

## State Horticultural Association of Pennsylvania | Research Grant Proposal, 2022

**Title:** Quantification of drought stress tolerance: A 3-dimensional approach using precision automated irrigation and topological data analysis.

### Personnel:

**Dr. Andrew M. Bierer** (Principal investigator), Research Soil Scientist, USDA-ARS Appalachian Fruit Research Station (AFRS), 2217 Wiltshire Rd, Kearneysville, WV 25430-2771. 1-304-725-3451 ext(326) | [Andrew.bierer@usda.gov](mailto:Andrew.bierer@usda.gov)

**Dr. Lisa Tang** (Co-Principal investigator), Research Horticulturalist, USDA-ARS Appalachian Fruit Research Station (AFRS), 2217 Wiltshire Rd, Kearneysville, WV 25430-2771.

**Duration of Project:** 2 years (April 2022 – January 2024)

### Justification:

Increased prevalence of climatic extremity in the last century has been linked with a high degree of confidence to human-induced climate change (IPCC, 2021). Uncharacteristic heat extremes, frequency of high intensity rainfall events, and concurrent heat and drought events have increased on a global scale. Study of climatic change in the Mid-Atlantic region of the United States show an increase in general “wetness”, e.g. soil moisture contents (Smith & Chang, 2020), mean annual precipitation (Polsky, Allard, Currit, Crane, & Yarnal, 2000), and notably, precipitation intensity (Karl & Knight, 1998). Despite this, periodic drought is common (Glenn, 1999; Holshouser; & Whittaker, 2002; Opoku et al., 2019) and is of concern for regional production of tree fruits (Bassett, 2013; Tworkoski & Fazio, 2015).. Additionally, increased early season temperatures may exacerbate frost kill while incidence of specific diseases may favor droughted or sodden edaphic environments. Clearly, an erratic climate presents challenging conditions for fruit growers in the Mid-Atlantic region.

Modernization of techniques utilized in abiotic stress research will promote the resolution and efficacy at which varietal selections are made for specific regions. Historically, drought stress imposed in horticultural research is accomplished by ceasing irrigation entirely until occurrence of stress symptoms or imposed for a pre-defined time period (Osmolovskaya et al., 2018), subjectively determined deficit irrigation rates (Atkinson, Policaprio, Webster, & Kingswell, 2000) or maintained at a fraction of the saturated media’s container mass (Granier et al., 2006). While exposing general plant tolerance to hydrologic stress, quantifiable levels of tolerance are exposed less frequently by this methodology. More precise identification of tolerance will improve variety selection for specific regions and management constraints. Standard quantifiable measures of drought stress include photosynthetic rate, stomatal aperture, and tissue water potential (Osmolovskaya et al., 2018). However, these metrics have a somewhat limited commercial adoption due to equipment requirements and lack of autonomy. Consequently, commercial orchards may manage water through irrigation scheduling tables or by growers’ experience. This empirical approach may have diminishing suitability considering increased annual variability and outlier conditions likely to become more prevalent under climate change. Responsive irrigation programs tolerant to a turbulent climate can be developed using

soil moisture sensors (He & Weber, 2020). Such programs are a viable water management alternative that are relatively inexpensive, scalable, and directly reflect edaphic hydrologic stress yet are not extensively used in horticultural drought stress research to quantify edaphic hydrologic tolerance.

Rooting depth, length, and density are believed to govern drought stress tolerance (Psarras & Merwin, 2000) but are intrinsically more difficult to study due to occlusion in media and complex branching. As a result, less is known about root system architecture (RSA) than above ground structure in response to the changes in environmental conditions, especially for woody species. It was recently suggested that above ground mesocosms provide an effective avenue for study of 3-D RSA for study of root growth and function when combined with photogrammetry and specialized sensor arrays (Dowd, McInturf, Li, & Topp, 2021). As explained by Dowd et al. (2021) wire lattice and specialized software provide a solution for obtaining 3-D RSA from 2-D photos using topological data analysis. Integration of sensor planes in the wire lattice allows for 3-D study of root activity and function associated with the variations in media conditions, which complement abiotic stress research. In this sense, mesocosms provide a realistic scale for studying root response to artificially imposed stresses, and therefore can benefit the evaluation of belowground traits during the variety selection for tree fruit and/or berry breeding. Precise regulation of the imposed stress at such a realistic scale provides a framework for identifying varietal response and performance under levels of stress, i.e., quantification of tolerance, and justifies the endeavor. Therefore, please consider the following proposal for a new approach to abiotic stress tolerance research with drought stress as the incipient abiotic condition of study.

### **Objective(s):**

#### *Year 1*

1. Construct two above-ground mesocosms with root architecture grid and integrated sensor planes; transplant apple trees in mesocosms for root establishment into the grid.
2. Assess the ability of the automated water management system to maintain soil matric potential and characterize plant physiology under baseline un-stressed conditions.

#### *Year 2*

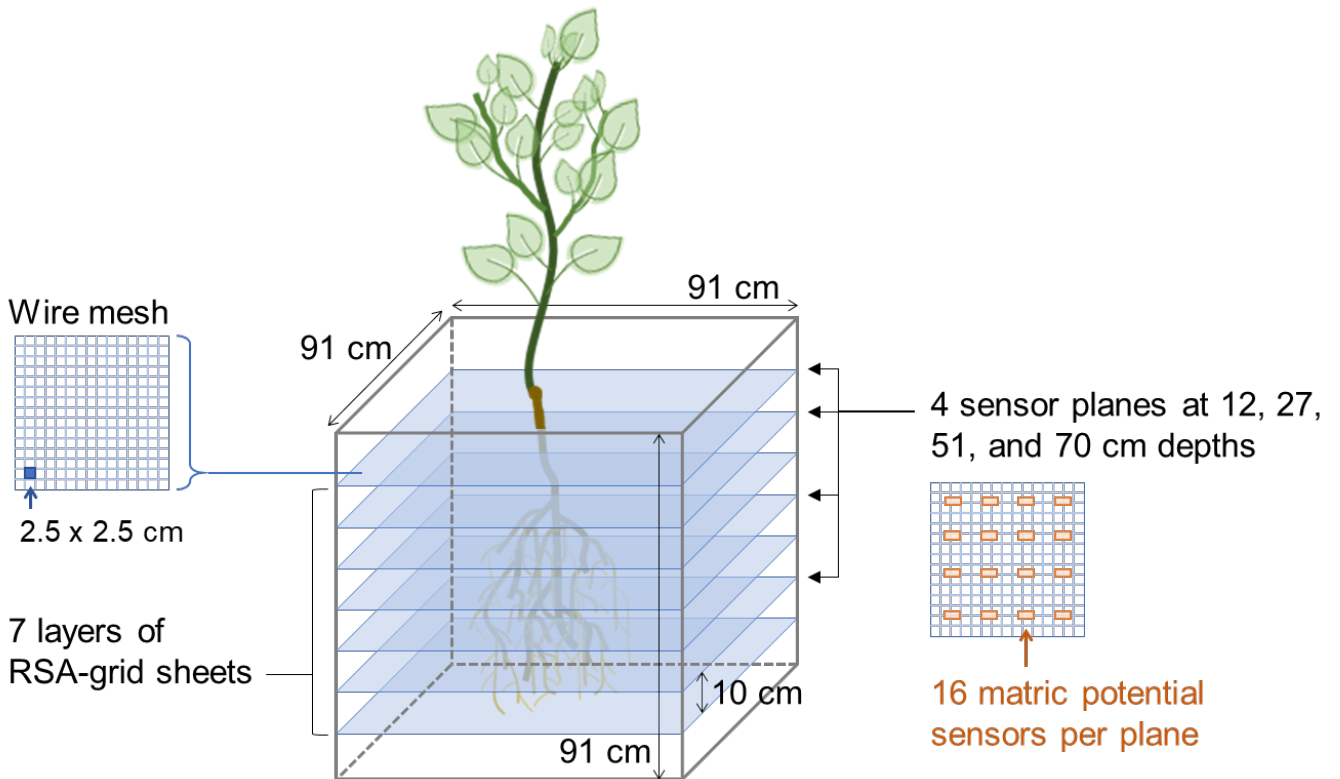
1. Determine aboveground response of apple trees to imposed hydrologic drought stress and assess capability of water management system for imposition of the stress.
2. Characterize RSA response through mesocosm deconstruction and reconstruction of 3-D RSA using photogrammetry.

### **Procedures:**

**Water Management System.** A water management system is currently under development for abiotic stress research at the Appalachian Fruit Research Station (AFRS) in Kearneysville, WV. The system is being designed to leverage cost efficient hardware in an open-source package for quantification of and response to edaphic hydrologic conditions. At the time of writing, the system “node” consists of a microcontroller platform (atmega 1284p) integrated with a

timekeeper (DS3231), micro-sd storage module, a multiplexed (CD74HC4067) array of 16 resistive soil water sensors (Watermark200ss), array of soil temperature sensors (DS18B20), 4 channel relay module, and 915 MHz radio transmission module. The program reads connected sensors, saves a user interpretable string, and transmits the data to a collector “gateway” for compilation with data from additional nodes. The gateway may trigger an irrigation event based on data received as specified by the user. The first formal test of the management system is set to occur in a greenhouse trial in early 2022.

**Mesocosm Design.** All research activities will take place at the AFRS in WV. Two mesocosms 0.91 x 0.91 x 0.91 m ID (3x3x3 ft) will be constructed (**Figure 1**). The RSA grid will be constructed from wire mesh (2.54 x 2.54 cm) sheets inserted into each mesocosm. The grid will consist of 7 sheets 10 cm apart vertically joined by a light frame for shape retention. Four sensor planes will be incorporated into each mesocosm at 11.5, 26.5, 51, and 70 cm depths. Each sensor plane will consist of one “node”, i.e., 16 matric potential sensors. When mesocosms are complete young apple trees will be transplanted, permitting natural root establishment into the RSA grid under optimal growing conditions for 1 year. At transplant, rooting parameters of interest will be quantified. Biometric measures will be collected on a regular interval.



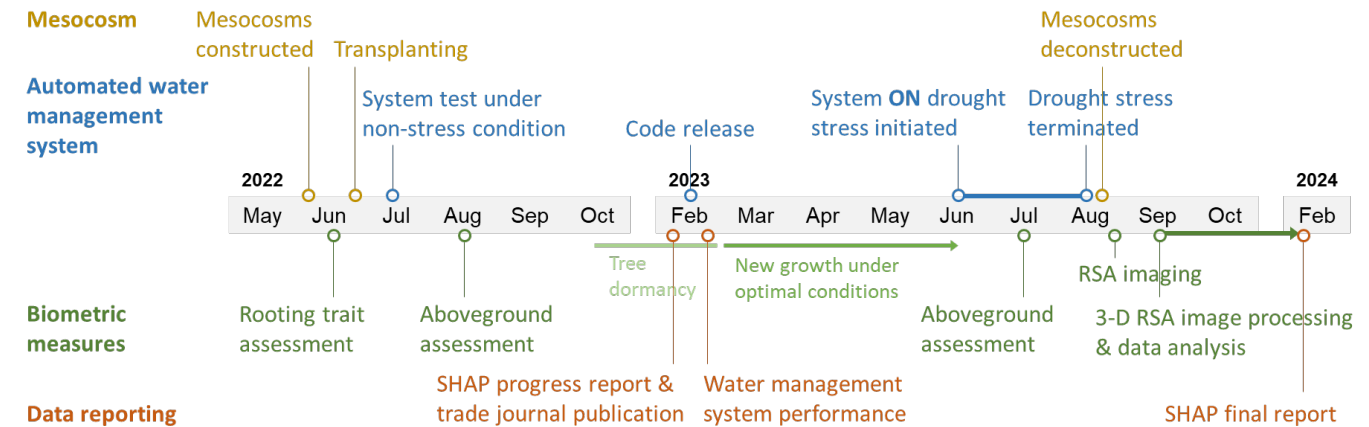
**Figure 1.** Illustration of mesocosm dimensions with root system architecture (RSA) sheets and integrated sensor planes.

**Drought Experiment and Biometric Measures.** Moderate drought treatment (determined by substrate matric potential curve) will be imposed in the second year to half of the apple trees for 2 months, while the other half are watered regularly. Aboveground portion of trees will be assessed for plant growth (tree height and trunk diameter) and the following traits related to plant

physiology. Photosynthetic parameters, including chlorophyll fluorescence, stomatal conductance, transpiration, carbon assimilation, and intrinsic water-use efficiency, will be measured with a chlorophyll fluorometer and portable infrared gas analyzer while leaf water potential will be quantified using a Scholander pressure chamber. The parameters will be measured bi-weekly as foliage density permits. Soil water potential &  $\theta_v$  will be continuously quantified through Watermark200ss sensors in designated planes. Rooting parameters considered include root number, root length, mean root diameter, root length production, and root growth velocity. Rooting parameters will be measured where applicable at transplant and after deconstruction of mesocosms. Upon deconstruction of mesocosms, RSA will be imaged using a digital single-lens reflex camera in a framed studio. Thereafter, 3-D RSA will be determined using Pix4-D<sup>®</sup> software to process the images. A timeline summary is presented below (**Figure 2**).

**Projected Outcomes.** **1.** Development and release of an open-source tool for automation of irrigation scheduling to improve farm efficiency. **2.** A new methodology for quantification of plant edaphic hydrologic tolerance and 3-dimensional root response will improve variety selection for specific regions.

**Figure 2.** Project timeline indicating construction of mesocosms, stage of water management system and periods of data collection and reporting.



**Project Budget (2 mesocosms):**

	Salaries	Hourly Wages	Fringe Benefits	Supplies	Travel	Misc.	Total
Cost							
Year 1	0	6426	2699	6155	500	0	15780
Year 2	0	6953	2920	1275	500	0	11648
Total	0	13379	5619	7430	1000	0	27428

1 student (2022 & 2023 G.S. 1 step-1&2, ~\$13.17 h<sup>-1</sup> x 40 h wk<sup>-1</sup> x 12 wks, fringe benefit at 42% of salary)

Mesocosms (\$1000), Sensor Node equipment (\$4550), Trees (\$420), Lattice (\$185), pix-4D license (\$425 month<sup>-1</sup> x 3 months), Travel twice to Mid-Atlantic Fruit and Vegetable Convention (\$1000)

**Other Support:** Some required materials have been acquired under other funding sources; these are not reflected in the proposed budget. Other sources of support are not anticipated.

## Literature Cited

- Atkinson, C. J., Policaprio, M., Webster, A. D., & Kingswell, G. (2000). Drought tolerance of clonal Malus determined from measurements of stomatal conductance and leaf water potential. *Tree Physiology*, 20, 557-563.
- Bassett, C. L. (2013). Water Use and Drought Response in Cultivated and Wild Apples. In *Abiotic Stress - Plant Responses and Applications in Agriculture*.
- Dowd, T., McInturf, S., Li, M., & Topp, C. N. (2021). Rated-M for mesocosm: allowing the multimodal analysis of mature root systems in 3D. *Emerg Top Life Sci*, 5(2), 249-260. doi:10.1042/ETLS20200278
- Glenn, D. M. (1999). Analysis of Trickle and Pulse Microsprinkler Irrigation of Processing Apples. *Journal of Tree Fruit Production*, 2(2), 11-17. doi:10.1300/J072v02n02\_02
- Granier, C., Aguirrezabal, L., Chenu, K., Cookson, S. J., Dauzat, M., Hamard, P., . . . Tardieu, F. (2006). PHENOPSIS, an automated platform for reproducible phenotyping of plant responses to soil water deficit in Arabidopsis thaliana permitted the identification of an accession with low sensitivity to soil water deficit. *New Phytol*, 169(3), 623-635. doi:10.1111/j.1469-8137.2005.01609.x
- He, L., & Weber, D. (2020). Updates on soil moisture-based irrigation for orchards. *Fruit Growers News*.
- Holshouser, D. L., & Whittaker, J. P. (2002). Plant population and row-spacing effects on early soybean production systems in the Mid-Atlantic USA. *Agronomy Journal*. doi:<https://doi.org/10.2134/agronj2002.6030>
- IPCC. (2021). *Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. Retrieved from
- Karl, T. R., & Knight, R. W. (1998). Secular trends of precipitation amount, frequency, and intensity in the United States. *Bulletin of the American Meteorological Society*, 79(231-242), 1. doi:10.1175/1520-0477(1998)079<0231:STOPAF>2.0.CO;2
- Opoku, J., Kleczewski, N. M., Hamby, K. A., Herbert, D. A., Malone, S., & Mehl, H. L. (2019). Relationship Between Invasive Brown Marmorated Stink Bug (Halyomorpha halys) and Fumonisin Contamination of Field Corn in the Mid-Atlantic U.S. *Plant Dis*, 103(6), 1189-1195. doi:10.1094/PDIS-06-18-1115-RE
- Osmolovskaya, N., Shumilina, J., Kim, A., Didio, A., Grishina, T., Bilova, T., . . . Wessjohann, L. A. (2018). Methodology of Drought Stress Research: Experimental Setup and Physiological Characterization. *Int J Mol Sci*, 19(12). doi:10.3390/ijms19124089
- Polsky, C., Allard, J., Currit, N., Crane, R., & Yarnal, B. (2000). The Mid-Atlantic region and its climate: past, present, and future. *Climate Research*, 14, 161-173.
- Psarras, G., & Merwin, I. A. (2000). Water stress affects rhizosphere respiration rates and root morphology of young "matsu" apple trees on m.9 and mm.111 rootstocks. *Journal of the American Society for Horticultural Science*, 125(5). doi:<https://doi.org/10.21273/JASHS.125.5.588>
- Smith, R. K., & Chang, D.-C. (2020). The utilization of a recursive algorithm to determine trends of soil moisture deficits in the Mid-Atlantic United States. *Climatic Change*, 163(1), 217-235. doi:10.1007/s10584-020-02898-w
- Twooski, T., & Fazio, G. (2015). Effects of Size-Controlling Apple Rootstocks on Growth, Abscisic Acid, and Hydraulic Conductivity of Scion of Different Vigor. *International Journal of Fruit Science*, 15(4), 369-381. doi:10.1080/15538362.2015.1009973