in spring and a rapid, short

growth flush in early fall. Contrarily, the low temperatures and low rainfall in Mediterranean regions limit the tree growth to only one flush in summer (Bach 2002; Orlandi et al. 2013). Higher air temperatures can lead to morphological changes in olives, as evidenced by enhanced secondary shoot growth in ‘Arbequina’ plants exposed to 37°C (Benlloch-González et al. 2016). Under warmer conditions, ‘Arbequina’ and ‘Coratina’ olives allocated more resources to vegetative growth rather than fruit production (Miserere et al. 2022). In contrast, ‘Frantoio’ and ‘Arbequina’ olives allocated more resources to productive structures, consequently producing higher yields (Paoletti et al. 2021).

Increased thermal amplitude can disrupt developmental and physiological processes (Sherif 2015; Benlloch-González et al. 2018). For instance, high temperatures severely affected flower development and pollen germination in ‘Koroneiki’ and ‘Manzanillo’ olives grown in Egypt (Sherif 2015). In vitro assessments of pollen viability revealed detrimental effects of heat stress in 47 of 54 olive cultivars (Wortmann et al. 2024). Furthermore, researchers in Argentina observed a 26-d advance in flowering in several olive cultivars across a latitudinal gradient (Hamze et al. 2022), demonstrating the effect of changing environmental conditions on olive reproductive phenology (Hamze et al. 2022; Wortmann et al. 2024). Similarly, ‘Nocellara’, ‘Nociara’, and ‘Picholine’ olives showed differences in their alternate bearing trends (Giuffrè 2017), indicating a genotype-environment interaction.

In addition to differences in response to environmental conditions, management practices from transplanting to harvest significantly influence tree performance. The establishment phase following transplanting is critical and often stressful for most plant species. However, transplant stress can be ameliorated by using appropriate transplanting techniques. A common horticultural practice is the use of large transplant sizes to shorten the time needed for trees to mature and reach their full yield potential (Arnold 2005). Nevertheless, compared with small transplants, large transplants require longer periods of time to recover from root damage related to the transplanting process (Struve 2009; Watson 2005). The impact of transplant size has been observed in various tree species. A study conducted on red maples (*Acer rubrum* L.) in Alabama showed that small transplants produced greater shoot elongation and height increase than large transplants, besides exhibiting higher levels of gas exchange and leaf water potential (Lauderdale et al. 1995). Transplant size also influences significantly on evapotranspiration in *Washingtonia robusta* H. Wendl, *Pinus brutia* Ten var. *eldarica*, and *Parkinsonia florida* (Benth. ex A. Gray) S. Wats (Devitt et al. 1995).

Damage or stress to the roots, which often occurs during transplanting, reduces the photosynthetic capacity of the plant, thereby decreasing its growth potential (Lauderdale

et al. 1995). In this regard, soil temperature, moisture, pH, microorganisms, and nutrient availability influence root development and architecture (Bao et al. 2014; Paez-Garcia et al. 2015; Zheng et al. 2024). Moreover, root growth and development are subject to internal plant characteristics and their interaction with the environment (Watson and Himelick 1982). Consequently, root length, diameter, depth, and distribution in the soil profile can vary significantly from cultivar to cultivar, affecting the adaptation and establishment capacity of fruit trees (Zheng et al. 2024).

Studying root architecture in situ, under field conditions, can be a tedious and labor-intensive process. Fortunately, the use of rhizotrons has enabled researchers to observe roots within the rhizosphere (Rewald and Ephrath 2013). Minirhizotrons, in particular, facilitate the repetitive observation of a fixed section of the root system by inserting a camera, without causing damage to root structures (Hendrick and Pregitzer 1996). This technique has allowed the study of root architecture of several fruit crop species such as peaches (*Prunus persica* L.) (Abrisqueta et al. 2017), apples [*Malus domestica* (Suckow) Borkh] (An et al. 2017), citrus [*Citrus × sinensis* (L.) Osbeck] (Zhou et al. 2018), olives (Othman and Leskovar 2019), and others. Despite some limitations, the use of minirhizotrons is a reliable nondestructive technique to study roots under controlled or field conditions (Głuszek et al. 2013).

Current knowledge of olive production in the southeastern United States is limited, and existing guidelines are primarily based on information from other regions. Evaluating cultivar performance and establishment strategies is critical to determine the viability of cultivating olive trees under challenging environmental and edaphic conditions. Thus, this study aimed to analyze the impact of cultivar selection, transplant strategies, and environmental conditions on the adaptation and early establishment of olive trees to the warm and humid subtropical climate of the southeastern United States.

Materials and Methods

Three olive cultivars were evaluated in two research orchards located at different latitudes in Florida, established using large and small transplant sizes.

Orchard establishment. Experimental orchards were established in Jul 2017 at the University of Florida's Plant Science Research & Education Unit [Citra, FL (29°24'37.6"N, 82°08'32.5"W); USDA Plant Hardiness Zone 9a, average minimum winter temperatures −3.9 to −6.7 °C; soil order: Entisols; soil series: Candler sand] and at the University of Florida's West Florida Research and Education Center [Jay, FL (30°46'41.7"N, 87°08'51.5"W); USDA Plant Hardiness Zone 8b, average minimum winter temperatures −6.7 to −9.4 °C; soil order: Ultisols; soil series: Fuquay loamy sand]. Within each location, 150 trees were planted in a randomized complete block design (five blocks) with a split-plot restriction on randomization at a spacing of 3.5 (within row) by 3.8 m (between row) (750 trees/ha) in 0.28 ha. This plant distribution corresponds to the super high-density production system for olives (Vossen 2007). Two transplant sizes (small, average height: 65 cm, average trunk diameter at 15 cm from soil: 6.73 mm; large, average height: 160 cm, average trunk diameter at 15 cm from soil: 10.27 mm) were the main plot factor and three cultivars (Arbequina, Arbosana, and Koroneiki) the subplot factor within each block. Additional trees were used as border rows completely surrounding the orchard to eliminate border effects. Trees were obtained from a local nursery. Before transplant, the small transplants were 2 years old and grown in 2.46-L containers, and the large transplants were 3 years old and grown in 3.78-L containers. Trees were pruned in Mar 2018 to remove dead wood. The experiment was conducted from Jul 2017 to May 2019. Meteorological data were recorded using an automated weather station located within 2000 m of the experiment. Overall, mean and maximum soil temperatures were higher in Citra compared with Jay, although minimum temperatures were similar. Precipitation in Citra and Jay was similar, although precipitation peaks occurred in different months for both locations. Relative humidity was similar in Citra and Jay during most of the experiment (Supplemental Fig. 1).

Orchard management. Trees were pruned to a central leader and trained with the aid of a two-wire trellis system consisting of PVC pipes supported by metal posts. To maintain a unique central leader, the main stem was attached to the PVC pipe using a tapener tool. Additional competing branches were pruned regularly. A 1.5-m-wide vegetation-free strip was maintained around the trees by applying 0.3 kg·ha^{−1} granular pre-emergent herbicide [Chateau SW (flumioxazin – 51%); Valent U.S.A. LLC, Walnut Creek, CA, USA] at the beginning of spring and 1 kg·ha^{−1} of glyphosate when needed. All trees received irrigation twice a week from May to November, via a single micro sprinkler (MAB36L1;

Maxijet, Dundee, FL, USA) located 0.4 m from the tree main stem, delivering 10 L of water per tree per irrigation event. Additionally, irrigation was adjusted in response to prolonged periods of drought or excessive precipitation. Insects were controlled with applications of 3 kg·ha^{−1} of the insecticide *Bacillus thuringiensis* as foliar spray. For each location, composite soil samples from each experimental block were collected in Oct 2017 and Oct 2018 at depths of 0 to 15 and 16 to 30 cm for nutrient analysis (Waters Agricultural Laboratories Inc., Camilla, GA, USA). Nutrients were extracted using a Mehlich 3 extractant solution (Mehlich 1984). Based on the soil analyses results, macronutrient fertilization (Supplemental Table 1) was split into six applications, monthly, from March to August by manually broadcasting the fertilizer within a 50-cm radius around the trees. Micronutrient fertilization was done by foliar application of a commercial micronutrient blend (9.24 L·ha^{−1}) (Soar Citrus Mix; Chemical Dynamics, Plant City, FL, USA) and boron (3.33 kg·ha^{−1}) (Beau-Ron; Drexel Chemical Company, Memphis, TN, USA) in Aug 2018.

Shoot growth measurements. The tree height (TH) was measured to the nearest centimeter throughout the experiment. The stem diameters at 0 and 15 cm above the soil surface were measured with a caliper (IP-54 Electronic Caliper; Fowler Company Inc., Newton, MA, USA) and used to calculate the trunk cross-sectional area (TCA) with the following formula:

$$TCA = \pi r^2, \quad [1]$$

where r (radius) is equivalent to 0.5 times the measured diameter.

The number of primary branches (NPB) growing from the main stem were counted. To assess tree canopy development, one vigorous branch above 5-cm length located in the lower (0–65 cm from the soil surface), middle (65–130 cm from the soil surface), and upper (130 cm or more from the soil surface) portion of each tree was selected and identified with a plastic zip-tie to be tracked throughout the duration of the experiment. For consistency purposes, selection priority was given to branches closer to the midpoint of each section. Branch length and number of secondary branches growing from the selected branches were also counted. The number of secondary branches in the lower branch (NSB) was used as an estimator of intracanalopy density.

Based on the length measurements of the primary branches, the canopy volume (CV) was calculated by the addition of the following sectional volumes:

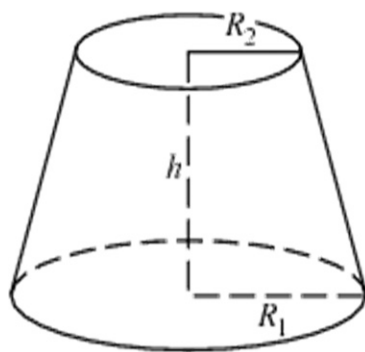
1. In trees in which all three sections were present (lower, middle, and upper), the volumes of the lower and middle sections were calculated as a frustum, with the following formula (NASA 2015):

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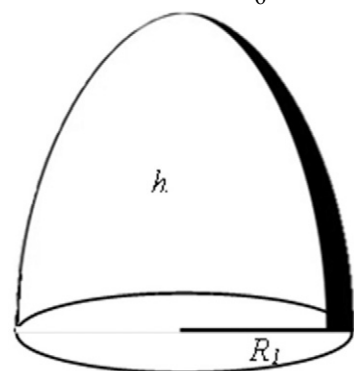
$$\text{volume} = \frac{\pi h}{12} [(2R_2)^2 + (4R_2R_1) + (2R_1)^2], \quad [2]$$



where R_1 and R_2 are the length of the branches in the corresponding section, and h is the height of the same section.

Also, the volume of the upper section was calculated as an elliptical cone, with the following formula (NASA 2015):

$$\text{volume} = \frac{\pi(2R_1)^2}{6} h, \quad [3]$$



where R_1 is the length of the branch in the corresponding section, and h is the height of the same section.

2. In trees in which all the sections were not present, the calculation of the volume was adjusted according to the conditions of the tree. The same formulas were used.

Root growth measurements. Minirhizotron acrylic tubes (200 cm long, outer diameter 6.0 cm, inner diameter 5.4 cm, wall thickness 0.3 cm) were installed in the experimental olive orchard at Jay, FL, after tree transplant. A hydraulic powered coring machine (Giddings Machine Company, Windsor, CO, USA) was used to place one tube directly under and parallel to the row, in the middle of each subplot (30 tubes total), ~50 cm apart from the base of the closest tree, forming a 60° angle with the soil surface. A cap was used to cover the tip of the tube inserted in the soil, to prevent water infiltration. Similarly, a piece of PVC pipe was used to cover the acrylic tube above the level of the soil to avoid water infiltration and sunlight penetration, which could have negatively impacted root development. A BTC 100X video camera and the BTC I-CAP image capture software (Bartz Technology Corporation, Carpinteria, CA, USA) were used to capture soil profile images at increments of

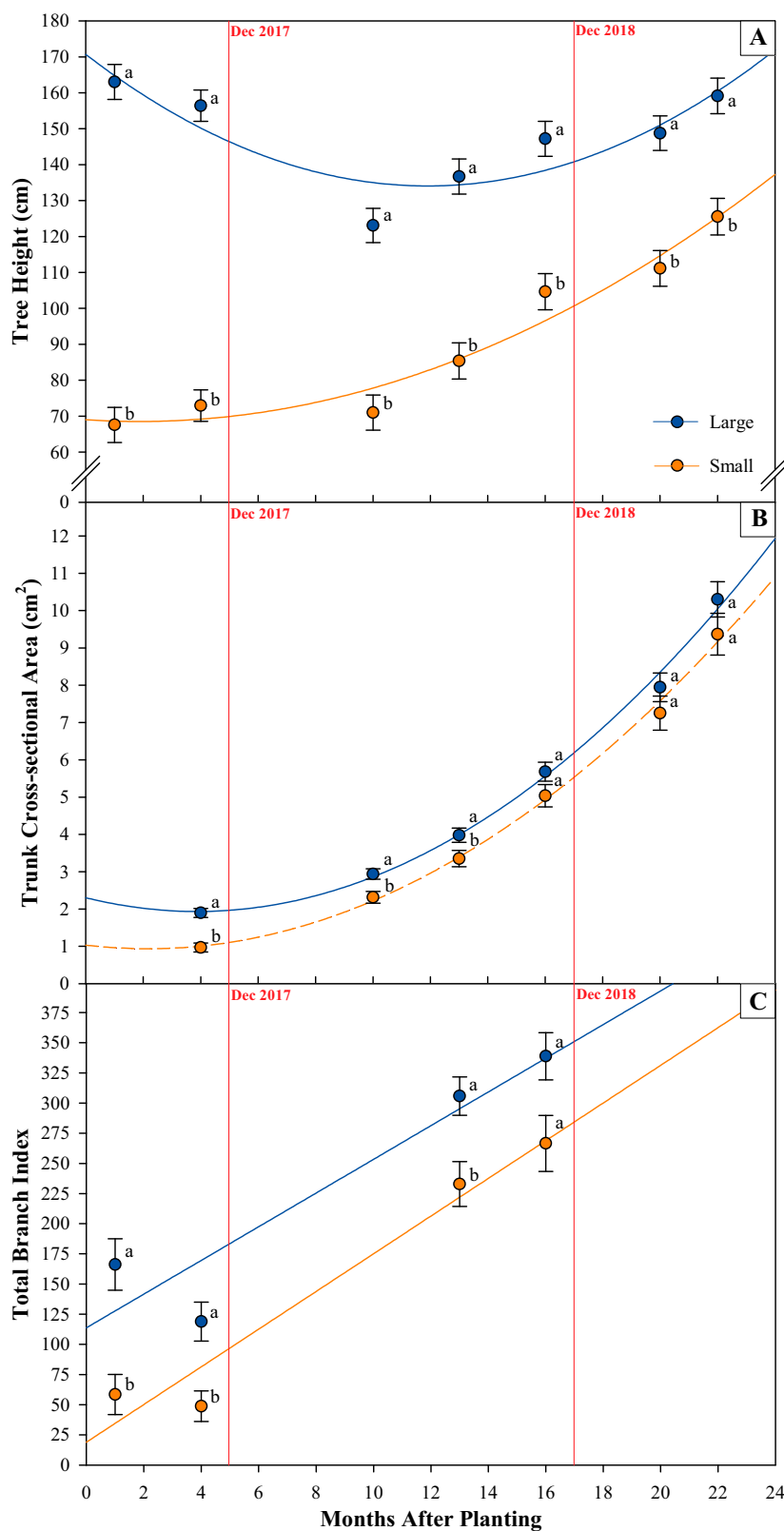


Fig. 1. (A) Tree height (cm), (B) trunk cross-sectional area (cm²), and (C) total branch index (the product of the number of primary branches and the number of secondary branches) over time for small and large transplants. Means and standard error bars correspond to the average values for ‘Arbequina’, ‘Arbosana’, and ‘Koroneiki’ olive trees planted in orchards established at Citra and Jay, FL. Trees were planted in Jul 2017 (0 months after planting). Means within each date labeled with the same letter were not statistically different (LSMeans statement, Simulation adjustment; $P < 0.05$).

13.5 mm, obtaining 66 to 79 frames per tube. For consistency purposes, only the initial 66 frames of each tube were analyzed.

The first image collection was performed in Nov 2017, and imaging was repeated every month from Apr 2018 to Mar 2019.

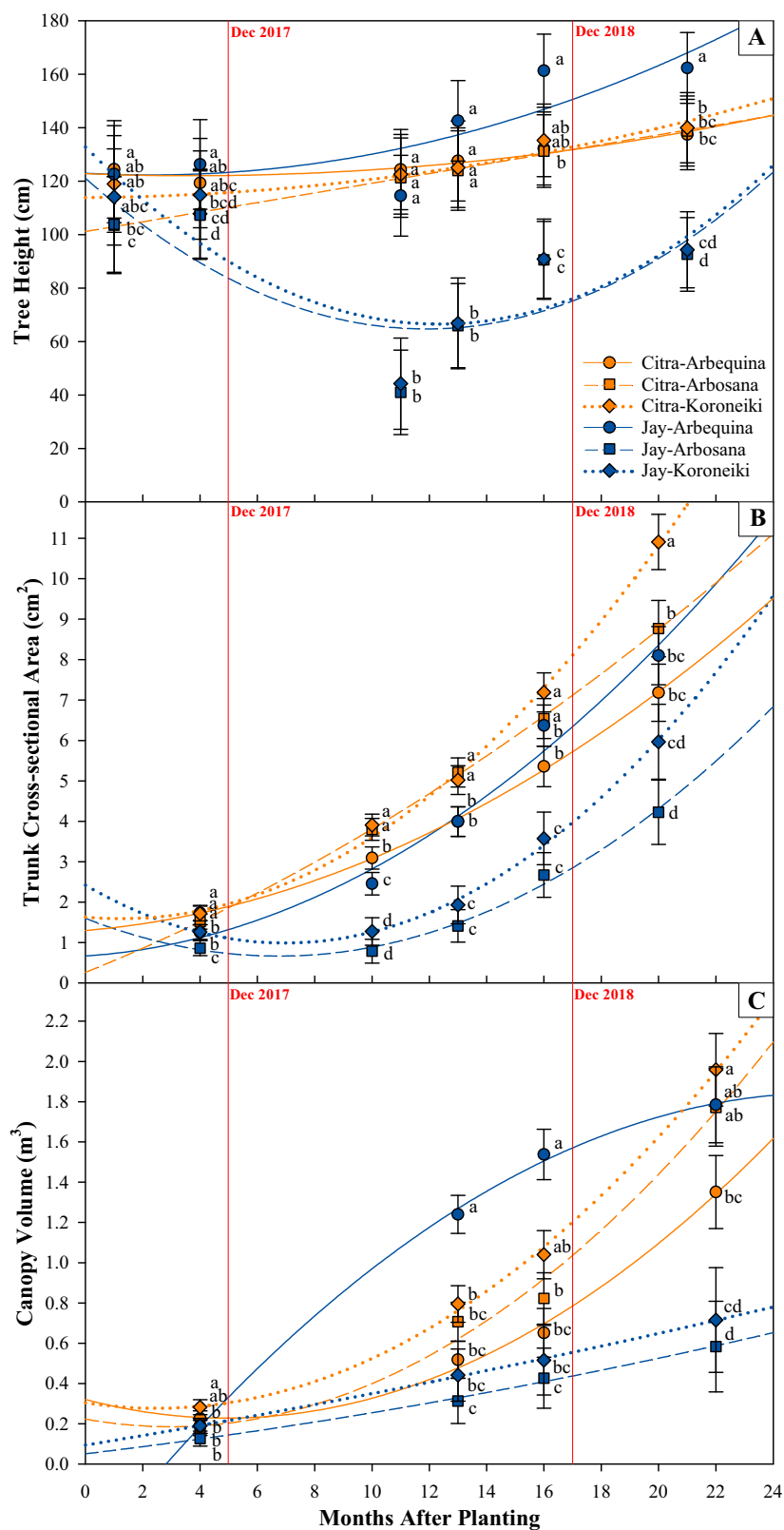


Fig. 2. (A) Tree height (cm), (B) trunk cross-sectional area (cm²), and (C) canopy volume (cm³) over time for 'Arbequina', 'Arbosana', and 'Koroneiki' olive trees planted in orchards established at Citra and Jay, FL. Means and standard error bars correspond to the average values for small and large transplants. Trees were planted in Jul 2017 (0 months after planting). Means within each date labeled with the same letter were not statistically different (LSMeans statement, Simulation adjustment; $P < 0.05$).

Once images were collected, root segments within each image frame were manually traced using the software WinRHIZO

Tron (Regent Instruments Inc., Quebec, Canada). From the analysis conducted by the software, the variable total root length (TRL)

was calculated by summing the linear length of every individual root segment. The average root diameter (ARD) was measured by the software. The variable total surface area (TSA) was estimated by calculating the product of cross-sectional circumference (πd) and TRL. In addition, TRL, ARD, and TSA were calculated for each of four consecutive segments of 20 cm to further analyze the effect of soil depth on root growth, in addition to the effect of transplant size and olive cultivar.

Statistical analysis. All response variables were analyzed using generalized linear mixed model procedures, as implemented in SAS PROC GLIMMIX (SAS/STAT version 14.1; SAS Institute, Cary, NC, USA). Most responses exhibited a normal distribution. Count (branch number and number of basal shoots) data were analyzed as normal data by obtaining mean values for each subplot. Data on shoot and root growth were collected between Jul 2017 and May 2019. An analysis of covariance (ANCOVA) was performed on these data, where the time factor was modeled as a covariate, a quantitative effect nested within the interaction among cultivar, size, and location. For each response variable, a polynomial fit was initially tested. Subsequently, simpler models were also fit, and the best model was selected based on the corrected Akaike Information Criterion (AICc). Pairwise comparisons of the means were completed, adjusting the data with the Simulation test within the LSMeans statement ($P < 0.05$) of SAS. When root growth responses were analyzed considering transplant size and olive cultivar as fixed factors, covariate time was the only significant factor in the model. Consequently, an analysis of variance (ANOVA) was performed to determine variations over time for root growth responses. In addition, a second ANOVA was conducted considering soil depth, transplant size, and cultivar as factors to further assess their effects on root growth.

Results

Shoot growth measurements. Shoot growth data showed variations throughout the experiment (Supplemental Table 2). Data over time [months after transplant (MAP)] is presented for each variable. Initial transplant size influenced TH over time, but the effects of initial transplant size on TH were independent of orchard location or cultivar (Fig. 1A). Small transplant size trees were shorter than large transplant size trees throughout the experiment. However, numerical differences between large and small transplant sizes reduced throughout this study. The orchard location and cultivar also influenced TH (Fig. 2A). Although slight differences were observed at the beginning of the experiment, these were not attributed to location. Some minor reductions in TH (dieback) were evident 4 MAP, and this was most notable for 'Arbosana' and 'Koroneiki' olives planted at Jay. Reduction continued in 'Arbosana' and 'Koroneiki' olives after the 2017–18 Winter, and the lowest tree heights were evident 11 MAP. 'Arbequina'

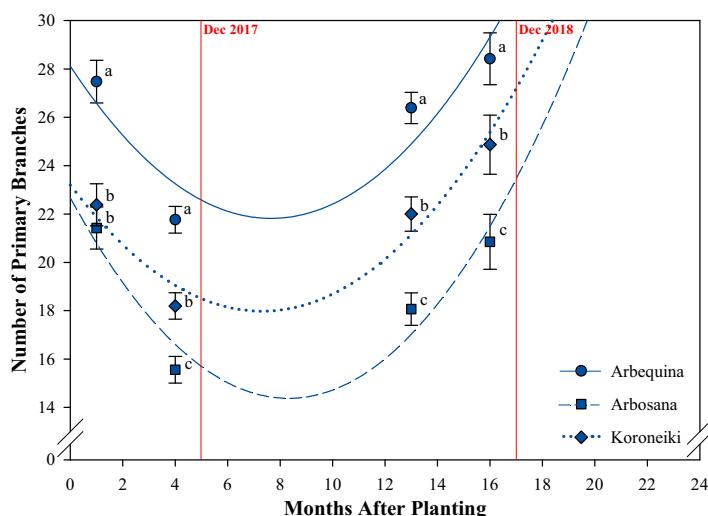


Fig. 3. Number of primary branches over time for ‘Arbequina’, ‘Arbosana’, and ‘Koroneiki’ olive trees. Means and standard error bars correspond to the average values for small and large transplants planted in orchards established in Citra and Jay, FL. Trees were planted in Jul 2017 (0 months after planting). Means within each date labeled with the same letter were not statistically different (LSMeans statement, Simulation adjustment; $P < 0.05$).

had a greater TH over time than ‘Arbosana’ and ‘Koroneiki’ at Jay. However, the rate of height increases for trees that overcame

winter cold damage was similar to that shown by other cultivars. The differences in TH over time among the cultivars at Citra were minor,

and trees were increasing in height similarly from 13 MAP until the last date of data collection.

Initial transplant size also influenced TCA over time, but the effects of initial transplant size on TCA were independent of orchard location or cultivar (Fig. 1B). Small transplant size trees showed smaller TCA than large transplant size trees up to 13 MAP. However, TCA did not differ between 16 MAP and 22 MAP, regardless of tree transplant size. Similar to TH, the differences in TCA over time were influenced by the main effects of orchard location and cultivar (Fig. 2B). These differences were notable at 4 MAP, as TCA for trees at Citra was greater than TCA for trees at Jay. For trees at Jay, TCA was smaller for ‘Arbosana’ than for either ‘Koroneiki’ or ‘Arbequina’. In most cases, TCA increased from the initial values measured at 4 MAP until the final measurement at 20 MAP. The exceptions were ‘Arbosana’ and ‘Koroneiki’ at Jay, which did not show TCA increases between 4 and 10 MAP. During the 2018 growing season, TCA for both ‘Arbosana’ and ‘Koroneiki’ increased at both locations, whereas TCA for ‘Arbequina’ at Jay was not significantly different from trees at Citra by 13 MAP. ‘Koroneiki’ trees in Citra showed the highest TCA by 20 MAP. ‘Arbosana’ and ‘Koroneiki’ at Citra exhibited higher TCA than all trees at Jay 20 MAP, while TCA among ‘Arbequina’ olives did not differ between locations 20 MAP.

CV over time was influenced by orchard location and cultivar (Fig. 2C). CV continuously increased throughout the study. At 4 MAP, ‘Koroneiki’ trees at Citra exhibited greater values than all other trees, except ‘Arbequina’ at Citra. Also, ‘Koroneiki’ at Jay showed the highest CV at that location after the 2017–18 Winter. Differences tended to increase during the 2018 growing season. However, all trees in Citra showed similar CV values during the 2018 growing season, although ‘Koroneiki’ trees exhibited higher CV than ‘Arbosana’ after the 2018–19 Winter at that location.

The NPB was influenced over time by olive cultivar, but this effect was independent of location and transplant size (Fig. 3). ‘Arbequina’ olives had higher NPB than ‘Arbosana’ and ‘Koroneiki’ throughout the study. The NPB for ‘Arbosana’ was lower than the NPB for ‘Koroneiki’ throughout the study, except at 4 MAP. NPB decreased in all cultivars from 0 to 4 MAP but an increasing trend was evident thereafter during the 2018 growing season. The NPB also varied over time depending on the orchard location and initial transplant size (Fig. 4A). At the time of transplant (0 MAP), large trees exhibited significantly higher NPB than small trees. From 0 to 4 MAP, large trees showed a decrease in NPB, but the reduction in NPB for small trees was notably smaller. Interestingly, although all trees were presumably similar and from the same sources, the random allocation of trees to sites resulted in large trees at Jay having ~7 more primary branches than equally sized trees at Citra. This initial difference between large trees was no longer observed

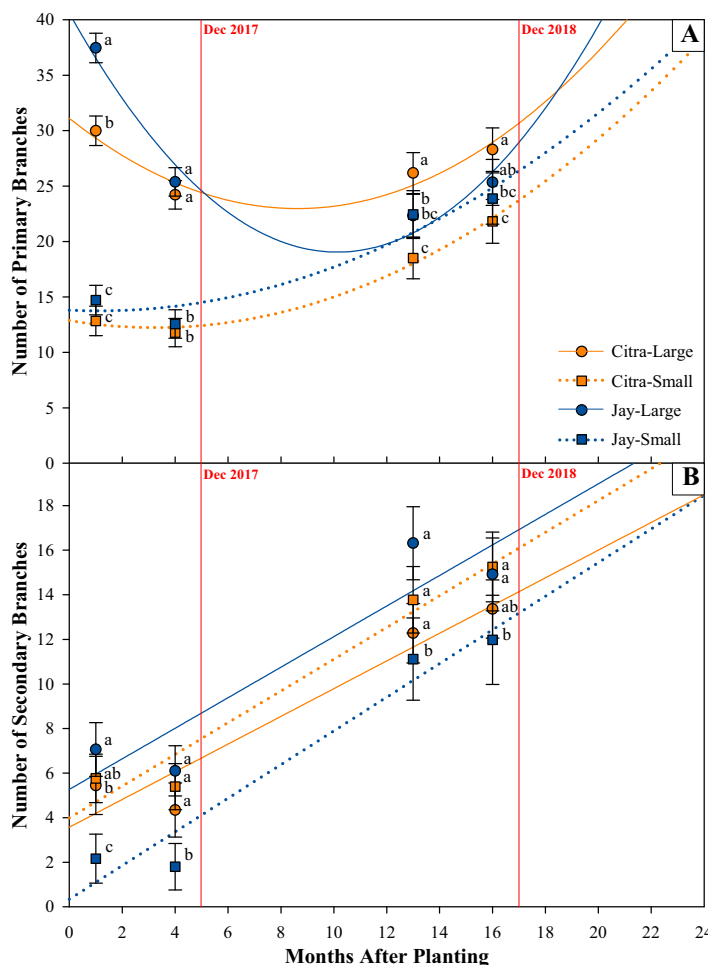


Fig. 4. (A) Number of primary branches and (B) number of secondary branches over time for small and large transplants planted in orchards established in Citra and Jay, FL. Means and standard error bars correspond to the average values for ‘Arbequina’, ‘Arbosana’, and ‘Koroneiki’ olive trees. Trees were planted in Jul 2017 (0 months after planting). Means within each date labeled with the same letter were not statistically different (LSMeans statement, Simulation adjustment; $P < 0.05$).

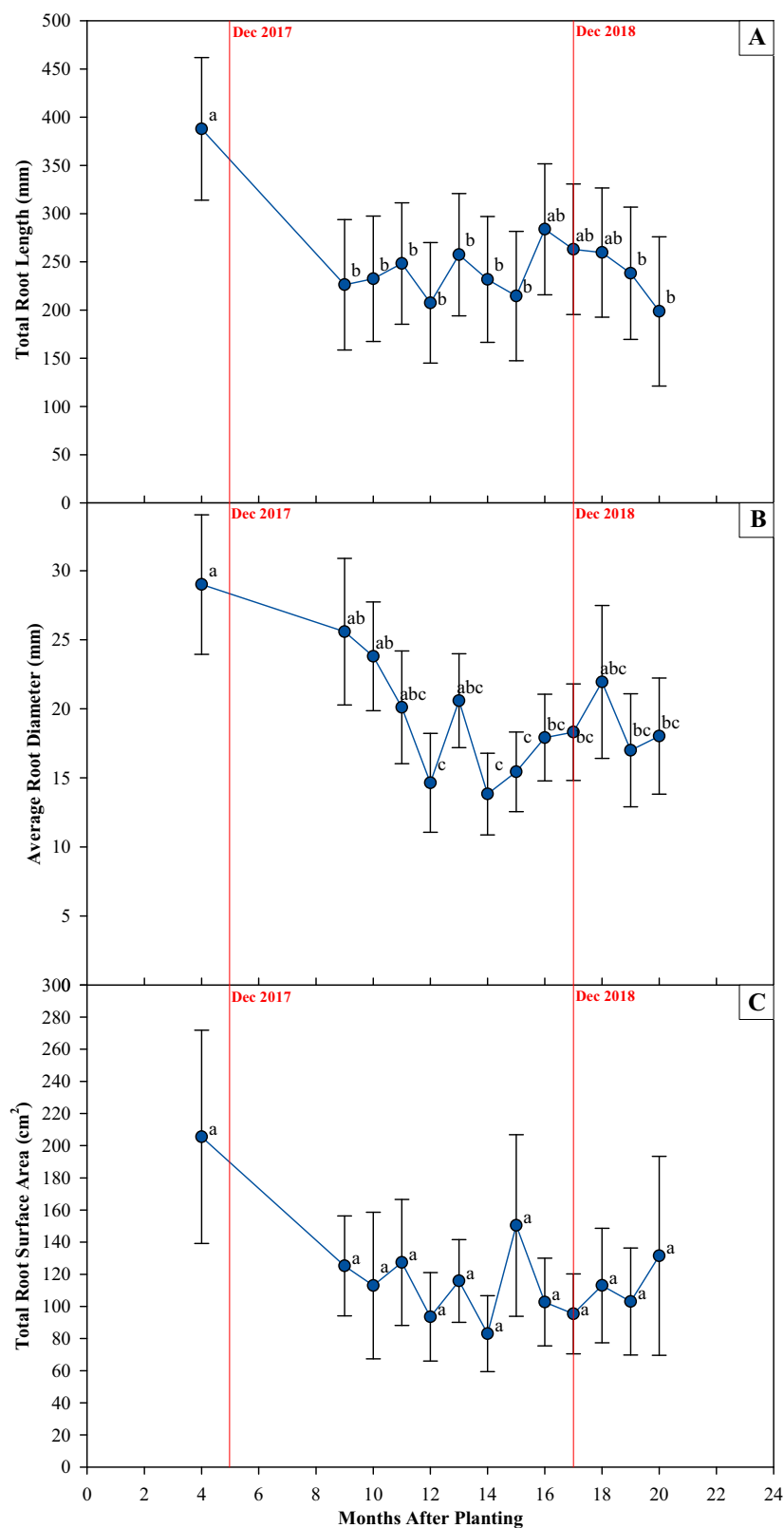


Fig. 5. (A) Total root length (mm), (B) average root diameter (mm), and (C) total root surface area (cm²) over time. Means and standard error bars correspond to the average values for small and large transplants of 'Arbequina', 'Arbosana', and 'Koroneiki' olive trees planted in an orchard in Jay, FL. Trees were planted in Jul 2017 (0 months after planting). Means within each root variable labeled with the same letter were not statistically different (LSMeans statement, Simulation adjustment; $P < 0.05$).

4 MAP. Large trees continued to have higher NPB per tree (13 more primary branches, on average) than small trees at both locations

4 MAP. From 4 to 13 MAP, an increase in NPB was noted in small trees. Large trees showed no significant changes in NPB at

Citra, but a slight decrease occurred at Jay. This decrease in NPB of trees at Jay is likely a reflection of cold damage to canopies during winter. At 13 MAP, large trees in Citra exhibited the highest NPB, four more primary branches than those in Jay. From 13 to 16 MAP, all trees showed an increase in NPB. Ultimately, the response for large trees was similar for both locations, although more primary branches per tree were evident for large trees at Citra. NPB for small trees was similar for both locations throughout the evaluation period. Ultimately, changes over time did not increase NPB differences among large and small trees at Jay, and large and small trees at Citra also exhibited similar NPB.

Transplant size and orchard location influenced the NSB per primary branch over time, but the effects of cultivar did not contribute to this response (Fig. 4B). Interestingly, no reductions in NSB were observed, and this response continued to increase throughout the experiment. Initially (0 MAP), no differences in NSB were evident among large trees at both locations. All trees at Citra exhibited similar NSB, but differences were evident for trees at Jay. At 4 and 13 MAP, both large and small transplant sizes in Citra and large trees at Jay showed no differences in NSB, but small trees at Jay had fewer NSB than all other trees. Presumably, the reduced NSB in small trees at Jay is an effect of cold damage experienced by those plants during the 2017–18 Winter. A similar NSB pattern remained at the last evaluation (17 MAP), with large trees at both locations, small trees in Citra showing no differences, and small trees at Jay showing NSB similar to large trees in Citra.

Total branch index (TBI) is the product of NPB and NSB and provides a hint of the combined changes in primary and secondary branch numbers over time. TBI increased continuously over time and was influenced by initial transplant size, but the effect was independent of orchard location and olive cultivar (Fig. 1C). TBI for large trees was higher than TBI for small trees throughout the experiment until 16 MAP, when TBI for large and small transplant sizes showed no differences.

Root growth measurements. Root growth data, including TRL, ARD, and TSA, were not influenced by transplant size or olive cultivar when depth was not considered as a factor (Supplemental Table 3). The highest TRL (Fig. 5A), ARD (Fig. 5B), and TSA (Fig. 5C) were observed 4 MAP, during the first evaluation. After the 2017–18 Winter (between 4 and 9 MAP), all variables show a reduction. Small numerical changes in TRL, ARD, and TSA variables were noticed from 9 MAP until the last date of observation at 20 MAP.

Interestingly, root growth data indicate the measured variables were influenced by transplant size, olive cultivar, and depth when depth was considered as a factor (Supplemental Table 4). Soil depth influenced TRL over time independent of initial transplant size or olive cultivar (Fig. 6A). Trees showed the highest TRL at 20- to 40-cm

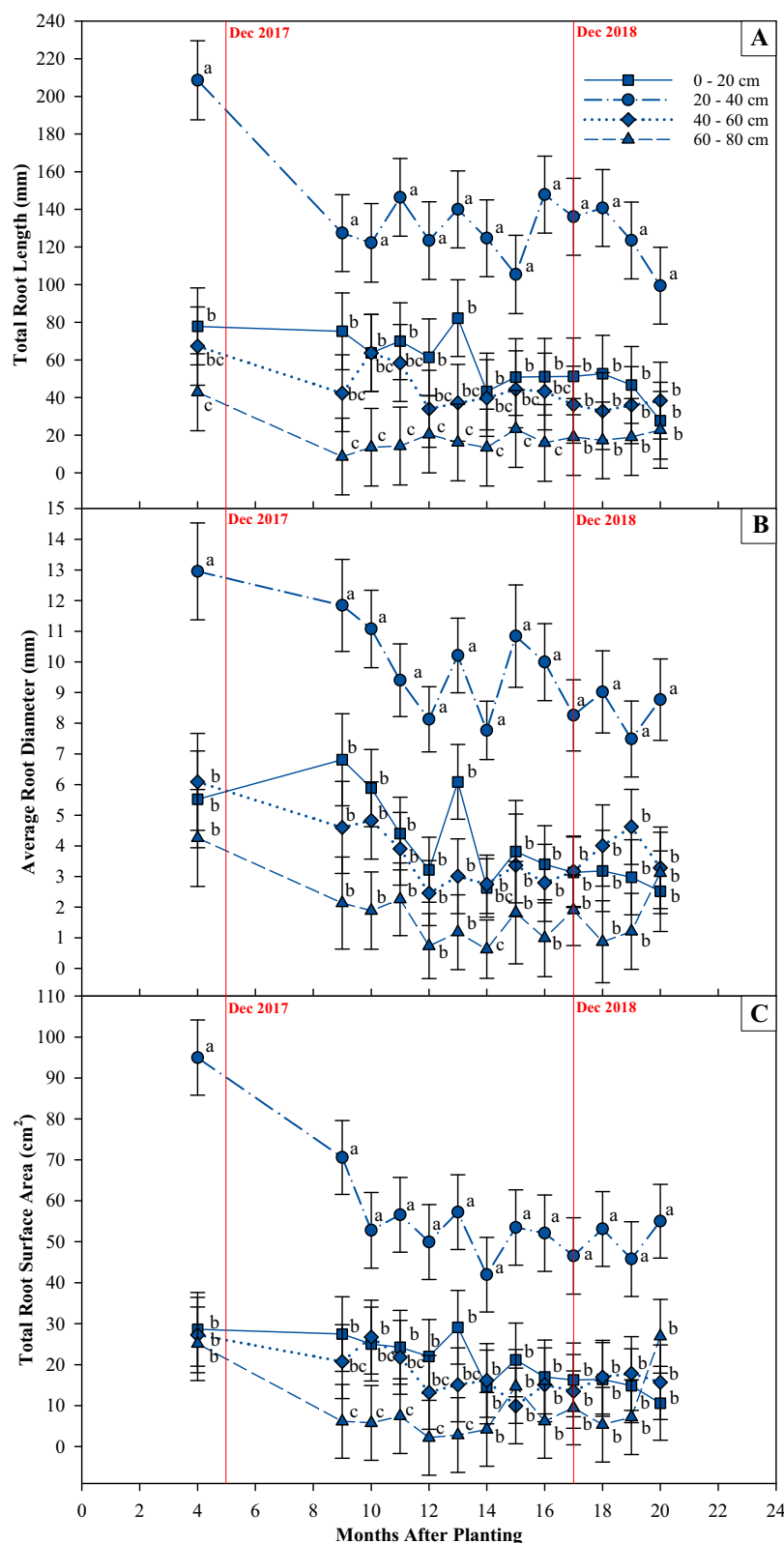


Fig. 6. (A) Total root length (mm), (B) average root diameter (mm), and (C) total root surface area (cm²) over time as affected by soil depth within 0- to 20-cm, 20- to 40-cm, 40- to 60-cm, and 60- to 80-cm depth segments. Means and standard error bars correspond to the average values for small and large transplants of 'Arbequina', 'Arbosana', and 'Koroneiki' olive trees planted in an orchard in Jay, FL. Trees were planted in Jul 2017 (0 months after planting). Means within each date labeled with the same letter were not statistically different (LSMeans statement, Simulation adjustment; $P < 0.05$).

depth throughout the experiment, and the lowest TRL was evident at 60- to 80-cm depth, although from 17 to 20 MAP the 60- to 80-cm

segment exhibited similar TRL compared with the 0- to 20- and 40- to 60-cm segments. From 4 to 9 MAP, roots at all depths exhibited a

slight, nonsignificant decreasing trend, although this trend was more pronounced at depths of 20 to 40 cm.

Soil depth also influenced ARD over time independent of initial transplant size or olive cultivar (Fig. 6B). Similar to TRL, roots exhibited the highest ARD at 20- to 40-cm depth throughout the experiment. ARD did not differ among the 0- to 20-, 40- to 60-, and 60- to 80-cm depth segments. From 4 to 9 MAP, roots in most of the segments showed a slightly decreasing trend, except for roots at 0- to 20-cm depth. Transplant size and olive cultivar also influenced ARD over time independent of depth (Fig. 7). Initially, at 4 MAP, the only difference in ARD was between small 'Arbosana' and 'Koroneiki' trees. All trees showed similar ARD from 9 to 19 MAP. By 20 MAP, the greatest ARD was observed on small 'Arbequina' olives, and it only differed between small 'Arbequina' and small 'Arbosana' trees at 20 MAP.

Similar to TRL and ARD, soil depth influenced TSA over time independent of initial transplant size or olive cultivar (Fig. 6C). During the study, trees showed the highest TSA at 20- to 40-cm depth. All other segments showed similar response, although TSA at 60- to 80-cm depth was smaller than in all other segments from 9 to 13 MAP. From 4 to 9 MAP, TSA at all depths showed a slight decreasing trend, although it was more evident at 20- to 40-cm depth. Olive cultivar also influenced TSA over time independent of transplant size and depth (Fig. 8). Although the analysis indicates cultivar as a significant factor, TSA for 'Arbequina' was higher than TSA for 'Koroneiki' only at 4 MAP. No other differences in TSA were observed during the study.

Discussion

Small transplants grew at a higher rate than large transplants, which can be inferred from the continuous numerical reduction in TH difference between both groups. However, large transplant size trees remained taller than small transplant size trees during the study. Similar growth rate patterns when comparing small and large size transplants have been previously reported in *Fagus sylvatica* L., *Ulmus laevis* Pall., *Fraxinus excelsior* L., *Acer pseudoplatanus* L., *Robinia pseudoacacia* L., and *Quercus rubra* L. (Ivetic et al. 2016). The reduction in differences between large and small transplant sizes was also observed in the TCA. Lauderdale et al. (1995) noticed that small red maple transplants exhibited higher shoot elongation compared with large transplants during the first year of their experiment. This increase in shoot elongation was reflected in a trunk diameter expansion during the second year, which aligns with our results.

The decreasing trend in TH at the beginning of the experiment in 'Arbosana' and 'Koroneiki' olives at Jay indicated that the upper portion of those trees may have suffered sufficient transplant stress to damage the main stem. Trees were transplanted in

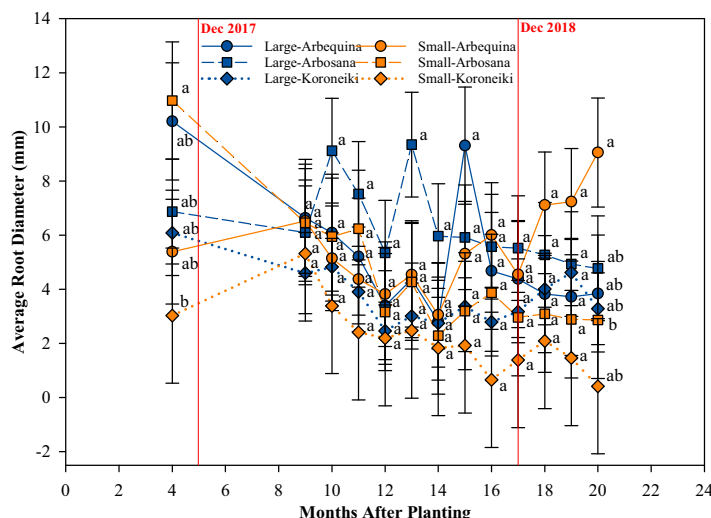


Fig. 7. Average root diameter (mm) over time for small and large transplants of 'Arbequina', 'Arbosana', and 'Koroneiki' olive trees. Means and standard error bars correspond to the average values for root segments distributed within 0- to 20-cm, 20 to 40-cm, 40- to 60-cm, and 60- to 80-cm depth. Trees were planted in Jul 2017 (0 months after planting). Means within each date labeled with the same letter were not statistically different (LSMeans statement, Simulation adjustment; $P < 0.05$).

Jul 2017, during summer in Florida, which provided favorable conditions for vegetative growth in healthy trees. However, the high average maximum temperatures (36°C) during Summer 2017 may have stressed the newly transplanted trees (Benlloch-González et al. 2018; Larcher 2000; Niinemets 2010; Teskey et al. 2015; Zida et al. 2023) before root systems expanded beyond the original container root volume. Although taller transplants typically exhibit higher growth rate, shorter transplants can be advantageous under stressful conditions because they are less susceptible to water stress, as observed by Grossnickle and MacDonald (2018). In addition, the differences between maximum and minimum temperatures increased rapidly in Sep 2017,

particularly at Jay, which recorded minimum average temperatures 7°C lower than those at Citra, although maximum temperatures remained similar. This continuously increasing gap between maximum and minimum temperatures may have further stressed and slowed the development of the actively growing upper portion of 'Arbosana' and 'Koroneiki' trees (Taiz et al. 2022). In addition, the fact that height reduction was practically not observed in 'Arbequina' olives suggests that this cultivar is more tolerant to transplant stress than 'Arbosana' and 'Koroneiki'.

Although all cultivars showed a reduction in NPB after transplanting, the decline was more pronounced in large trees, further indicating that transplant stress had a greater impact

on large transplant size trees compared with small transplant size trees, consistent with previous findings (Chance et al. 2017). The ability of small transplant size trees to quickly overcome transplant stress may be linked to their higher adaptive capacity under stressful environments, as suggested by previous research (Grossnickle and MacDonald 2018; Lauderdale et al. 1995; Watson 2005). In addition, roots are susceptible to stress after transplant due to the high quantity of resources used to adapt to new environments (Dror et al. 2020). Transplant stress can impair root function, reducing the plant's ability to transport soil water to aboveground tissues, particularly to the most distant parts of taller trees. Although root growth differences related to transplant size were not detected in our study, likely due to an insufficient number of minirhizotrons and other limitations of this method (Franco and Abrisqueta 1997), greater root growth in small transplant size trees compared with large transplant size has been previously reported (Watson 2005).

The low temperatures during the 2017–18 Winter (from 4 to 8 MAP) seemed to have caused significant stress on the trees, particularly in the orchard at Jay. Although only one 'Koroneiki' tree died after winter at Citra, mortality was high in 'Arbosana' and 'Koroneiki' at Jay (data not shown), especially among small transplants of these cultivars. 'Arbosana' (Wang et al. 2018) and 'Koroneiki' (Vossen 2007) olive trees are considered sensitive to low temperatures compared with 'Arbequina', which is consistent with the observations of this study. In fact, 'Arbequina' is considered one of the most cold-hardy cultivars, able to resist temperatures down to -6°C with no signs of damage (Therios 2009). From Dec 2017 to Apr 2018, the average minimum temperatures at Jay were 3°C lower than those at Citra. In Jan 2018, the coldest month throughout the study, temperatures dropped to -8°C in Jay and -4°C in Citra. During that month, nine nights below -4°C were recorded at Jay, whereas only one was recorded at Citra. According to Vossen (2007), young olive trees can be seriously injured when exposed to temperatures below -4°C . On the other hand, small branches of mature trees can suffer significant damage at temperatures below -5.5°C . This is consistent with the observations of this study and explains the higher tree damage observed at Jay. In addition, low temperatures can be lethal for mature trees when they fall below -9.5°C .

The greater CV observed in 'Arbequina' olive trees at Jay after the 2017–18 Winter suggests a higher recovery capacity after severe cold damage compared with 'Arbosana' and 'Koroneiki'. Interestingly, the greater increase in TH in 'Arbequina' may indicate a more elongated canopy architecture for this cultivar. In the evaluations conducted after 10 MAP (May 2018), responses indicated that all surviving trees recovered from winter stress and resumed vegetative growth. The positive increases in growth continued through our last observations, even after the 2018–19 Winter. It is important to highlight

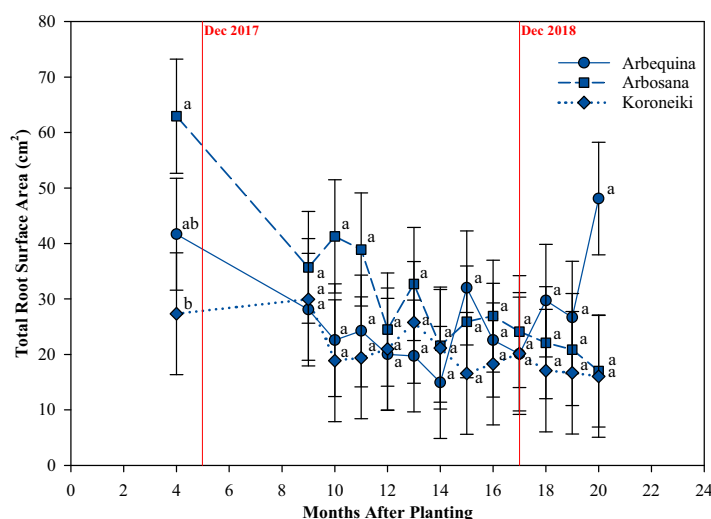


Fig. 8. Total root surface area (cm^2) over time for small and large transplants of 'Arbequina', 'Arbosana', and 'Koroneiki' olive trees. Means and standard error bars correspond to the average values for root segments distributed at depths of 0 to 20 cm, 20 to 40 cm, 40 to 60 cm, and 60 to 80 cm. Trees were planted in Jul 2017 (0 months after planting). Means within each date labeled with the same letter were not statistically different (LSMeans statement, Simulation adjustment; $P < 0.05$).

that the temperatures during the second winter after planting were not as low as those in the first winter. However, the damage from the 2017–18 Winter demonstrated the importance of orchard location, initial transplant size, and cultivar selection on tree survival, recovery, and vegetative growth capacity.

To better understand the impact of cultivar selection and transplant strategies on early orchard establishment, the development of the root systems was also monitored using minirhizotrons. However, the limited number of minirhizotrons installed in the orchard, along with the inherent limitations of minirhizotrons as a root growth assessment technique (Franco and Abrisqueta 1997), may have reduced our ability to detect differences related to cultivar. In addition, no differences in root growth were observed between large and small transplant sizes at the beginning of root image collection or at any other time point.

The significant reduction in TRL observed from 4 to 9 MAP can be attributed to stress caused by the 2017–18 Winter. A similar trend was noted in ARD and TSA after the winter. Rainfall is reduced during winter in north Florida. In addition, irrigation systems were turned off and drained in Nov 2017 to prevent overwatering and irrigation system damage. Besides canopy damage from low winter temperatures, scarce rainfall likely exacerbated stress on the olive trees, contributing to the decrease in TRL and declining trends in ARD and TSA. Similarly, Eissenstat et al. (2000) reported higher root mortality in non-watered apple trees compared with watered ones, with comparable responses observed in peach trees by Abrisqueta et al. (2008). Despite these challenges, root growth stabilized between 9 MAP and the final observation date (20 MAP). These results align with the observations of Fernández Luque et al. (1992) in ‘Manzanillo’ olives.

The depth of the root system was an important factor influencing root growth. This has been previously reported in other studies using minirhizotrons (Anderson et al. 2003; Fernández Luque et al. 1992; Gluszek et al. 2013; Searles et al. 2009). Approximately 75% of the root system was concentrated in the upper 60 cm of soil, in concert with previous observations (Sibbett and Ferguson 2005; Therios 2009). TRL, ARD, and TSA were higher at 20- to 40-cm depth, which agrees with previous studies in ‘Manzanillo’ olives (Fernández Luque et al. 1992; Searles et al. 2009) and grapes (Anderson et al. 2003). Previous olive research has shown that most of the root growth concentrates in the upper portion of the soil, within the first 50 cm, especially when irrigation is available. In agreement with our observations, higher root density in upper soil horizons has been observed in different fruit trees such as apple (Ma et al. 2013a), peach (Paltineanu et al. 2016), walnut (*Juglans regia* × *nigra* L.) (Cardinael et al. 2015), and jujube (*Ziziphus jujube* Mill.) (Ma et al. 2013b), as well as in other tree species (Watson and Himelick 1982).

The availability of irrigation during the growing season may have contributed to the concentration of most of the root systems within the 20- to 40-cm depth segment. Ample water supply around the microsprinklers could also explain why only minor changes in root TRL, ARD, and TSA were observed from 9 to 20 MAP, rather than a significant expansion of the root system. In addition, split fertilizer applications were made during the growing season. According to Othman and Leskovar (2019), olive root development might be detrimentally influenced by N fertilization. These authors observed that root growth and density were negatively affected by increasing N applications, which suggests that the availability of N may have decreased root growth.

Conclusion

Our results also suggest that small transplants are better suited to withstand transplant stress when establishing olive orchards in the southeastern United States. Moreover, their faster growth rate may help offset initial size disadvantages compared with large transplants in the long term. However, our study also showed that small transplants, particularly ‘Arbosana’ and ‘Koroneiki’, are more vulnerable to cold damage from low winter temperatures. In contrast, ‘Arbequina’ olive trees demonstrated greater tolerance to transplant stress, as well as better cold hardiness and post-winter recovery. This cultivar may be particularly well-suited for subtropical regions where extended periods of temperatures as low as -4°C are expected. These findings reinforce the importance of cold hardiness as a critical trait for subtropical olive production. On the other hand, the root growth decline observed during winter (from 4 to 9 MAP) may be attributed to cold temperatures and soil moisture limitations. However, root growth stabilized after winter, with most roots concentrated in the upper 60 cm of soil, particularly at 20- to 40-cm depth. This distribution is consistent with previous studies and may be associated with irrigation and fertilization practices. Overall, this study highlights the influence of cultivar selection, transplant size, and environmental conditions on the adaptation and early establishment of olive trees grown under a warm and humid subtropical climate. Further research is needed to explore the long-term effects of these factors on tree productivity, root development, cold tolerance, and canopy recovery after environmental stress.

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