Title: Identifying belowground response to water deficits and developing tools for mitigating drought stress in apple trees

Personnel:

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Duration of project: 2 years (May 2022 – January 2024)

Justification:

For the Mid-Atlantic areas, despite known for abundant precipitation, some predictive models forecasted an increase in agricultural drought due to increased surface and groundwater flow and evapotranspiration (Kang and Sridhar, 2018). Such a trend presents a threat to fruit production, including apple (*Malus* x *domestica*), in the region. Water-deficit stress not only results in reduced apple yield and fruit quality but can also lead to tree mortality especially for young plantings that are sensitive to adverse growing conditions. Given the role of roots in water acquisition and sensing soil water deficits, a better understanding of how apple roots respond to the environment with limited water is fundamental for strategy development to enhance apple production in the presence of challenging climates.

In apple, fine roots are responsible for taking up water and dissolved minerals in the soil (Lavely et al., 2020). Distribution of fine roots, which is determined by various component traits including root depth, length, angle, and branching, influences the contact between roots and soil and therefore is indicative of drought resilience and associated mechanisms. However, due to the difficulty in preserving architectural traits during uprooting, much remains unclear about the distribution of apple fine roots alone and with respect to soil moisture depletion. As severe stress often causes dieback of small-diameter roots in apple (Wells and Eissenstat, 2001), the capability of trees to produce new absorptive roots upon re-watering following a drought episode is pivotal to maintaining normal physiology and fruit production. Therefore, post-drought-stress recovery in apple needs to be investigated in the context of stress resilience.

Apple trees with marginally more fine roots tended to have better internal plant water status (in terms of leaf water potential) when irrigation was interrupted (Tworkoski et al., 2016), indicating an association between fine root biomass and tolerance to water deficits. This further suggests manipulating root growth may be a potential avenue to enhance drought resilience in apple. Relevantly, a synthetic analog of plant hormone strigolactone (SL) was recently demonstrated to increase fine root density in grapevine (*Vitis vinifera*) (Jiu et al., 2022). Thus, whether this plant growth regulator (PGR) has additive effects on apple fine roots, as observed in grapevine, and thereby confers apple trees' tolerance to water-deficit stress awaits determination.

This proposal aims to characterize apple fine root distribution in response to the changes in soil moisture when treated with SL, a root-promoting PGR. The goal is to fill in the gap between belowground traits and whole-tree physiology in apple regarding drought resilience. Results generated from the PGR studies will provide a novel, precautionary tool to mitigate drought stress in apple trees. Additionally, the findings from the research efforts will help clarify the dynamics between root growth patterns and soil water availability. This information has critical implications for the effects of water-deficit stress on belowground activities that can influence apple tree development as well as orchard sustainability, such as root turnover, nutrient extraction, and carbon sequestration. Ultimately, the new knowledge developed from the proposed work will benefit the development of field strategies to maintain a healthy root system, which is the key to enhancing productivity of apple trees in the face of extreme climate events in the Mid-Atlantic region.

Objectives:

Year 1 (May 2022 – Jan 2023)

1. Characterize root distribution of apple trees during soil water depletion and after the relief of stress by re-watering.
2. Evaluate the effects of strigolactone (SL) on fine root production of apple trees.

Year 2 (May 2023 – Jan 2024)

3. Assess the effects of SL on apple fine roots in response to water-deficit stress.

Procedures:

Plant materials and growing conditions.

Three independent experiments (to meet the proposed objectives 1, 2, and 3, respectively) will be conducted using first leaf 'Gold Rush' apple grafted on 'Budagovsky 9' in a controlled greenhouse at the Appalachian Fruit Research Station, Kearneysville, WV. Specialized growing containers ("rhizoboxes") (**Figure 1**), instead of conventional pots, will be used to preserve root system architecture. Rhizoboxes are constructed from scratch using polycarbonate sheets to create an inner dimension of 60-cm height by 60-cm width with 5-cm depth (24" h × 24" w × 2" d) and covered with aluminum foil to avoid light penetration and temperature increase at the root zone. Inside the rhizobox, there is a pinboard-like matrix of needles inserted at 2.5 cm by 2.5 cm spacing to enable roots stay in place during excavation. Prior to the start of all experiments, apple trees will be grown in rhizoboxes under optimal conditions for at least 2 months (May – July) to allow the establishment of root system in the box.

Soil sensors will be inserted at different depths of the rhizobox to monitor volumetric water content and temperature in real time during the experiments. In addition, to document the condition of growing media when tree data are collected, potting substrate in the rhizobox will be collected at excavation to determine moisture content, pH, and bulk density.

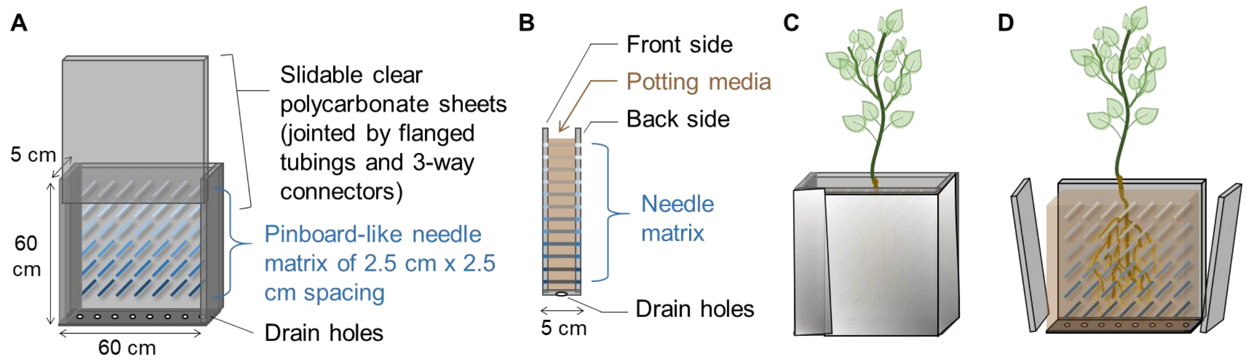

Figure 1. Rhizobox. A, Prototype construction; **B**, longitudinal section of a rhizobox; **C**, tree in a rhizobox covered with aluminum foil; **D**, rhizobox disassembly for root excavation.

Experiment setup and treatment conditions.

Experiment 1 (objective 1): In Aug 2022, tree data will be collected (aboveground measurement and destructive root sampling) as pre-treatment control in the beginning of Experiment 1. For water-deficit treatment, irrigation will be withheld for 2 weeks followed by 2 weeks of re-watering, whereas trees of well-watered treatment are irrigated regularly for 4 weeks (**Figure 2**). Tree will be assessed and sampled in both treatments at the end of week 2 and 4.

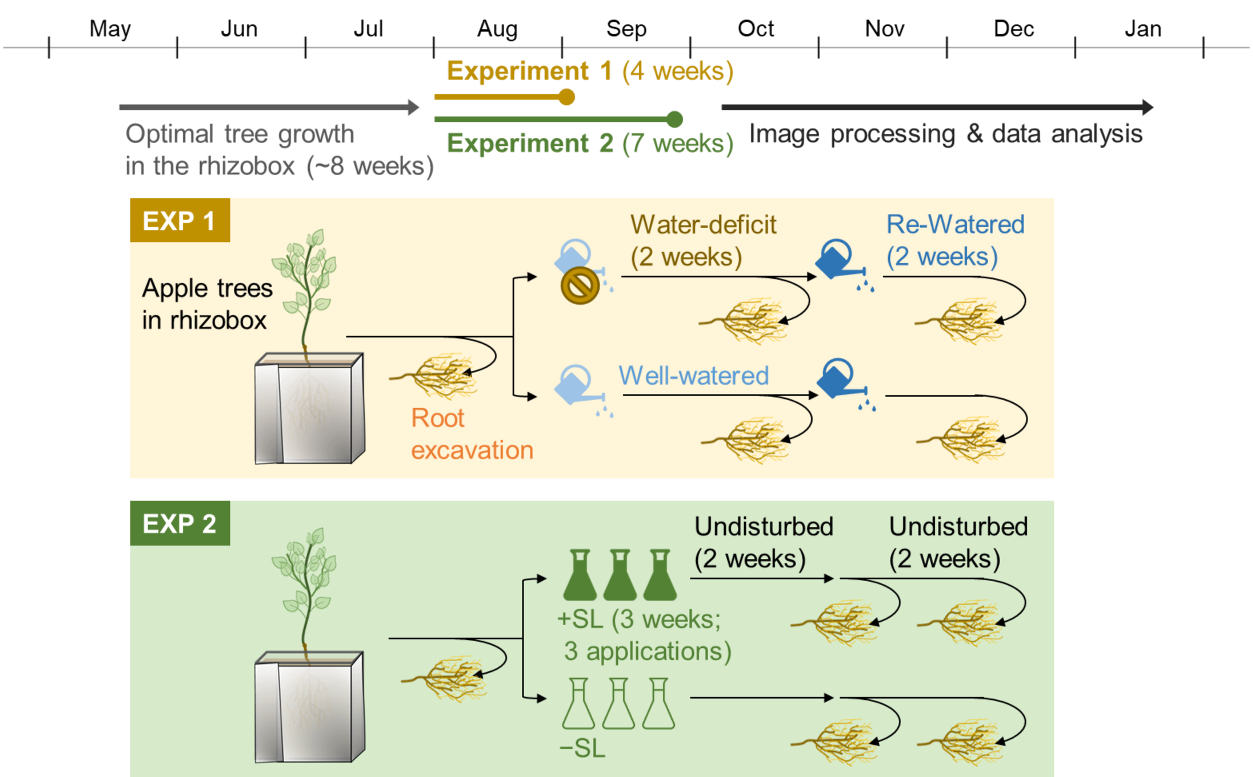

Figure 2. Experimental schemes for year 1 (2022). Experiments (EXPs) 1 and 2 are depicted in the yellow and green color blocks, respectively, with anticipated time indicated below the timeline; strigolactone, SL.

Experiment 2 (objective 2): After pre-treatment collection (Aug 2022) for Experiment 2, SL will be applied weekly to half of the apple trees via root drench for 3 weeks (+SL treatment), and the rest are receiving the same volume of solution containing no SL (-SL treatment) (**Figure 2**). After the third application, trees are left undisturbed (without additional PGR or stress) for 4 weeks; tree will be evaluated and sampled 2 and 4 weeks following the last PGR application.

Experiment 3 (objective 3): Experiment 3 is a factorial design (2 PGR × 2 stress treatments) and comprises two phases starting in Aug 2023. In *Phase I*, apple trees will be applied with or without SL weekly for 3 weeks (+SL and -SL treatments, respectively) and left undisturbed for 2 weeks. In *Phase II*, within each PGR treatment, half of the trees will not receive water for 2 weeks followed by re-watering for 2 weeks (water-deficit treatment), and the other half will be irrigated for 4 weeks (well-watered treatment). At the end of *Phase I* and at weeks 2 and 4 (last day of the stress period and re-watering/watering period, respectively) of *Phase II*, tree data will be collected from each unique PGR × stress treatment.

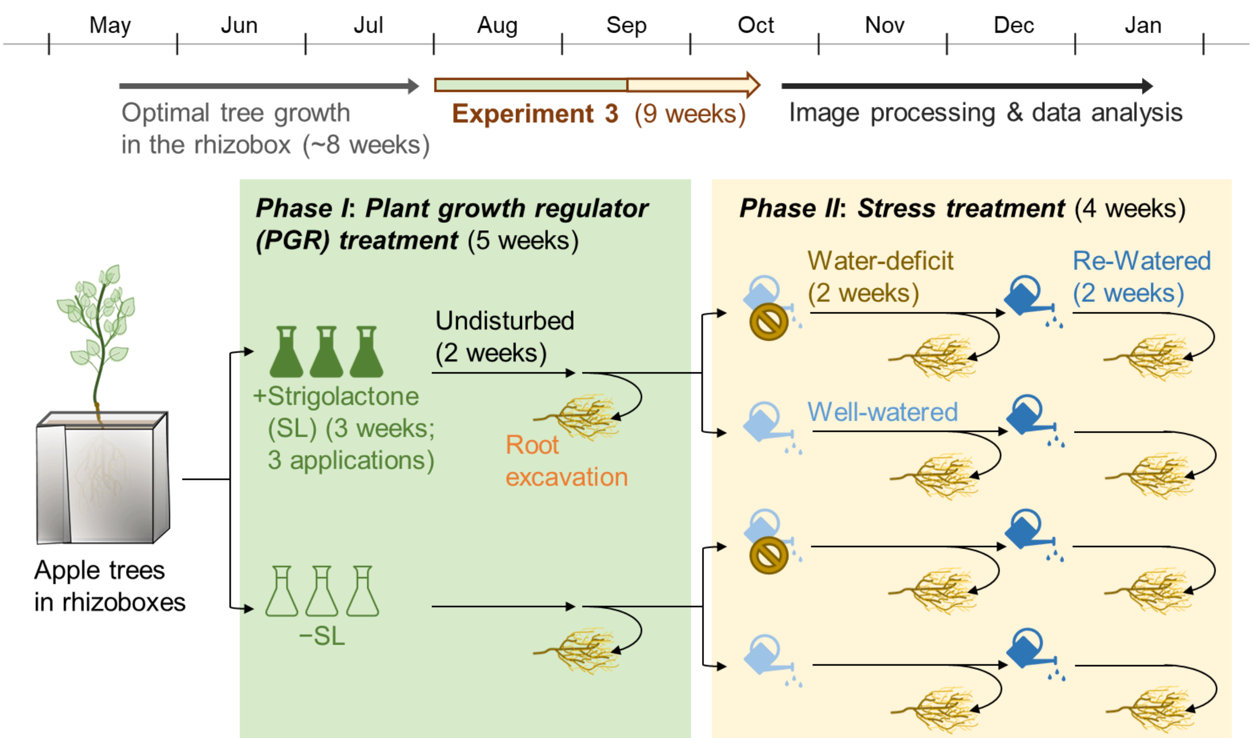

Figure 3. Experimental schemes for year 2 (2023). Experiment 3 (2 PGR × 2 stress treatments) with two phases is depicted with anticipated time indicated below the timeline.

Aboveground and belowground tree data collection

In Experiments 1 through 3 of this proposal, tree data collection refers to both aboveground assessment (nondestructive) and belowground sampling (destructive). One day before root sampling, all trees will be evaluated for aboveground growth (trunk diameter and tree height), physiology (leaf water potential and photosynthetic parameters), and stress response (leaf rolling and abscission). For belowground excavation, rhizoboxes of trees will be dissembled (**Figure 1D**) followed by removal of potting substrate by an air compressor, leaving the root system confined by the needle matrix for photograph. Next, roots at different vertical depths will be separated and rinsed thoroughly for high-resolution scanning. Root photographs and scans will be processed and analyzed using RhizoVision Explorer software to obtain root angle, diameter, and length. Finally, roots and the aboveground portion will be dried to determine the biomass allocation. The procedures described herein will be used on all occasions of tree data collection/processing in this project.

Budget:

Total requested: $28,102

Salaries	$0	
Hourly wages	$6,307	1 summer student (efforts: $13.14/hour x 40 hours/week x 12 weeks, with benefit at 42% of federal salary)
Fringe benefits	$2,649	
Supplies	$9,040	Trees ($450), rhizobox materials ($6,000), potting media ($465), PGR applications ($290), tools and supplies for root excavation and scan ($635), and dual-light sourced scanner ($1,200)
Travel	$0	
Miscellaneous	$0	
Year 1 total	**$17,996**	

Salaries	$0	
Hourly wages	$6,307	1 summer student (efforts: $13.14/hour x 40 hours/week x 12 weeks, with benefit at 42% of federal salary)
Fringe benefits	$2,649	
Supplies	$1,150	Trees ($450), potting media ($465), PGR applications ($235)
Travel	$0	
Miscellaneous	$0	
Year 2 total	**$10,106**	

Other support: There is no other funding source for this proposed project.

References:

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