FRUIT TREE RESPONSES TO WATER STRESS: AUTOMATED PHYSIOLOGICAL MEASUREMENTS AND ROOTSTOCK RESPONSES

by

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ABSTRACT

Fruit tree responses to water stress:
Automated physiological measurements and rootstock responses.

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Fruit orchards are increasingly established in high density plantings utilizing dwarfing rootstocks to maximize yields. While widely planted, there is limited analysis of physiological responses of dwarfing rootstocks to water stress. In the Intermountain West growers would benefit from rootstocks that are adapted to calcareous soils, extreme temperatures and low rainfall. Growers also face increasing pressure on water resources from a growing population and climate change. To implement precision irrigation, reliable and easily automated methods are needed to determine orchard water stress. In a series of studies, I evaluated the use of dendrometers and sap flow sensors for detecting water stress. I also examined drought tolerance of four rootstock cultivars using a weighing lysimeter system.

Measurements of a sap flow index (SFI), maximum daily trunk shrinkage (MDS), and mid-day stem water potential (Ψ_{stem}) showed phenological changes in response to environmental drivers in apple. Correlations of MDS and SFI to Ψ_{stem} were also improved by controlling for phenological stage. Correlations were strongest early in the season and declined as the season progressed, which may be due to leaf age and fruit set. Overall MDS was judged to have the greatest potential for irrigation scheduling due to its high correlation to Ψ_{stem}.

In a separate study, four rootstocks were evaluated for their resilience to water stress using a weighing lysimeter system. In the first study, trees sourced from a peach orchard maintained by Navajo farmers in South Eastern Utah were evaluated against the commercial rootstock Lovell. Navajo trees had greater leaf area and dry weight after a series of drought events indicating greater overall drought tolerance. In the second study two commercially available dwarfing cherry rootstocks, Krymsk® 5 and 6, were compared to determine drought resilience. Krymsk® 6 showed greater leaf level transpiration rates and depleted available water faster. This is likely due to a higher percentage of roots as a function of total biomass. While Krymsk® 6 showed greater vigor throughout the trial, it had a less conservative water use strategy suggesting a tradeoff between growth and water conservation during drought.

(159 pages)

PUBLIC ABSTRACT

Fruit tree responses to water stress: automated physiological measurements and rootstock responses.

William Wheeler

New orchard plantings utilize trees grafted to dwarfing rootstocks planted close together to facilitate larger harvests. These dwarfing rootstocks have not been comprehensively studied for their ability to withstand drought. This is of special importance in the Intermountain West which has limited rainfall. Additionally, orchard growers face competition for water from a growing population and increased uncertainty in rainfall from climate change. My research examined the use of dendrometers, which measure changes in trunk diameter, and sap flow sensors, which measure how quickly sap moves, as methods to inform growers about tree water status. I also used a weighing lysimeter system to measure tree water use in four different rootstocks as they were subjected to drought.

In a field study, I placed dendrometers and sap flow sensors in a high-density apple orchard. As the trees progressed through the season and put on fruit the responses from the sensors changed. By separating data from the sap flow sensors and dendrometers into seasons, their ability to predict tree water status improved. I found that overall dendrometers would be the best way to automate measurements of tree water status.

In my first rootstock trial I examined responses of peach trees grown from seeds from an orchard maintained by Navajo farmers in South Western Utah, and a commercially available rootstock. I found that the Navajo peach trees put on more growth than the commercial trees despite drought, which suggests that they may be useful for rootstock development. In my second rootstock trial I examine the commercially available Krymsk® 5 and 6 series dwarfing cherry rootstocks for responses to drought. I found that the Krymsk® 6 rootstocks had higher rates of transpiration and used water faster than the Krymsk® 5 allowing them to put on growth quickly even during drought. Krymsk® 5 rootstocks have a lower percentage of their biomass in their roots which may have helped to conserve water.

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

Global population is expected to peak around 11 billion by 2100 (United Nations et al., 2019). The pressure to feed this population with a finite amount of arable land and limited freshwater resources present significant challenges for the future. These challenges will be compounded by increasing uncertainty due to climate change in precipitation patterns and frequency of extreme weather (Elliott et al. 2014). Agricultural producers also face social pressure from an increasingly informed consumer base to manage production in ways that minimize environmental impacts and maximize food quality and safety. The need for more sophisticated tools to manage crop production and conserve resources is greater than ever.

Agricultural irrigation currently accounts for approximately 70% of the global freshwater withdrawals with competition for these freshwater resources expected to increase in the future (Foley et al., 2011). Irrigation management has direct economic impacts for fruit tree producers with irrigation scheduling affecting fruit quality and yield as well as costs associated with pumping and water usage. Most new orchard plantings in the US are occurring in the more arid Western half of the country necessitating greater irrigation management (Schaible and Aillery, 2017). New orchard plantings are also increasingly moving to higher densities with dwarfing rootstocks to maximize production per hectare (Morandi et al. 2018; Robinson, 2006). Dwarfing rootstocks increase precocity and reduce costs associated with pruning and harvesting while shortening return on investment times compared to full sized trees (Cline, 2019; Forner-Giner et al.

2014). In the field, vigorous rootstocks may be less susceptible to drought because large rootzone volumes allow greater access to soil moisture (Tworkoski and Fazio 2015). However, studies indicate that dwarfing rootstocks exhibit greater stomatal regulation and ABA production which, when controlling for root zone volume, making them more resilient to drought (Atkinson et al. 2000). This is of interest not only due to increasing demand on water resources from a growing population and climate change but also for the implementation of deficit irrigation to improve orchard management and fruit quality (Naor, 2006). Deficit irrigation has been shown to improve crop quality while generating significant water savings (Fereres and Evans, 2006). However, to maximize efficiency while not impacting yield and fruit size, deficit irrigation needs to be precisely timed and the amount of stress imposed carefully regulated. Current irrigation management methods available to growers are sufficient to maintain orchard crops under well-watered conditions but are not precise enough to allow for implementation of regulated deficit irrigation.

The use of heat as a tracer to measure sap flow and velocity have been extensively studied over the past seventy years. Several methodologies exist which utilize heated needles inserted into the trunk of the tree to introduce either a continuous or timed pulse of heat. By accounting for the thermal properties of the tree and measuring the rate of heat dissipation, sap velocity can be determined. Studies in both ecological and agricultural settings have used these techniques to estimate single tree and stand level transpiration (Edwards and Warwick, 1984, Granier et al., 1990). There has been limited testing of heated needles sensors for scheduling irrigation, but the literature that is available has been promising with several authors reporting to have used sap flow sensors to maintain orchard corps under well-watered conditions. (Fernández et al., 2008; Nadezhdina and Cermak, 1997). It remains to be determined however if the use of heat as a tracer for sap flow would be a sensitive enough indictor for the implementation of deficit irrigation. Additionally, sap flow sensors require extensive technical expertise to accurately calibrate the system in order to obtain absolute values of sap flow (Taylor et al. 2013). Absolute measurements are necessary to determine exact transpirative water losses but relative rates of sap flow have been shown to be well correlated to tree transpiration rates (Burgess et al. 2001). Because transpiration rates and sap flow decline under both water stress and low evaporative demand from the environment, relative rates of sap flow may provide a good indication of tree transpiration rates once they are corrected to environmental demand (Burgess et al. 2001).

Diurnal trunk diameter variation has also been proposed as an easily automatable measure of plant water status for irrigation scheduling (Goldhamer and Fereres, 2001). Stem diameter typically peaks near sunrise as trees recharge water used during the day and reaches a minimum a few hours after solar noon when evaporative demand is the greatest (Ginestar and Castel, 1995). The difference between the maximum and minimum trunk diameters in a 24-hour period, or the maximum daily shrinkage (MDS), has been shown to be well correlated to stem water potential (Fernández and Cuevas, 2010). To date the use of dendrometers for irrigation scheduling has been limited in part due to the high degree of variability between measurements (Ortuño et al. 2010). This variability may be related to the use of point dendrometers which, due to the localized nature of the measurement is sensitive to positioning errors. Band dendrometers capable of measuring changes in trunk circumference sample a larger area of the trunk and thus reduce position errors (Corell et al. 2014). These types of sensors have been used extensively in forestry settings but have seen limited use in horticulture settings.

Accurate indicators of tree water stress could improve orchard irrigation application and water use efficiencies. Plant based measures of tree water status, such as sap flow and trunk diameter variations, are widely regarded as the best candidates for orchard irrigation scheduling. Further characterization of these type of easily automatable plant based measures of water status is needed to help bring them to commercialization. Determination of drought tolerant rootstocks could also help orchard water use efficiency and buffer against water stress. Comprehensive screening for drought tolerance of widely used dwarfing rootstocks and continued evaluation of new rootstock candidates can help orchard managers make informed decisions when putting in new plantings. Here we evaluate the use of a sap flow index and band dendrometer as measures of plant water stress. We also evaluate four different rootstocks for drought tolerance using a weighing lysimeter system.

1.2 Literature review

Irrigation scheduling is the most common and one of the most difficult tasks to accurately assess for fruit tree growers. Precaution is the guiding principle when faced with uncertainty about tree hydration status or the need to apply irrigation. Underwatering often results in acute drought stress characterized by loss of leaf turgor and wilting, which is easily visible to the grower. Prolonged drought affects fruit size, overall yields, and flower development ultimately impacting crop value (Lopez et al., 2012). The effects of overwatering are subtler: increased potential for disease and pests, promotion of excessive vegetative growth, and contribute to runoff of agricultural chemicals and fertilizers (Goldhamer et al., 2006). These long-term effects are harder to discern and conceptualize when contrasted with symptoms of acute drought. This leads to the tendency by irrigation managers to chronically over apply irrigation water. In addition, irrigation scheduling is often based on the informed intuition of the grower, calander applications (set time intervals) or energy balance modeling used to estimate potential evapotranspiration. While these methods can be effective for maintaining soils at well-watered conditions, they do not allow for implementation more sensitive practices such as deficit irrigation.

1.2.1 Regulated deficit irrigation

Regulated deficit irrigation (RDI) was first outlined in Chalmers et al. (1981) as a means to control vegetative growth in peach orchards. It has been shown in a number of crops to be an effective method of not only limiting vegetative growth and associated pruning costs, but also improving fruit quality while minimally impacting yield (Fereres and Soriano, 2006). Successful implementation requires precise irrigation timing and real-time knowledge of the amount of stress experienced by the crop as drought stress quickly impacts yields. Water savings up to 75% have been reported with minimal yield reduction when deficit irrigation is correctly implemented (Einhorn and Caspari, 2003). Phases of fruit development is often used in scheduling RDI, with stone and pome fruits following different development patterns. Pome fruit development follows a sigmoidal growth pattern and is generally broken into two stages when considering RDI application: stage I – Flowering and fruit set and stage II – fruit cellular expansion. When RDI has been applied to citrus during stage I, crop load was affected while during stage II fruit size is generally reported to be reduced (Domingo et al., 1996). However, RDI during fruit development is not commonly practiced in pome fruits as fruit development proceeds rapidly after fruit set and any reductions in irrigation reduce yields (Naor et al., 2008). In stone fruit development follows a double sigmoidal pattern which has been characterized in three stages: Stage I, fruit set and fruit cellular division; Stage II, seed development, referred to as pit hardening or the lag stage; and stage III rapid cellular expansion and fruit maturation (Lopez et al., 2012). Stages vary in length depending on cultivar and are more evident in late-maturing cultivars than early-maturing cultivars (Intrigliolo and Castel, 2005). It is hypothesized that RDI implemented during stage II of stone fruit development will limit vegetative growth and minimally impact fruit size

(Johnson and Handley, 2000). In addition to application of RDI during fruit development, researchers have also documented significant water savings and no impacts on production during the post-harvest period (Johnson et al. 1996). Regulated deficit irrigation during post-harvest has the potential to generate large water savings due to the amount of time spent in post-harvest and has minimal impact on fruit development, though there is question to the impact on flower set the following year (Girona et al., 2005). To fully implement RDI, growers need a reliable indicator of tree water status.

1.2.2 Irrigation scheduling methodology

Techniques for precision irrigation scheduling can broadly be broken into three categories: Evapotranspirative modeling, soil moisture measurements, and plant-based measurements (Jones, 2004). These techniques provide a more rigorous approach to quantifying crop water availability and subsequent irrigation scheduling than traditional approaches such as time interval (calendar), grower intuition, or trial and error experience.

Evapotranspiration modeling:

Evapotranspirative modeling is based on the principle of energy conservation and works through accurate accounting of energy fluxes in a system. Because water requires a large amount of energy (2.45 MJ $°C^{-1}$) to undergo a phase change from liquid to gas, transpiration rates are largely proportional to energy input (Allen et al., 1998). On a macro-scale we can consider the primary energy input into agronomic systems as electromagnetic radiation from the sun (Monteith, 1973). When modeling agronomic systems we can use the simplifying assumption that this incoming solar energy is equal to the latent and sensible heat energy transfers plus storage of energy within the system:

$$R_n = \lambda ET + H + G$$

Where R_n is net radiation, λET is latent heat, H is sensible heat, and G is thermal storage. These terms account for the major fluxes of energy with additional exchanges of energy, such as those used in metabolic activities, ignored due to the small fraction of total energy transfer (Allen et al., 1998). Rearranging this equation to solve for latent heat transfer allows for the estimation of potential water loss from the system:

$$\lambda ET = R_n - H - G$$

Calculations of evapotranspirative losses of water from the system can then be determined from accurate measurements or models of the remaining parameters. Numerous models exist to compute potential evapotranspiration (ET) (e.g. Priestly and Taylor 1972, Hargraves and Samani 1985, Shuttleworth and Wallace 1985) with the most widely used being the FAO - 56 Penman-Monteith equation. The Penman-Montieth is a one-dimensional aerodynamic model that requires measurements of radiation, air temperature, air humidity and wind speed to derive the potential ET for a hypothetical grass reference crop approximately 0.12 m in height (ET_o). This reference ET_o can then provide the basis for estimation of crop water needs by applying an empirically developed crop coefficient to scale to individual crop water requirements (Naor, A., 2006). Crop coefficients must be rigorously developed and can vary based on climate, soil type, planting density, canopy management in perennial crops, crop load and plant growth stage and are not available for every application (Allen et al., 2005; Juhász et al. 2013). Modifying crop coefficients for orchard crops can be problematic due to the tall, dispersed nature of tree crops, which leads to a high coupling of tree transpiration to the bulk properties and movement of air (Annandale and Stockle, 1994). Reference Penman – Montieth calculations assume values for a homogenous grass-like crop which has a large boundary layer due to canopy effects. This in turn serves to decouple the modeled crop canopy from bulk atmospheric conditions causing deviations in modeled ET_o vs actual orchard ET even when using crop coefficients (Jarvis and McNaughton, 1986). More accurate ET measurements can be attained using parameters specific to orchard crops and attempts to account for the influence of bulk air properties and reduced boundary layer effects have been published (most notably by Jarvis and McNaughton (1986) who provide a comprehensive discussion of stomatal versus environmental factors in determining transpiration). However, implementation of these models require technical expertise that is often not available to commercial growers. Because of this commercial orchard operations that do use ET modeling based approaches still rely on the use of calculated potential ET_o and crop coefficients to estimate water loss and irrigation application.

Soil water measurements:

Soil water is widely quantified in two ways: as the potential energy of water relative to an idealized state, and as the percentage of water in the root zone on a volumetric basis. There are six measurement methods that are commonly used in research and irrigation scheduling: measurements using tensiometers, resistance blocks, neutron probes, dielectric sensors, heat dissipation sensors, and gravimetric determination of water content.

Gravimetric determination

Gravimetric determination of soil moisture is accomplished by weighing a moist soil core at the time of coring and again after oven drying. Using the dry bulk soil density and the change in mass after drying, the volumetric soil moisture content can be calculated (Schmugge et al., 1980). This is an inexpensive and relatively straight forward way of determining soil moisture provided the user has access to reliable soil property information, a drying oven, and accurate scales. This technique is relatively time intensive as soil cores must often be dried for 24 to 48 hours before and accurate reading can be taken. This can limit the utility of this method when rapid assessment of plant water availability is needed. In addition, taking a large number of soil cores over the course of a season can also be overly intrusive and destructive.

Tensiometers

Tensiometers consist of a water filled tube with a porous ceramic cup at one end which is buried in the soil. As the soil dries the adhesive and cohesive forces of water increase the tension in the soil water which subsequently translates to water within the tensiometer through the porous ceramic cup. This suction is measured either with a vacuum gauge at the top of the sensor or an electronic pressure transducer. Tensiometer measurements are limited by the need to maintain the hydraulic continuity between the ceramic cup and the soil. This limits utility to wetter conditions within the atmospheric pressure range. However, in highly irrigated agricultural settings this may not be of concern. Tensiometers are also not well suited to largely clay soils that do not allow drainage or coarse sandy soils that drain quickly (Migliaccio et al., 2012). Other limitations include the need for frequent maintenance to refill the water within the tensiometer, and the relatively slow response time of the instrument (Muñoz-Carpena et al., 2004).

Resistance blocks

Resistance blocks measure the electrical resistance between two electrodes imbedded in a porous material such as ceramic or gypsum. Because water is a better conductor of electricity than air, the wetter the resistance block and surrounding soil the lower the resistance read by the sensor. Resistance measurements can then be calibrated to the water potential of the soil. Resistance blocks work under a larger range of soil matric potentials than tensiometers, however they are not as sensitive and have a slow response time (Werner, 2002). The sensors are relatively maintenance free and inexpensive however they are temperature sensitive and salinity can increase conductance impacting accuracy. In addition, sensors made of gypsum have been reported to degrade over one to three years (Goodwin, 2009).

Neutron probes

Neutron probes consist of a fast neutron emitter from a decaying radioactive source that is lowered through guide tubes in the soil. The emitted neutrons are slowed by hydrogen, predominantly from water, and the probe is configured to detect these slow neutron particles. The density of this slow neutron cloud in the soil is proportional to the water content. Neutron probes can be extremely accurate with proper calibration and can sense soil moisture for a large area. However, calibration must be done on a site by site basis increasing the technical difficulty of this methodology. Additional challenges to using neutron probes include the operator expertise, licensing, and security necessary because of the use of a radioactive source (Muñoz-Carpena et al., 2004).

Electromagnetic sensors

Common electromagnetic techniques to determine soil water content include time domain reflectometry, capacitance, impedance and frequency domain sensing. Sensors utilizing these techniques take advantage of the high dielectric of water compared to air and mineral soil to measure soil water content. As electrical waves emitted from buried sensors propagate through the soil, the composition of the soil changes the frequency, amplitude, impedance, and time of travel (Jones et al., 2005). Soil water content can then be determined through soil specific calibration. These types of sensors can be left in place for long periods of time and can measure a wide range of soil moisture contents in all soil types. Electromagnetic sensors can be susceptible to air gaps around the metal prongs of the sensor which will cause inaccuracies in the sensor output. Salinity can also impact sensor accuracy as salts modify permittivity (Jones et al., 2005) These types of sensors also need to be calibrated for the specific soil type they are being used in, and only sense a relatively small area of soil (Robinson et al., 1999).

Heat dissipation

The use of heat dissipation to measure soil moisture content is based on the high heat capacity of water and its ability to alter heat transfer in a porous material such as soil (Dias et al. 2013). Soil moisture can be determined by releasing a controlled burst of heat from a heating element and monitoring the change in temperature over time. Because properties such a density, porosity, and organic content affect the thermal properties of the soil or soilless media, proper calibration of the sensors is required to ensure accurate determination of soil moisture (Kluitenberg et al. 1995).

Soil moisture measurements face two challenges for irrigation scheduling, first they are an indirect measure of plant water status. Soil moisture readings are not by themselves indicative of water stress with different crops able to tolerate different soil moisture extremes. The second is that all methods are localized measurements which can be problematic particularly in field settings due to the heterogeneity of soil composition and water distribution. Localization of readings is also an issue in the use of soil moisture sensors for orchard irrigation scheduling as trees have extensive root systems and it is difficult to assess the degree of hydration in the entire root zone. Additional sensors dispersed throughout the root zone can help provide a more robust picture of the soil moisture content, but this quickly increases the cost.

Plant-based measurements:

Water potential

Transpiration requires a continuous stream of water to drive photosynthesis and cool the plant. Because atmospheric demand can outpace the uptake of water by the roots, plants rely on storage in their tissues. This draw-down of stored water creates increased tensive forces on the conductive vasculature of the plant and subsequently the individual leaves. Measurements of stem and leaf water potentials are commonly used as indicators of plant water stress. Leaf water potential can be assessed using either a variable pressure chamber or chilled mirror system. Using variable pressure chamber, leaves are removed from the canopy and placed in the chamber. Pressure is slowly increased in the chamber until sap can be observed leaving the petiole of the leaf. In a chilled mirror system, a disk is cut out of the leaf which is to be sampled and quickly placed into a sample cup and placed inside the chilled mirror system. The temperature in the chamber is reduced until dew forms on the chilled mirror, at which point the saturation vapor pressure is reached. Water potential can then be determined by relating the saturation vapor pressure of the air in equilibrium with the sample using the Kelvin equation (Bittelli, 2010). Assessment of stem water potential is done indirectly by determining water potential of leaves attached to the main stem. Individual leaves that are attached to the main stem are enclosed in reflective Mylar to minimize transpiration, which allows them to come to equilibrium with the stem water potential. Stem leaf water potential can then be determined as described above with the leaf at equilibrium with the stem. The pressure needed to cause this backward flow of sap from the leaf at equilibrium is equal to the stem water potential (Ritchie and Hinckley, 1975). Pressure chambers have been used to determine leaf water in wine grape production but is labor intensive and previously has not easily been automated. More recently several companies have come out with automated stem water potential measurements. These sensors consist of a sensor body with a liquid reservoir that is invasively positioned in direct contact with the plants vasculature. A pressure sensor is used to detect changes in pressure in the liquid reservoir which is then related to the stem water potential (Stroock et al., 2018)

Trunk diameter

Trunk diameter variation is composed of four components: irreversible radial growth, cellular dehydration/rehydration, thermal expansion/contraction of the woody matrix, and the expansion/contraction of dead conducting elements due to transpiration driven tension (Dauedet et al., 2005). Thermal contraction of the woody matrix can usually be ignored and effects due to growth and transpiration account for most diameter variation (Ortuno et al. 2010). Water storage in the stem, branches, roots and leaves accounts for between 10 to 50% of tree daily transpirational water demand (Köcher et al 2013). This storage allows for rapid response to changing transpiration demands without relying on water uptake from the roots. During the day, when environmental demand is the greatest, water is withdrawn from the phloem and related tissues and living tissues of the outer xylem causing branch and stem diameter to decline (Čermárk et al., 2007). At night water uptake from the roots allows for rehydration of tissues and expansion of trunk diameter. Stem diameter typically peaks near sunrise and reaches its nadir a few hours after solar noon when evaporative demand is the greatest, the difference between these extremes is referred to as the Maximum Daily Shrinkage (MDS) (Ginestar and Castel, 1995). Sensitivity of dendrometers as a water stress indicator compared to other measurements in the literature has been mixed, with results varying with tree species and age (Fernandez and Cuevas (2010). In addition, previous research has found stem diameter variation measurements are influenced by phenological stage, fruit load, tree size, and crop management techniques (Fernandez and Cuevas, 2010). Under well-watered conditions MDS is considered a good indication of daily transpiration (Ortuno et al. 2010). As trees become water limited MDS values increase and are well correlated to stem water potentials until a species-specific threshold below which any further reduction in stem water potential is associated with an increase in MDS values (Ortuno et al. 2010). This is attributed to depletion of stored water in the phloem and cambium and the increase in transversal resistance of water movement from these tissues to the xylem (Intrigliolo et al., 2010). Reductions in MDS values during periods of limited water availability therefore may be an indication of severe drought stress in the tree. In peaches trunk diameter variations have been reported to be a more sensitive indicator of water stress than stem and leaf water potentials (Goldhamer et al. 1999) and sap flow measurements (Remorini and Massai 2003). However, in plum (Intrigliolo and Castel 2006), almond (Nortes 2008), and apple (Doltra et al. 2007) stem water potential was reported to be more sensitive to water stress than maximum daily trunk shrinkage. In young rapidly growing trees however total daily growth (TDG), calculated as the difference between maximum trunk diameters from one day to the next, appears to be better correlated to tree hydration status (Moriana and Fereres, 2002; Nortes et al., 2005).

Both MDS and TDG have been the focus of previous efforts for the use of stem diameter measurements in irrigation scheduling. The use of stem diameter measurements in a ratio of deficit irrigated trees to well-watered controls has been used to successfully control irrigation in peach (Li et al. 1989), almond (Goldhamer and Fereres, 2001; Goldhamer and Fereres, 2004), lemon (Garcia-Orellana et al., 2007; Ortuno et al., 2010), and clementine (Velez et al. 2007). Most irrigation scheduling researchers have used linear variable differential transformers (LVDT) which need to be corrected for temperature effects. LVDT type sensors must be in direct contact with the living tissues to be accurate, thus necessitate the removal of a portion of the bark. Sensors need to be well shielded to minimize interference from rain, wind and excessive thermal loading from the sun (Fernández 2017). Band dendrometers have greater use in forestry applications and incorporate changes in the circumference of the stem (Corell et al. 2014). Band dendrometers have historically been less accurate compared to point dendrometers however continued developments have improved technology (Drew and Downs 2009). Band dendrometers also require less maintenance and less correction for thermal effects (Corell et al. 2014).

Infrared canopy measurements

Due to the large latent heat of vaporization of water, transpiration is the major determinant of canopy temperature. As plants become water-limited transpiration declines reducing evaporative cooling of the crop and causing the canopy to heat up. This principle has been the focus of numerous researchers looking at canopy temperature measurements since the 1970s. Recent advancements in infrared imaging have made possible the widespread application of the technology to crop canopy temperature monitoring for irrigation scheduling (Maes and Steppe, 2012). Spatial and temporal variation in canopy temperatures under similar soil moisture conditions can be observed due to differences in meteorological conditions and crop morphology (Gomez-Bellot et al. 2015). Canopy temperatures must be normalized to account for these variations and have utility as an irrigation scheduling tool. Idso et al. (1981) and Jackson et al. (1981)

are attributed with developing three methodologies for computing a Crop Water Stress Index (CWSI) that normalizes for environmental and crop characteristics. These indexes utilized canopy temperature difference from air temperature under current conditions and compare those values to canopy temperature differences to air temperature measured under non-water stressed conditions. The indexes also attempt to correct for background temperature by evaluating temperature differences from air from a non-transpiring reference crop. This approach has successfully been used in arid and semi-arid environments however it is challenging to implement in humid or cloudy climates (Jones, 2004).

Sap flow sensors

The concept of using heat as a way to detect sap flow in trees was first proposed by Huber in 1932. Empirical methods were used to relate heat transport to sap flow until Marshall (1958) conducted the first quantitative analysis based on first principals. This theoretical framework assumed an infinitesimally small line source heater inserted perpendicularly to the tree trunk that releases an instantaneous pulse of heat. The movement of this heat can then be accounted for using mathematical models for idealized solids. Convection in a three dimensional isotropic solid can be accounted for using the following partial differential equation:

$$\frac{dT}{dt} = k\left(\frac{d^2T}{dx^2} + \frac{d^2T}{dy^2} + \frac{d^2T}{dz^2}\right)$$

Where T is the temperature (K); t is time (s); k is the diffusivity (m^2 s^{-1}); and x, y and z are spatial coordinates. Because diffusivity can be defined as: k = λ/ρc where ρ and c represent the density (kg m^{-3}) and specific heat capacity (J s^{-1} m^{-1} K^{-1}) respectively and λ the thermal conductivity (W m^{-1} K^{-1}). The formula can then be rearranged to account for the greater thermal conductivity of wood along the grain (vertically) than across the grain (azimuthally) (Green et al., 2003):

$$\rho_w c_w \frac{dT}{dt} = \frac{d}{dx}\lambda_x \frac{dT}{dx} + \frac{d}{dy}\lambda_y \frac{dT}{dy}$$

Where ρ_w and c_w represent the density (kg m^{-3}) and specific heat capacity (J s^{-1} m^{-1} K^{-1}) of fresh wood respectively and λ the thermal conductivity (W m^{-1} K^{-1}) in the x and y plane. Marshall then accounted for heat convection by sap flow on a 2-dimensional plane perpendicular to the x-axis using the following formula:

$$au\rho_s c_s \frac{d^2T}{dx^2}$$

Where a is the area through which the sap is flowing; u the velocity of the sap (m s^{-1}); ρ_s represents the density (kg m^{-3}) of sap and c_s the specific heat capacity of sap (J s^{-1} m^{-1} K^{-1}). Combing both of these formulas to account for the conduction and convection of heat through the wood matrix and accounting for the amount of heat released by the heating element gives us the following formula:

$$\rho_w c_w \frac{dT}{dt} = \frac{d}{dx}\lambda_x \frac{dT}{dx} + \frac{d}{dy}\lambda_y \frac{dT}{dy} - au\rho_s c_s \frac{dT}{dx} + Q$$

Where Q is the internal heat released from the heating element (W m^{-3}). With this theoretical understanding of heat movement, we can then turn to the mathematical solution for temperature rise from a line heater injected into a theoretically infinite non-moving medium as given by Carslaw and Jaeger (1959):

$$\Delta T = \frac{Q}{4\pi\kappa t} e^{-\frac{x^2+y^2}{4\kappa t}}$$

Where ΔT is the temperature rise (°C); Q is the internal heat released from the heater (W m^{-3}), x is the vertical distance from the heater (m) and y the azimuthal distance. Finally, we can modify this formula to account for convective movement of heat by sap:

$$\Delta T = \frac{Q}{4\pi\kappa t} e^{-\frac{\left(x-\frac{\rho_s c_s}{\rho_w c_w} aut\right)^2+y^2}{4\kappa t}}$$

This solution equation and Marshall's discussion and analysis form the basis of several methods for determining heat pulse velocity and sap velocity through the tree. In addition to the above methods which utilize short pulses of heat there are two methods based on the continual application of heat: the heat field deformation (HFD) method (Nadezhdina et al., 1998) and the thermal dissipation probe (TDP) (Granier, 1985). In both of these methods sap flow is empirically linked to measured heat dissipation. In addition, a number of non-invasive methods are also used in herbaceous and small diameter woody stems but are not feasible for larger stems due to the necessary heater size and energy requirements. Fernández et al. (2017) comprehensively reviewed the methods to determine sap flow from heat velocity measurements.

Methods for determining heat pulse velocity

Currently there are three widely used heat pulse-based methods for the determination of sap flow: Compensation heat pulse (CHP), Heat Ratio Method (HRM) and maximum temperature rise (T-Max).

The CHP method (Marshall, 1958) uses two temperature probes asymmetrically spaced around a line heater with the upstream probe closer to the heater. Following a brief heat pulse, the time delay for both up and downstream temperature sensors to reach an equal temperature rise is used to calculate the heat pulse velocity (V_h):

$$V_h = \frac{(x_D + x_U)}{2t_Z}$$

Where x_U and x_D are the distances from the line heater to the upstream and downstream needle respectively, and t_Z is the time for temperatures of the upstream and downstream points to become equal (Green et al., 2003). This technique has the advantage of not needing to determine thermal diffusivity however it performs poorly under low flow or reverse flow conditions, such as those which occur in roots or at night (Green et al. 2009).

The HRM builds off Marshall's initial work and includes revisions proposed by Hogg et al. (1997) and Burgress et al. (2001). This method uses two temperature probes spaced equidistant from the line heater on the same plane. The determination of heat velocity is based on the ratio of the increase in temperatures from the upstream and downstream sensors following a heat pulse from the line heater as shown below:

$$V_h = \frac{2k}{x_u + x_d} \ln \frac{\Delta T_d}{\Delta T_u}$$

While the HRM has been observed to be accurate for low and reverse sap flux measurements, it performs poorly under high flow rates with maximum heat velocities detectable peaking at 45 cm/h (Burgess and Dawson, 2008). Another limitation of this method is accurate determination of diffusivity (k) cannot be made without destructively harvesting the tree (Vandegehuchte and Steppe, 2012).

Finally, the T-Max method proposed by Cohen (1981) uses a single temperature sensor downstream of a line heater. Following a short heat pulse the time in seconds for the temperature downstream to reach the maximum is used to calculate heat velocity:

$$V_h = \frac{\sqrt{x_d^2 - 4kt_M}}{t_M}$$

In which x_d is the distance downstream of the sensor from the line heater, t_M is the time for the maximum temperature rise to occur and k is the thermal diffusivity which can be estimated under zero flow conditions using the following equation:

$$k = \frac{x_d^2}{4t_M}$$

However, the accuracy of this method to determine diffusivity has been questioned due to the need for zero flow conditions for calibration that do not commonly occur in natural or agronomic settings (Forest, 2019; Vandegehuchte and Steppe, 2012). Additionally, the above equation for heat velocity is derived from the solution for an instantaneous line source of heat along an axis. Systems to determine sap flow typically use a heat pulse lasting between 1 and10 seconds (Marshall, 1958; Forster, 2019). Kluitenberg and Ham (2004) proposed an improved calculation to account for non-instantaneous release of heat:

$$V_h = \sqrt{\frac{4k}{t_0}\ln\left(1 - \frac{t_0}{t_m}\right) + \frac{x^2}{t_m(t_m - t_0)}}$$

Where t_0 is the heat pulse duration in seconds. The T-Max method is limited at low rates of flow with minimum heat velocities detectable at approximately 10 cm/h (Forester, 2019).

The Dual Method Approach (DMA) has been suggested by Forster (2020) to resolve limitations of measurement in low and high rates of flows by combining the HRM and T-Max methods. The use of this method necessitates using a three-needle probe with needles equidistant from each other. Computation of the HRM can be accomplished using the upstream and downstream measurements and the T-Max method using the downstream only measurements. The transition between the two methods is based on the Péclet equation which produces a dimensionless number that describes the ratio of conductive and convective heat transfer (Wang et al. 2002). To determine the Péclet number using a three needle sap flow sensor the following equation can be used:

$$\beta = \ln\left(\frac{\Delta T_{d,max}}{\Delta T_{u,max}}\right)$$

Where β is the Péclet number and $\Delta T_{d,max}$ and $\Delta T_{u,max}$ are the difference between the initial temperature of the woody matrix and the maximum temperature rise following a heat pulse downstream (d) and upstream (u). A Péclet number equal to or less than one indicates that the temperature rise upstream of the heater is equal to or greater than the downstream rise due to low rates of sap flow. Under low rates of flow conductive heat transfer through the woody matrix dominates and the HRM should be used. Values above one indicates the temperature rise is greater downstream due to conductive heat transfer from high rates of sap flow, thus the T-Max should be used. This approach provides a theoretical basis to transition between the two methods, eliminating the need for post-hoc statistical analysis to define thresholds that have hampered earlier attempts to resolve measurement of low and high rates of flow.

Wound correction

Drilling and subsequent insertion of probe needles interrupts sap flow and wounding size has been shown to decrease heat velocity readings by 50% to 90% (Green et al., 2003; Swanson and Whitfield, 1981). The extent of wounding has been found to be both a function of mechanical damage and species-specific anatomical structure. Barrett et al. (1995) reported that longitudinal shearing caused by torque from the drill bit produced damage up to 0.85 mm from the site of the wound. Damage to vessels located on the transversal plane to the wound was reported to extent up to 0.3 mm and thought to be caused predominately by crushing from the drill bit. Xylem vessel length has also been reported to affect wounding size with some species. Green and Clothier (1988) reported that damage to kiwi xylem vessels can extend up to 0.5 mm past the initial wound. Wounding size can be used to correct heat pulse measurements, either empirically or through physical principles, using an equation in the form shown below (Cohen et al., 1981; Green et al., 2003):

$$V_c = a_0 V_H + a_1 V_H^2 + a_2 V_H^3$$

Where V_c is the corrected heat pulse velocity; V_H is the raw heat pulse velocity and a_{0-2} are correction factors derived from numerical solutions to the combined equation for conduction and convection in the tree trunk (Swanson and Whitfield, 1981). The above equation accounts not only for wounding effects but also thermal heterogeneity caused by the insertion of the probes into the system.

Conversion of heat pulse velocity

In all of the heat pulse methods, heat velocity is initially derived and sap flow must be calculated by accounting for the density and specific heat of both the sap wood and sap as well as the moisture content of the sap wood (Barrett et al, 1995):

$$V_s = \frac{V_c \rho_w (C_w + mC_s)}{(\rho_s C_s)}$$

Where V_s is the sap velocity; V_c is the wound corrected heat velocity; ρ_w and ρ_s are the densities (kg m^{-3}) of sapwood (ρ_w = oven dry sapwood weight / fresh sapwood volume) and sap (assume that of water 997 kg m^{-3}) respectively; C_w is the specific heat capacity of sap wood (1200 J kg^{-1} °C^{-1} (Becker and Edwards 1999)) and C_s is the specific heat capacity of water (4182 J kg^{-1} °C^{-1} at 20 °C); and m is the moisture content of the sap wood (kg water kg^{-1} dry wood). Sap velocity is then used to compute sap flow by multiplying flow rate by the estimated conducting area (sapwood).

Anatomical and physiological considerations

Rates of flow through the conducting area is influenced by species wood anatomy which in angiosperms can broadly be divided in to ring porous and diffuse porous. Ring wood species, which include peach, cherry and apricot, have a bimodal distribution of vessel diameters. Annual growth rings are broadly broken into early season, large diameter vessels and late season, small diameter vessels (Bush et al., 2010). This leads to an asymmetrical Gaussian distribution of sap velocity with peak velocities typically reported to occur in the outer 20-30% of the sapwood, closest to the cambium tissue (Alvarado-Barrientos et al., 2013; Chang et al., 2014). Diffuse porous species, such as apple and pear, have a narrow range of normally distributed vessel diameters which are symmetrically distributed in the annual growth rings as the season progresses. This in turn leads to a symmetrical distribution of sap velocities across the sapwood with peak velocities occurring roughly in the center of the conducting tissue (Woodcock, 1989). This sap velocity profile, when normalized for sapwood depth, is relatively uniform within species and has been suggested to be linked with species specific anatomical and structural characteristics (Caylor and Dragoni, 2009). In a series of papers, Caylor and Dragoni (Caylor and Dragoni, 2009; Dragoni et al., 2009) argue that the sap velocity profile is largely stable over the course of a season under normal conditions and can be decoupled from stem conductance, the latter being correlated with photosynthetically active photon flux density (PPFD), net radiation (R_n), and vapor pressure deficit (VPD). Several studies have reported reductions in peak sap velocity under severe drought stress. Researchers also noted a shift of peak velocities toward the heartwood with severe drought stress (Cermak and Nadezhdina, 1998; Ford et al., 2004). Based on these findings Nadezhdina et al. (2007) suggested that analysis of the shape of the sap wood profile might be a realizable indicator for irrigation scheduling. However, studies in olive, apple, and Asian pear did not show significant variation in sap velocity profiles under a range of soil water availability and atmospheric demand (Fernández et al. 2008a). Changes in the velocity profile have also been reported after selective pruning by Fiora and Cescatti (2008), in which lower branches are more hydraulically connected to inner sapwood while upper branches more connected to outer sapwood. Daily and seasonal patterns have also been observed in radial patterns of sap wood causing peak sap velocity to shift in magnitude and depth relative to the heartwood (Dragoni et al., 2009). Cohen (1981) reported that differences in sap flow could vary up to 10% across the cross-sectional area of the sap wood and that differences were the greatest in the morning and afternoon hours due to sun angle. For these reasons, integration of point measurements of sap flow over the entire sapwood can lead to errors in estimation of transpiration (Clearwater et al., 1999). These problems have partly been overcome by utilizing multiple probe integration at various sapwood depths and points around the tree (Cohen et al., 2008; Dragoni et al., 2009; Wullschleger and King, 2000). However, accurate absolute measurements are difficult to obtain even by experienced researchers. Even with accurate calibration, sap flow rates can vary widely between trees though the general pattern of response to environmental conditions is consistent (Molina et al., 2016). Dragoni et al. (2005) found that four different measurements of sap flow in apple resulted in positive linear correlations to measured transpiration for all, however large variations in the slope of response were observed. Variation in orchard soil heterogeneity, microclimates and genetic variability in individual tree hydraulic conductivity have all be cited as reasons for this variability (Cueavas et al., 2013).

There has been significant discussion in the literature about iso/anisohydric water use strategies and how it effects the interpretation of sap flow readings. Previous reports commented that in plants exhibiting isohydric (water conserving) or near-isohydric behavior, sap flow rates may not always be well correlated to plant water status as trees down regulate transpiration during drought (Fernandez, 2014). Similarly, anisohydric plants maintain high rates of transpiration and thus sap flow under drought conditions making high rates of sap flow possible even during water stress. Recently however, the use of iso/anisohydry to describe species specific water relations strategies has been called into question. Specifically, the limited degree to which stomatal regulation has been observed to be correlated to stem water potential undercuts the mechanistic underpinnings of the classification system (Martinez-Vilalta and Garcia-Forner, 2017). Other researchers have highlighted the dominant effect environmental factors play in determining water potential when compared to genotype (Feng et al., 2019). Researchers advocate consideration of more biologically robust parameters to define plant water use strategies such as maximum tolerated stem water potentials, carbon assimilation rates, and hydraulic conductance (Hochberg et al., 2018). Regardless of how future researchers choose to describe plant water use strategies, the use of sap flow measurements must take into account these strategies and how they may alter sap flow readings in relation to plant water status.

Relative and absolute measures of sap velocity

Forster (2017) analyzed 104 peer-reviewed studies published in the literature where heat pulse velocity measurements were compared to independent measures of plant water use (weighing lysimeters, gas exchange, potometers). He concluded that only a small portion of the studies (<20%) were within 5% of plant water use as measured by the secondary method. The average error rate for studies was 34% with the majority of studies underestimating plant water usage. Underestimation of flow is believed to be due to the disruption of sap flow by the probe and differences in thermal properties between the probe and the surrounding woody matrix (Kluitenberg and Ham, 2004; Sanson and Whitfield, 1981). Adding to the challenge of determination of absolute sap flow is the need for accurate calibration which is technically challenging and requires precise measures of thermal parameters of the sampled tree and sensor. These parameters include: sapwood density, sapwood moisture content, thermal diffusivity of the sapwood, area of conducting tissue, and possibly correction for needle misalignment (Taylor et al.

2013). In practice, physical properties of the woody matrix are often measured once at the beginning of the study through tree coring or alternatively at the end of the study through destructive harvest of the tree. Physical parameters are then applied post hoc to convert measured heat velocity to sap flow. Although some physical properties, such as moisture content, are dynamic and fluctuate slightly during the study, reasonable estimates can be obtained by applying them as constants. This means that the below equation can be simplified to contain wound corrected heat velocity, which is derived through measurement of change in temperature following a heat pulse, and a single constant representing the physical properties of the woody matrix:

$$V_s = V_c \frac{\rho_w (C_w + mC_s)}{(\rho_s C_s)} \rightarrow \; V_s = V_c C$$

Wound corrected heat pulse velocity (V_c) is therefore the responsive variable when looking at environmental or water status effects. In his review Forster (2017) did conclude that while absolute measures of volumetric sap flow were difficult to obtain, sap velocity measurements were well correlated with relative changes in transpiration rate. If the primary objective is to examine or leverage sap flow response to biotic or abiotic stressors, sap flow sensors could be reliably used without extensive calibration.

The use of relative measures is not without precedent: several researchers in the literature have used either sap flow indexes, normalized values or relative measures of sap flow in tree water use analysis. Nadezhdina (1999) successfully used a sap flow index based on the symmetrical temperature difference around a line heater to control irrigation of an apple orchard. Similarly, Ballester et al. (2013) found that a ratio of diurnal to nocturnal sap flow was well correlated to stem water potential and could be used in irrigation scheduling in citrus. Doronila and Forster (2015) used relative rates of sap flow for analyzing Eucalyptus responses to environmental variables. Finally, Burgess and Dawson (2008) used normalized rates of flow to analyze sap flow lags between the trunk and branches to determine capacitance. For the purposes of our research we are primarily interested in developing an index of tree hydration that could be easily implemented in commercial orchards. By removing the extensive technical calibration required to accurately determine sap flow and instead focusing on the pattern of underlying temperature response we remove barriers to use and simplify application.

Irrigation scheduling using sap flow sensors

Sap flow sensors have been used extensively to investigate fruit tree physiology and response to environmental conditions. A handful of researchers have also used these readings to schedule irrigation. In apple, a sap flow index, derived from the temperature difference between upstream and downstream sensors arranged symmetrically around a continuously powered line heater, was used to activate a mist system in Ukraine. The system compared pre-dawn sap flow index readings to those throughout the day. When day-time readings fell below pre-dawn values a misting system was activated to alleviate air drought (Nadezhdina and Cermak, 1997). Because this system used a continuously powered heater a large power supply is needed making it challenging to implement in field conditions. In grapefruit grown in Israel, a ratio of sap flow to potential transpiration calculated from meteorological data was used to schedule irrigation. When the ratio declined by 20% from its value under well-watered conditions irrigation was triggered. Researchers noted that the ratio on the first day after irrigation was stable through the early growing season but increased linearly towards the end of the summer. (Cohen 1991). Studies in Spain with olive compared sap flow in well-watered control trees to that of trees being irrigated by an automated system. Each day an irrigation dose was applied based on the difference between calculated transpirative losses in the well-watered and experimental trees (Fernández et al. 2008a, Fernández et al. 2008b). This method requires careful monitoring of well-watered controls for nitrogen leaching and soil anoxia (Fernández et al., 2008b). Consideration must also be given to deviating characteristics of the control trees under well-watered conditions over the course of the growing season which can result in increased vegetative growth and altered fruit set patterns compared to trees undergoing deficit irrigation (Fernández et al. 2008a). Both Cohen (1991) and Fernández et al. (2008b) noted that soil water loss was a function of both evaporation and transpiration with total ET exceeding transpiration losses measured via sap flow alone. When basing irrigation dose off of estimated water transpired from sap flow readings Fernandez et al. (2008b) noted that their system encountered difficulties as available soil water declined. This was attributed to the irrigation dose being determined by the calculated amount of water transpired. Researchers noted the system underapplied water due to the decreasing availability of soil water and decline in amount of water transpired which created a negative feedback loop. Ultimately researchers deemed it necessary to intervene to return soil water status close to field capacity and increase transpiration rates.

There are a number of challenges facing the use of sap flow or heat pulse sensors for automating irrigation in commercial settings. Variability in soil heterogeneity, microclimates and individual tree responses within orchards necessitates widespread instrumentation (Cohen, 1991; Cueavas et al., 2013; Fernández, 2014). This quickly increases the costs of any potential system. Additionally, users must have a high degree of technical expertise to install and maintain the system as well as process the data (Jones, 2004). This means that dedicated technicians must be either hired within the company or contracted to handle data collection, again increasing production costs. Finally, localized factors such as soil and crop hydraulic conductivities, crop specific drought tolerance, and rootstock/scion combinations can all affect sap flow responses to water stress (Horchberg et al. 2018; Fassio et al., 2009). This makes blanket recommendations for growers difficult to issue and further increases the technical capability needed to correctly implement these systems. For these reasons automating irrigation based on sap flow can be both time consuming and expensive (Fernandez, 2017). Greater development of user-friendly data processing and “plug and play” instillation could reduce the degree of technical expertise needed to utilize measurements. However, a robust sap flow index that reliably provides a sensitive indicator of crop water stress must be developed before barriers to user interface are addressed.

1.2.3 Dwarfing rootstocks

Dwarfing rootstocks have been used for centuries to control scion volume and height (Webster, 2004). Modern commercial fruit tree production is increasingly shifting to the use of dwarfing rootstocks to improve orchard efficiency and maximize yields per area. In addition to controlling canopy volume, dwarfing rootstocks improve disease resistance, flower number, precocity (early bearing), and fruit yield and quality (Atkinson and Else, 2001). Reduced canopy volume and improved lateral branching with scions grafted to dwarfing rootstocks improves efficiency and worker safety as shorter trees reduce the use of ladders and facilitate faster harvesting and pruning (Webster, 2004). Shorter more open canopies improve light penetration and fruit development and allow for more targeted spray applications reducing the environmental impact of new orchards. While individual tree yield is reduced with dwarfing rootstocks the more compact nature of the trees facilitates high density plantings which maximize yields per planting area (Foster et al., 2017).

Despite being widely planted and extensively researched the precise mechanisms by which rootstocks control vigor are not fully understood (Webster, 2002). Current understanding of mechanisms underlying dwarfing have identified changes in hydraulic conductivity and rootstock chemical signaling, in addition to potential sequestrating effects of the graft union itself and perception of environmental signals (Gautier et al., 2019; Gregory et al., 2013). It is likely however that interactions between these mechanisms also play a role in dwarfing and that the dominant mechanism varies with species (Warschefsky et al. 2016). Previous researchers have suggested that reduced hydraulic conductivity caused by misalignment of vessel elements and varying conductive tissue areas could provide an explanation for dwarfing in grafted scions (Atkinson et al. 2003; Gonçalves et al., 2007). While reduced hydraulic conductivity at the graft union has been found in some dwarfing rootstocks, evidence has been at best mixed with other studies pointing to higher rates of water and nutrient transportwhen normalizing for biomass (Tworkoski and Fazio, 2015, Webster, 2004). One of the best supported mechanisms, based on the ‘Hormone Message Concept’ (Jackson, 1993), proposes that disruption of hormone balance and production affects long distance signaling and scion development (Gautier et al., 2019). There exists multiple hypotheses on the precise functioning and signal compounds involved. The predominant theory suggests a feedback loop in which auxin transport from the shoots to the roots is either disrupted or out of balance. This reduction of auxin transport to the roots limits root growth and the production of cytokinin which subsequently reduces shoot growth (Aloni et al., 2010). This proposed mechanism has been supported by studies where auxin inhibition in vigorous rootstocks has produced similar effects in grafted scions as dwarfing rootstocks (van Hooijdonk et al., 2010). In addition to growth promoting hormones such as auxin, cytokinin, and gibberellins, inhibiting compounds such as abscisic acid, and ethylene have also been implicated in dwarfing capacity and shoot responses (Gregory et al., 2013). Despite numerous studies supporting these mechanisms, definitive evidence is still lacking however due in part to the complex nature of scion response to hormonal concentrations and delivery rates. Additional mechanisms which have been suggested include the sequestration of nutrients and chemical messengers at the graft union, and changes in perception of seasonal environmental signals related to dormancy (Gautier et al., 2019). In general, however, these mechanisms have either been found to have limited evidence supporting them, in the case of graft union sequestration, or have not had extensive investigation, in the case of environmental perception (Gautier et al., 2019; Webster, 2004).

Vigorous rootstocks have been reported to be more resistant to drought due to extensive root systems that can access greater soil volumes. However, dwarfing rootstocks can confer drought tolerance through greater stomatal regulation, increased ABA production, and reducing transpiration, vegetative growth, hydraulic conductance and leaf and stem water potentials (Atkinson et al. 2000; Hajagos and Végvári 2013; Martínez-Ballesta et al. 2010). While rooting volume may be an inherent limitation to dwarfing rootstock drought tolerance, commercial production will continue to shift to the planting of these rootstocks because of production benefits. Commercial growers can benefit from empirical observations of dwarfing rootstock responses to drought, but maximizing production requires a mechanistic understanding of these responses. Screening currently available dwarfing rootstock cultivars for drought response can simultaneously improve extension recommendations and theoretical understanding of rootstock responses.

1.3 Objectives and Hypotheses

In pursuing the overarching goal of improving orchard water use efficiency and drought tolerance I had two main objectives. For the first I evaluated easily automatable measures of sap flow and trunk diameter fluctuations to determine tree water status. For the second I compared the overall resilience to drought of commercially available and novel rootstocks using a weighing lysimeter system.

In my approach to the first objective I evaluated wound corrected heat pulse velocity as a simplified sap flow index that does not require extensive calibration for use. I also evaluated the use of band dendrometers to determine stem diameter fluctuations, which have been used extensively in forestry settings but had limited used in orchard irrigation scheduling. In testing my second objective I worked closely with commercial tree nurseries and state agricultural extension agents to identifying rootstock cultivars with the greatest potential for drought resilience. With my research I tested the following hypotheses:

1. Diurnal change in stem circumference (maximum daily shrinkage) will be correlated with stem water potential.
2. Heat pulse velocity will be well correlated to environmental drivers and stem water potential.
3. Rootstock cultivars will differ in their ability to recover from severe acute drought stress as measured in ability to recover to pre-drought daily transpiration rates.

4. Rootstock cultivars drawn from the Intermountain West will be better adapted to drought than commercially available rootstocks cultivars.

1.4 Literature Cited

Allen, R.G., L.S.Pereira, D. Raes, and M. Smith. 1998. Crop evapotranspiration: guidelines for computing crop water requirements Food and Agriculture Organization of the United Nations, Rome.

Allen, R.G., Pereira, L.S., Smith, M., Raes, D., Wright, J.L., 2005. FAO-56 Dual Crop Coefficient Method for Estimating Evaporation from Soil and Application Extensions. Journal of Irrigation Drain Engineering 131:2–13.

Aloni, B., Cohen, R., Karni, L., Aktas, H., Edelstein, M., 2010. Hormonal signaling in rootstock–scion interactions. Scientia Horticulturae 127:119–126.

Alvarado-Barrientos, M.S., Hernández-Santana, V. and Asbjornsen, H., 2013. Variability of the radial profile of sap velocity in Pinus patula from contrasting stands within the seasonal cloud forest zone of Veracruz, Mexico. Agricultural and Forest Meteorology, 168:108-119.

Annandale, J.G. and Stockle, C.O., 1994. Fluctuation of crop evapotranspiration coefficients with weather: a sensitivity analysis. Irrigation Science, 15:1-7.

Atkinson, C., Else, M., 2001. Understanding how rootstocks dwarf fruit trees. Compact Fruit Tree 34:46–49.

Atkinson, C.J., Policarpo, M., Webster, A.D. and Kingswell, G., 2000. Drought tolerance of clonal Malus determined from measurements of stomatal conductance and leaf water potential. Tree Physiology, 20:557-563.

Ballester, C., Castel, J., Testi, L., Intrigliolo, D.S. and Castel, J.R., 2013. Can heat-pulse sap flow measurements be used as continuous water stress indicators of citrus trees? Irrigation Science, 31:1053-1063.

Barrett, D., Hatton, T., Ash, J., and Ball, M. 1995. Evaluation of the heat pulse velocity technique for measurement of sap flow in rainforest and eucalypt forest species of south-eastern Australia. Plant, Cell & Environment 18:463–469.

Becker, P. and Edwards, W.R.N., 1999. Corrected heat capacity of wood for sap flow calculations. Tree Physiology, 19:767-768.

Bittelli, M., 2010. Measuring soil water potential for water management in agriculture: A review. Sustainability, 2:1226–1251.

Burgess, S.S., Adams, M.A., Turner, N.C., Beverly, C.R., Ong, C.K., Khan, A.A., and Bleby, T.M. 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. Tree Physiology, 21:589–598.

Burgess, S.S.O., and Dawson, T.E. 2008. Using branch and basal trunk sap flow measurements to estimate whole-plant water capacitance: a caution. Plant and Soil, 305:5–13.

Bush, S.E., Hultine, K.R., Sperry, J.S. and Ehleringer, J.R., 2010. Calibration of thermal dissipation sap flow probes for ring-and diffuse-porous trees. Tree Physiology, 30:1545-1554.

Caylor, K.K. and Dragoni, D., 2009. Decoupling structural and environmental determinants of sap velocity: Part I. Methodological development. Agricultural and Forest Meteorology, 149:559-569.

Čermák, J., Kučera, J., Bauerle, W.L., Phillips, N. and Hinckley, T.M., 2007. Tree water storage and its diurnal dynamics related to sap flow and changes in stem volume in old-growth Douglas-fir trees. Tree physiology, 27:181-198.

Chalmers DJ, Mitchell PD, Van Heek L. 1981. Control of peach tree growth and productivity by regulated water supply, tree density and summer pruning. Journal of the American Society of Horticulture Science, 106:307–312

Chang, X., Zhao, W. and He, Z., 2014. Radial pattern of sap flow and response to microclimate and soil moisture in Qinghai spruce (*Picea crassifolia*) in the upper Heihe River Basin of arid northwestern China. Agricultural and Forest Meteorology, 187:14-21.

Clearwater, M.J., Meinzer, F.C., Andrade, J.L., Goldstein, G. and Holbrook, N.M., 1999. Potential errors in measurement of nonuniform sap flow using heat dissipation probes. Tree Physiology, 19:681-687.

Cline, J.A., 2019. Planting density and size-controlling rootstocks influence the performance of Montmorency tart cherry (*Prunus cerasus* L.). Canadian Journal of Plant Science, 100:16-28.

Cohen, Y., 1991. Determination of orchard water requirement by a combined trunk sap flow and meteorological approach. Irrigation Science, 12:93-98.

Corell, M., Girón, I.F., Galindo, A., Torrecillas, A., Torres-Sánchez, R., Pérez-Pastor, A., Moreno, F. and Moriana, A., 2014. Using band dendrometers in irrigation scheduling: influence of the location inside the tree and comparison with point dendrometer. Agricultural Water Management, 142:29-37.

Cuevas, M.V., Martín-Palomo, M.J., Diaz-Espejo, A., Torres-Ruiz, J.M., Rodriguez-Dominguez, C.M., Perez-Martin, A., Pino-Mejías, R. and Fernández, J.E., 2013. Assessing water stress in a hedgerow olive orchard from sap flow and trunk diameter measurements. Irrigation Science, 31:729-746.

Daudet, F. A., Améglio, T., Cochard, H., Archilla, O., & Lacointe, A., 2005. Experimental analysis of the role of water and carbon in tree stem diameter variations. Journal of Experimental Botany. 56:135-144.

Dias, P.C., Roque, W., Ferreira, E.C. and Dias, J.A.S., 2013. A high sensitivity single-probe heat pulse soil moisture sensor based on a single npn junction transistor. Computers and Electronics in Agriculture. 96:139-147.

Domingo, R., Ruiz-Sánchez, M.C., Sánchez-Blanco, M.J. and Torrecillas, A., 1996. Water relations, growth and yield of Fino lemon trees under regulated deficit irrigation. Irrigation Science, 16:115-123.

Doronila, A.I. and Forster, M.A., 2015. Performance measurement via sap flow monitoring of three Eucalyptus species for mine site and dryland salinity phytoremediation. International Journal of Phytoremediation, 17:101-108.

Dragoni, D., Caylor, K.K. and Schmid, H.P., 2009. Decoupling structural and environmental determinants of sap velocity: Part II. Observational application. Agricultural and Forest Meteorology, 149:570-581.

Dragoni, D., Lakso, A.N. and Piccioni, R.M., 2005. Transpiration of apple trees in a humid climate using heat pulse sap flow gauges calibrated with whole-canopy gas exchange chambers. Agricultural and Forest Meteorology, 130:85-94.

Drew, D.M. and Downes, G.M., 2009. The use of precision dendrometers in research on daily stem size and wood property variation: a review. Dendrochronologia, 27:159-172.

Edwards, W.R.N. and Warwick, N.W.M., 1984. Transpiration from a kiwifruit vine as estimated by the heat pulse technique and the Penman-Monteith equation. New Zealand Journal of Agricultural Research, 27:537-543.

Einhorn, T. & Caspari, H. Partial rootzone drying and deficit irrigation of 'Gala' apples in a semi-arid climate. 2003. IV International Symposium on Irrigation of Horticultural Crops:197-204.

Elliott, J., Deryng, D., Müller, C., Frieler, K., Konzmann, M., Gerten, D., Glotter, M., Flörke, M., Wada, Y., Best, N., Eisner S., Balazs F., Folberth C., Foster I., Gosling S., Haddeland I., Khabarov N., Ludwig F., Masaki Y., Olin S., Rosenzweig C., Ruane A.C., Satoh Y., Schmid E., Stacke T., Tang Q., and Wisser D., 2014. Constraints and potentials of future irrigation water availability on agricultural production under climate change. Proceedings of the National Academy of Sciences 111:3239–3244.

Fassio, C., Heath, R., Arpaia, M.L. and Castro, M., 2009. Sap flow in ‘Hass’ avocado trees on two clonal rootstocks in relation to xylem anatomy. Scientia Horticulturae, 120:8-13.

Feng, X., Ackerly, D.D., Dawson, T.E., Manzoni, S., McLaughlin, B., Skelton, R.P., Vico, G., Weitz, A.P. and Thompson, S.E., 2019. Beyond isohydricity: the role of environmental variability in determining plant drought responses. Plant, cell & environment, 42:1104-1111.

Fereres, E., and Evans, R.G. 2006. Irrigation of fruit trees and vines: an introduction. Irrigation Science, 24:55–57.

Fereres, E., and Soriano, M.A. 2006. Deficit irrigation for reducing agricultural water use. Journal of Experimental Botany 58:147–159.

Fernández, J.E., 2017. Plant-based methods for irrigation scheduling of woody crops. Horticulturae, 3:35.

Fernández, J. 2014. Plant-based sensing to monitor water stress: Applicability to commercial orchards. Agricultural Water Management, 142:99–109.

Fernández, J.E., Green, S.R., Caspari, H.W., Diaz-Espejo, A. and Cuevas, M.V., 2008a. The use of sap flow measurements for scheduling irrigation in olive, apple and Asian pear trees and in grapevines. Plant and Soil, 305:91-104.

Fernández, J.E., Romero, R., Montaño, J.C., Diaz-Espejo, A., Muriel, J.L., Cuevas, M.V., Moreno, F., Girón, I.F., and Palomo, M.J. 2008b. Design and testing of an automatic irrigation controller for fruit tree orchards, based on sap flow measurements. Australian Journal of Agricultural Research 59:589–598.

Fernández, E., Cermak, J., Cohen, Y., Ferreira, M.I., Nadezhdina, N., Testi, L., and Steppe, K. 2017. Methods to estimate sap flow. ISHS Working Group on Sap Flow online publication; https://www.ishs.org/sap-flow/ishs-working-group-sap-flow-online-resources

Fernández, J.E. and Cuevas, M.V., 2010. Irrigation scheduling from stem diameter variations: a review. Agricultural and Forest Meteorology, 150:135-151.

Fiora, A. and Cescatti, A., 2008. Vertical foliage distribution determines the radial pattern of sap flux density in *Picea abies*. Tree Physiology, 28:1317-1323.

Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., Mueller, N.D., O'Connell, C., Ray, D.K., West, P.C., Blazer, C., Bennet E.M., Capenter R., Hill J., Monfreda C., Polasky S., Rockstrom J., Sheehan J., Siebert S., Tilman D., and Zaks D.P. 2011. Solutions for a cultivated planet. Nature, 478:337–342.

Ford, C.R., Goranson, C.E., Mitchell, R.J., Will, R.E. and Teskey, R.O., 2004. Diurnal and seasonal variability in the radial distribution of sap flow: predicting total stem flow in Pinus taeda trees. Tree Physiology, 24:951-960.

Forner-Giner, M.A., Rodriguez-Gamir, J., Martínez-Alcántara, B., Quinones, A., Iglesias, D.J., Primo-Millo, E. and Forner, J., 2014. Performance of Navel orange trees grafted onto two new dwarfing rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418). Scientia Horticulturae, 179:376-387.

Forster, A.M., 2017. How Reliable Are Heat Pulse Velocity Methods for Estimating Tree Transpiration? Forests 8:350

Forster, M.A., 2020. The importance of conduction versus convection in heat pulse sap flow methods. Tree Physiology, 40:683-694.

Foster, T.M., McAtee, P.A., Waite, C.N., Boldingh, H.L., McGhie, T.K., 2017. Apple dwarfing rootstocks exhibit an imbalance in carbohydrate allocation and reduced cell growth and metabolism. Horticulture research 4:1–13.

Gautier, A.T., Chambaud, C., Brocard, L., Ollat, N., Gambetta, G.A., Delrot, S., Cookson, S.J., 2019. Merging genotypes: graft union formation and scion–rootstock interactions. Journal of Experimental Botany 70:747–755.

García-Orellana, Y., Ruiz-Sánchez, M.C., Alarcón, J.J., Conejero, W., Ortuño, M.F., Nicolás, E. and Torrecillas, A., 2007. Preliminary assessment of the feasibility of using maximum daily trunk shrinkage for irrigation scheduling in lemon trees. Agricultural Water Management, 89:167-171.

Granier, A. 1985. Une nouvelle méthode pour la mesure du flux de sève brute dans le tronc des arbres. Annales des Sciences Forestières 42:193-200.

Granier, A., Bobay, V., Gash, J.H.C., Gelpe, J., Saugier, B., Shuttleworth, W.J., 1990. Vapour flux density and transpiration rate comparisons in a stand of Maritime pine (*Pinus pinaster* Ait.) in Les Landes forest. Agricultural and Forest Meteorology 51:309–319.

Ginestar, C. and Castel, J.R., 1995. Use of stem dendrometers as indicators of water stress in drip-irrigated citrus trees. In II International Symposium on Sensors in Horticulture 421:209-222.

Ginestar, C., Eastham, J., Gray, S. and Iland, P., 1998. Use of sap-flow sensors to schedule vineyard irrigation. I. Effects of post-veraison water deficits on water relations, vine growth, and yield of Shiraz grapevines. American Journal of Enology and Viticulture, 49:413-420.

Girona, J., Gelly, M., Mata, M., Arbones, A., Rufat, J. and Marsal, J., 2005. Peach tree response to single and combined deficit irrigation regimes in deep soils. Agricultural Water Management, 72:97-108.

Goodwin, I., 2009. Gypsum blocks for measuring the dryness of soil. Agnote AG0294. Department of Environment and Primary Industries. Melbourne, Victoria, Australia.

Goldhamer, D.A., Fereres, E., Mata, M., Girona, J., Cohen, M., 1999. Sensitivity of continuous and discrete plant and soil water status monitoring in peach trees subjected to deficit irrigation. Journal of the American Society for Horticultural Science 124:437–444.

Goldhamer, D.A. and Fereres, E., 2001. Irrigation scheduling protocols using continuously recorded trunk diameter measurements. Irrigation Science, 20:115-125.

Goldhamer, D.A. and Fereres, E., 2004. Irrigation scheduling of almond trees with trunk diameter sensors. Irrigation Science, 23:11-19.

Goldhamer, D.A., Viveros, M. and Salinas, M., 2006. Regulated deficit irrigation in almonds: effects of variations in applied water and stress timing on yield and yield components. Irrigation Science, 24:101-114.

Gómez-Bellot, M.J., Nortes, P.A., Sánchez-Blanco, M.J. and Ortuño, M.F., 2015. Sensitivity of thermal imaging and infrared thermometry to detect water status changes in Euonymus japonica plants irrigated with saline reclaimed water. Biosystems Engineering, 133:21-32.

Gonçalves, B., Correia, C.M., Silva, A.P., Bacelar, E.A., Santos, A., Ferreira, H., Moutinho-Pereira, J.M., 2007. Variation in xylem structure and function in roots and stems of scion–rootstock combinations of sweet cherry tree (*Prunus avium* L.). Trees 21:121–130.

Green, S.R. and Clothier, B.E., 1988. Water use of kiwifruit vines and apple trees by the heat-pulse technique. Journal of Experimental Botany, 39:115-123.

Green, S., Clothier, B., and Jardine, B., 2003. Theory and practical application of heat pulse to measure sap flow. Agronomy Journal 95:1371–1379.

Green S, Clothier B, Perie E. 2009. A re-analysis of heat pulse theory across a wide range of sap flows. In: Fernandez JE, Diaz-Espejo A, eds. 7th International Workshop on Sap Flow. Seville, Spain: Acta Horticultura:95–104.

Gregory, P.J., Atkinson, C.J., Bengough, A.G., Else, M.A., Fernández-Fernández, F., Harrison, R.J., Schmidt, S., 2013. Contributions of roots and rootstocks to sustainable, intensified crop production. Journal of Experimental Botany 64:1209–1222.

Hajagos, A., Végvári, G., 2013. Investigation of tissue structure and xylem anatomy of eight rootstocks of sweet cherry (*Prunus avium* L.). Trees, 27:53–60.

Harrison, N., Harrison, R.J., Barber-Perez, N., Cascant-Lopez, E., Cobo-Medina, M., Lipska, M., Conde-Ruíz, R., Brain, P., Gregory, P.J., Fernández-Fernández, F., 2016. A new three-locus model for rootstock-induced dwarfing in apple revealed by genetic mapping of root bark percentage. Journal of Experimental Botany 67:1871–1881.

Hochberg, U., Rockwell, F.E., Holbrook, N.M. and Cochard, H., 2018. Iso/anisohydry: a plant–environment interaction rather than a simple hydraulic trait. Trends in Plant Science, 23:112-120.

Huber, B. 1932. Beobachtung und Messung pflanzlicher Saftströme. Ber. deutsch. Bot. Ges. 50: 89-109.

Idso, S.B., Jackson, R.D., Pinter Jr, P.J., Reginato, R.J. and Hatfield, J.L., 1981. Normalizing the stress-degree-day parameter for environmental variability. Agricultural Meteorology, 24:45-55.

Intrigliolo, D.S., Castel, J.R., 2005. Effects of regulated deficit irrigation on growth and yield of young Japanese plum trees. The Journal of Horticultural Science and Biotechnology 80:177–182.

Jackson, M., 1993. Are plant hormones involved in root to shoot communication?, in: Advances in Botanical Research. Elsevier, pp. 103–187.

Jackson, R.D., Idso, S.B., Reginato, R.J. and Pinter Jr, P.J., 1981. Canopy temperature as a crop water stress indicator. Water Resources Research, 17:1133-1138.

Jarvis, P.G., and McNaughton, K.G. 1986. Stomatal Control of Transpiration: Scaling Up from Leaf to Region. In Advances in Ecological Research, A. MacFadyen, and E.D. Ford, eds. (Academic Press), 1–49.

Johnson, R.S. and Handley, D.F., 2000. Using water stress to control vegetative growth and productivity of temperate fruit trees. HortScience, 35:1048-1050.

Johnson, R.S., D.F. Handley, and T.M. DeJong. 1992. Long-term response of early maturing peach trees to postharvest water deficits. Journal of the American Society of Horticultural Science, 117:881–886.

Jones, H.G. 2004. Irrigation scheduling: advantages and pitfalls of plant-based methods. Journal of Experimental Botany, 55:2427–2436.

Jones, S.B., Blonquist, J., Robinson, D., Rasmussen, V.P., Or, D., 2005. Standardizing Characterization of Electromagnetic Water Content Sensors: Part 1. Methodology. Vadose Zone Journal 4:1048–1058.

Juhász, Á., Sepsi, P., Nagy, Z., Tőkei, L. and Hrotkó, K., 2013. Water consumption of sweet cherry trees estimated by sap flow measurement. Scientia Horticulturae, 164:41-49.

Kluitenberg, G.J., Bristow, K.L. and Das, B.S., 1995. Error analysis of heat pulse method for measuring soil heat capacity, diffusivity, and conductivity. Soil Science Society of America Journal, 59:719-726.

Kluitenberg, G.J., and Ham, J.M. 2004. Improved theory for calculating sap flow with the heat pulse method. Agricultural and Forest Meteorology, 126:169–173.

Li, S.H., Huguet, J.G. and Bussi, C., 1989. Irrigation scheduling in a mature peach orchard using tensiometers and dendrometers. Irrigation and Drainage Systems, 3:1-12.

Liu, C., Du, T., Li, F., Kang, S., Li, S., Tong, L., 2012. Trunk sap flow characteristics during two growth stages of apple tree and its relationships with affecting factors in an arid region of northwest China. Agricultural Water Management 104, 193–202.

Lliso, I., Forner, J.B., Talón, M., 2004. The dwarfing mechanism of citrus rootstocks F&A 418 and# 23 is related to competition between vegetative and reproductive growth. Tree physiology 24:225–232.

Lopez, G., Behboudian, M.H., Girona, J., Marsal, J., 2012. Drought in deciduous fruit trees: implications for yield and fruit quality, in: Plant Responses to Drought Stress. Springer, pp. 441–459.

Maes, W.H. and Steppe, K., 2012. Estimating evapotranspiration and drought stress with ground-based thermal remote sensing in agriculture: a review. Journal of Experimental Botany, 63:4671-4712.

Marshall, D.C. 1958. Measurement of sap flow in conifers by heat transport. Plant Physiology 33:385-396.

Martínez-Ballesta, M.C., Alcaraz-López, C., Muries, B., Mota-Cadenas, C., Carvajal, M., 2010. Physiological aspects of rootstock–scion interactions. Scientia Horticulturae 127:112–118.

Martínez-Vilalta, J. and Garcia-Forner, N., 2017. Water potential regulation, stomatal behaviour and hydraulic transport under drought: deconstructing the iso/anisohydric concept. Plant, Cell & Environment, 40:962-976.

Migliaccio, K.W., Olczyk, T., Li, Y., Muñoz-Carpena, R., and Dispenza, T. Using Tensiometers for Vegetable Irrigation Scheduling in Miami-Dade County. Extension bulletin ABE326. University of Florida Department of Agricultural and Biological Engineering. Gainesville, Florida

Molina, A.J., Aranda, X., Carta, G., Llorens, P., Romero, R., Savé, R., and Biel, C. 2016. Effect of irrigation on sap flux density variability and water use estimate in cherry (*Prunus avium*) for timber production: Azimuthal profile, radial profile and sapwood estimation. Agricultural Water Management 164:118–126.

Monteith, J.L., 1973. Principles of environmental physics, Edward Arnold, London.

Morandi, B., Manfrini, L., Lugli, S., Tugnoli, A., Micheli, A., Boini, A., Perulli, G., Bresilla, K. and Corelli Grappadelli, L., 2018, August. Physiological responses to rootstocks vigor in cherry: why dwarfing is efficient? In XXX International

Horticultural Congress IHC2018: International Symposium on Cultivars, Rootstocks and Management Systems of Orchards 1281:487-492.

Moriana, A. and Fereres, E., 2002. Plant indicators for scheduling irrigation of young olive trees. Irrigation Science, 21:83-90.

Muñoz-Carpena, R., Shukla, S. and Morgan, K., 2004. Field devices for monitoring soil water content (Vol. 343, pp. 1-24). University of Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, EDIS.

Nadezhdina, N., and Cermak, J. 1997. Automatic control unit for irrigation systems based on sensing the plant water status. Anais Do Instituto Superior de Agronomia 46: 149–157.

Nadezhdina, N., Čermák, J., Nadezhdin, V. 1998. Heat field deformation method for sap flow measurements. p. 72-92. In: J. Čermak and N. Nadezhdina (eds.), Measuring Sap Flow in Intact Plants, IUFRO Publications, Publishing House of Mendel University, Brno (Czech Republic).

Nadezhdina, N., Nadezhdin, V., Ferreira, M.I., and Pitacco, A. 2007. Variability with xylem depth in sap flow in trunks and branches of mature olive trees. Tree Physiology 27:105–113.

Naor, A., 2006. Irrigation Scheduling and Evaluation of Tree Water Status in Deciduous Orchards, in: Janick, J. (Ed.), Horticultural Reviews. John Wiley & Sons, Inc., Oxford, UK, pp. 111–165.

Naor, A., Naschitz, S., Peres, M., Gal, Y., 2008. Responses of apple fruit size to tree water status and crop load. Tree Physiology 28:1255–1261.

Nortes, P.A., Pérez-Pastor, A., Egea, G., Conejero, W. and Domingo, R., 2005. Comparison of changes in stem diameter and water potential values for detecting water stress in young almond trees. Agricultural Water Management, 77:296-307.

Ortuño, M.F., García-Orellana, Y., Conejero, W., Pérez-Sarmiento, F. and Torrecillas, A., 2009. Assessment of maximum daily trunk shrinkage signal intensity threshold values for deficit irrigation in lemon trees. Agricultural Water Management, 96:80-86.

Ortuño, M., Conejero, W., Moreno, F., Moriana, A., Intrigliolo, D., Biel, C., Mellisho, C., Pérez-Pastor, A., Domingo, R., and Ruiz-Sánchez, M. 2010. Could trunk diameter sensors be used in woody crops for irrigation scheduling? A review of current knowledge and future perspectives. Agricultural Water Management, 97:1–11.

Ritchie, G.A. and Hinckley, T.M., 1975. The pressure chamber as an instrument for ecological research. In Advances in Ecological Research,9:165-254. Academic Press.

Robinson, D.A., Gardner, C.M.K. and Cooper, J.D., 1999. Measurement of relative permittivity in sandy soils using TDR, capacitance and theta probes: comparison, including the effects of bulk soil electrical conductivity. Journal of Hydrology, 223:198-211.

Robinson, T., 2006, August. The evolution towards more competitive apple orchard systems in the USA. Acta Horticulturae, 772:491-500

Schaible, G.D., and Aillery, M.P. 2017. Challenges for US Irrigated Agriculture in the Face of Emerging Demands and Climate Change. In: Competition for Water Resources: 44–79.

Schmugge, T., Jackson, T., McKim, H., 1980. Survey of methods for soil moisture determination. Water Resources Research 16:961–979.

Stroock, A.D., Lakso, A.N., Pagay, V., Santiago, M. and Sessoms, D., Cornell University, 2018. Mems microtensiometer. U.S. Patent Application 15/689,584.

Swanson, R., and Whitfield, D. 1981. A numerical analysis of heat pulse velocity theory and practice. Journal of Experimental Botany, 32:221–239.

Taylor, N.J., Ibraimo, N.A., Annandale, J.G., Everson, C.S., Vahrmeijer, J.T., Gush, M.B., 2013. Are sap flow measurements useful for determining water use of fruit orchards when absolute values are important? Acta Horticulturae:77–83.

Tworkoski, T. and 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, 15:369-381.

United Nations, Department of Economic and Social Affairs/Population Division 2019. World population prospects Highlights, 2019 New York.

van Hooijdonk, B.M., Woolley, D.J., Warrington, I.J., Tustin, D.S., 2010. Initial alteration of scion architecture by dwarfing apple rootstocks may involve shoot-root-shoot signaling by auxin, gibberellin, and cytokinin. The Journal of Horticultural Science and Biotechnology 85: 59–65.

Vandegehuchte, M.W. and Steppe, K., 2012. Improving sap flux density measurements by correctly determining thermal diffusivity, differentiating between bound and unbound water. Tree Physiology, 32:930-942.

Wang, Q., Ochsner, T.E. and Horton, R., 2002. Mathematical analysis of heat pulse signals for soil water flux determination. Water Resources Research, 38:27-1.

Warschefsky, E.J., Klein, L.L., Frank, M.H., Chitwood, D.H., Londo, J.P., von Wettberg, E.J.B., Miller, A.J., 2016. Rootstocks: Diversity, Domestication, and Impacts on Shoot Phenotypes. Trends in Plant Science 21:418–437.

Webster, A.D., 2004. Vigour mechanisms in dwarfing rootstocks for temperate fruit trees. Acta Horticulturae 658:29–41.

Webster, T., 2002. Dwarfing rootstocks: past, present and future. Compact Fruit Tree, 35:67-72.

Werner, H., 2002. Irrigation management: Using electrical resistance blocks to measure soil moisture. Extension bulletin FS 899. South Dakota State University. Brookings, South Dakota.

Woodcock, D.W., 1989. Distribution of vessel diameter in ring-porous trees. Aliso: A Journal of Systematic and Evolutionary Botany, 12:287-293.

Wullschleger, S.D. and King, A.W., 2000. Radial variation in sap velocity as a function of stem diameter and sapwood thickness in yellow-poplar trees. Tree Physiology, 20:511-518.

CHAPTER 2

SAP FLOW SENSOR AND DENDROMETER MEASUREMENTS OF HIGH DENSITY APPLE IN RELATION TO ENVIRONMENTAL DEMAND

ABSTRACT

Successful automation of plant-based water stress measurements has the potential to greatly advance precision irrigation in orchard crops. This study was conducted to determine how automated measurements of tree physiology responded to environmental conditions and water stress in a high-density apple orchard. Three physiological measurements, maximum daily trunk shrinkage (MDS) from a band dendrometer, sap flow index (SFI) derived from a heat pulse sap flow sensor and midday stem water potential (Ψ_{stem}) were monitored in a 6th leaf high-density apple orchard. Measurements were taken on scions 'Aztec Fuji' (AF) and 'Scilate (Envy™)' (SE) which were trained to a central leader and grafted to the dwarfing rootstock Malling 9 Selection NIC™ 29 (M.9). Physiological measurements were compared to environmental parameters over the course of a growing season in which several dry downs were imposed. Sap flow index was most highly influenced by environmental drivers followed by MDS and Ψ_{stem}. Sap flow was highly correlated to ET_r suggesting that planting density in high density orchards decouple orchard ET to a greater extent from bulk air properties especially later in the season as leaf area indexes reach a maximum. Controlling for phenological stage of growth improved correlations of MDS and SFI to Ψ_{stem}. Trunk circumferential growth plateaued in the AF scions midway through the season while SE circumferential growth

continued to increase late into the season. Stem water potential measurements had the least tree to tree variability followed by MDS and SFI. Stem water potentials reached a minimum of approximately -2.0 MPa, during which time leaf curling and low soil moisture content was observed. Maximum daily shrinkage was linearly correlated to Ψ_{stem} and despite higher measurement variability has potential to replace labor intensive Ψ_{stem} measurements.

2.1 Introduction

Commercial apple production in many areas of the world is almost exclusively accomplished through grafting of genetically different fruit bearing scions to well adapted rootstocks. Newly established orchards have shifted toward dwarfing rootstocks and high planting densities (Robinson, 2008). One of the most common and widely planted rootstocks is M.9 due to its highly dwarfing nature, precociousness and high fruit set (Fallahi et al., 2002). Dwarfing rootstocks, because of their reduced root volumes are more prone to drought and necessitate careful irrigation management (Gonçalves et al. 2006). Drought quickly reduces yields and can lead to tree mortality while over application of irrigation can promote excessive vegetative growth, reduced pathogen resistance and leaching of nutrients from the rootzone (Bonany and Camps, 1996). Precise understanding of tree water status can inform irrigation timing, ensure tree health and maximize productivity. A reliable, intuitive and inexpensive method to accurately determine orchard water stress is needed to help orchard producers advance precision irrigation methodology.

Evapotranspiration (ET) modeling is widely used by commercial orchard managers to estimate crop water loss. Numerous models exist to compute potential evapotranspiration (ET) (e.g. Priestly and Taylor 1972, Hargraves and Samani 1985, Shuttleworth and Wallace 1985) with the most widely used being the FAO - 56 Penman-Monteith equation. Reference evapotranspiration values for a grass (ET_0) or alfalfa (ET_r) like crop are commonly reported from an extensive network of private and government monitored weather stations in North America. These reference values are then multiplied by empirically derived crop coefficients to estimate specific crop water losses. While this methodology has been shown to be effective in many annual crops, results in orchard crops have been mixed (Dzikiti et al. 2018; Naor et al. 2008). The elevated nature of orchard canopies and relatively low orchard planting densities compared to reference crops has been cited as reasons for divergence of orchard ET from modeled ET with modifying crop coefficients (Jarvis 1985). For these reasons, the use of reference models as an accurate predictor of tree water status has been questioned (Annandale and Stockle 1994).

Soil moisture measurements have also been proposed as a relatively inexpensive and intuitive method for controlling irrigation in commercial orchards. However, soil moisture status remains an indirect measure of plant tissue water which has been found to be a function of both soil water availability and environmental demand (Jones, 2004). In addition, soil heterogeneity and the large volume of soil tree roots can occupy combined with the relatively localized nature of soil moisture measurements requires extensive instrumentation to accurately capture soil moisture availability within the orchard (Pardossi et al. 2009).

Direct plant-based measurements of water stress have long been considered the best approach for automating irrigation in orchard crops (Jones, 2004). Midday stem water potential (Ψ_{stem}) is considered a reliable indicator of peak water stress for fruit trees (Doltra et al. 2007; Naor, 1999). However, the use of Scholander type pressure chambers to determine stem water potential is labor intensive, time consuming and cannot easily be automated. Additional plant indicators are needed that can be easily automated and interpreted for accurate deficit irrigation.

Sap flow sensors can provide a direct, near instantaneous, measure of sap flow which, when extensively calibrated, can estimate tree transpiration (Burgess et al. 2001). Accurate calibration of these sensors however can be technically challenging and prone to error even for experienced researchers (Forester 2017). Normalized values of sap flow, sap flow indexes or relative measures of heat velocity have been found to be as well correlated to environmental variables as absolute measures due to the underlying mechanics of sap flow sensors (Ballester et al. 2012; Burgess and Dawson, 2008; Forster 2017). If the primary objective is to analyze and leverage sap flow responses to biotic or abiotic stressors, sap flow sensors could be reliably used to estimate transpiration responses without extensive calibration.

Diurnal trunk diameter variation has also been proposed as an easily automatable measure of plant water status for irrigation scheduling (Goldhamer and Fereres, 2001). Stem diameter typically peaks near sunrise as trees recharge water used during the day and reaches a minimum a few hours after solar noon when evaporative demand is the greatest (Ginestar and Castel, 1995). The difference between the maximum and minimum trunk diameters in a 24 hour period, or the maximum daily shrinkage (MDS), has been shown to be well correlated to stem water potential (Fernández and Cuevas, 2010). To date, the use of dendrometers for irrigation scheduling has been limited in part due to the high degree of variability between measurements (Ortuño et al. 2010). This variability may be related to the use of point dendrometers which, due to the localized nature of the measurement is sensitive to positioning errors. Band dendrometers capable of measuring changes in trunk circumference sample a larger area of the trunk and thus reduce position errors (Corell et al. 2014). These types of sensors have been used extensively in forestry settings but have seen limited use in horticulture settings.

We examined the interactions of SFI derived from a heat pulse sap flow sensor, MDS from a band dendrometer, and midday Ψ_{stem} to environmental conditions under prolonged periods of drought in a high-density apple planting. Based on preliminary research, we hypothesized that SFI would be well correlated to water stress with declining SFI values with increasing water stress. We also hypothesize that MDS from band dendrometers would be well correlated to Ψ_{stem}. Finally, we hypothesize variability between MDS measurements will be similar to those of Ψ_{stem} making them a good candidate for irrigation scheduling.

2.2 Materials and Methods

2.2.1 Experimental field

Research was conducted at the Utah State University Research Farm located in Kaysville, UT (41° 01’ 21” N by 111° 55’ 51”W, elevation 1325 m) during the 2020 growing season. The region has a semi-arid continental climate under the Köppen classification system with average annual rainfall of 380 mm. Annual average pan evapotranspiration is 995 mm (57-year average). Sampling was done in a 0.5 ha, 6th leaf high density apple (*Malus* x *domestica* Borkh.) planting with 1.5 m in row spacing and approximately 3 m between rows with rows oriented north to south. Trees were trained to a tall spindle system with trunk diameters averaging 6-7 cm, 30 cm above the soil surface (Robinson et al., 2011). The orchard was established to examine the potential relationship between initial graft union strength and subsequent drought tolerance (Adams, 2016) and these goals ran concurrently with our trial. The trial was organized in a randomized complete block consisting of a 2x2 factorial of scion and rootstock combinations with each block replicated five times with six trees per block. Fruiting scions consisted of ‘Scilate (Envy™)’ (SE) (White, 2008) and ‘Aztec Fuji’ (AF) grafted in combination with M.9 and Geneva® 41 (G.41) rootstocks. For the purposes of this trial the G.41 treatments were not considered. Irrigation was withheld until midday stem water potential from an average of twenty randomly selected trees (one per block) dropped below -1.5 MPa after which between 20 and 33 mm of irrigation water was applied. Later in the season irrigation quantity was increased to 50 to 80 mm when it was noted that readings from soil moisture sensors buried 80 cm below the surface continued to decline despite irrigation application. Trees were irrigated with mico-spray emitters with a 2 m overlapping spray radius with an approximate application rate of 3.4 mm hr^{-1}. Soils were a well-drained Kidman series fine sandy loam. Four CR1000 dataloggers (Campbell Scientific, Logan UT, USA) were used to collect data from sap flow, dendrometer, and soil moisture (Fig. 2.3.1).

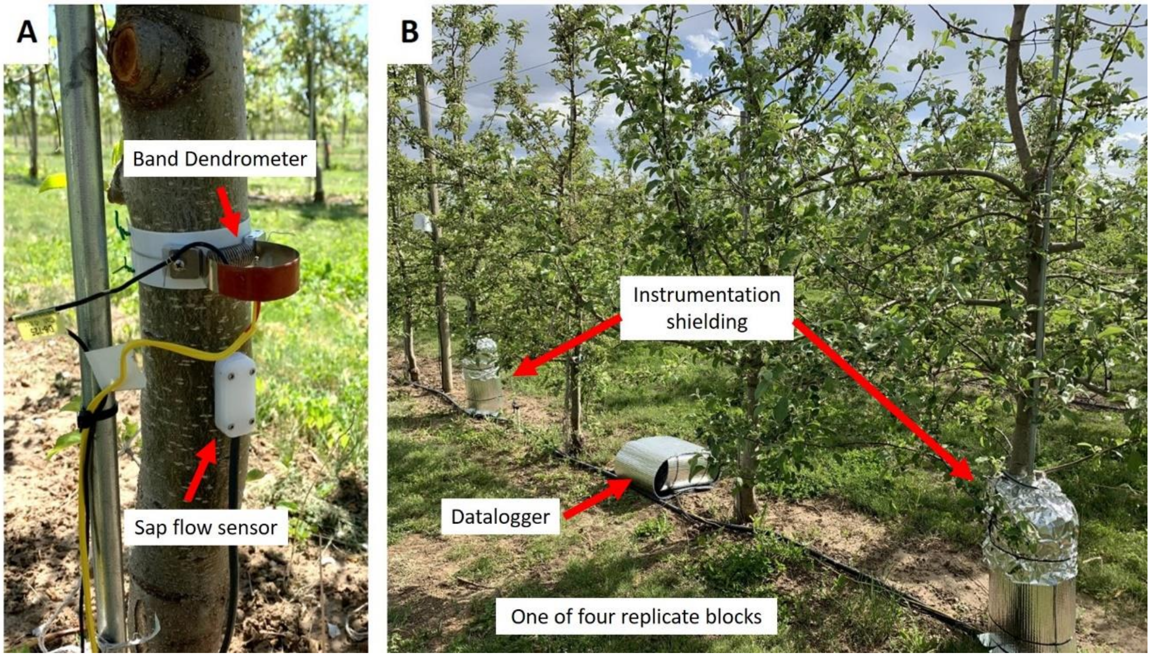

Fig. 2.2.1 Representative instrumentation setup in a high-density apple block. A) Sap flow sensor and dendrometer. B) Datalogger (center) and sensor shielding (left and right of center box)

2.2.2 Stem water potential

Midday (12:00 – 14:00) stem water potential (Ψ_{stem}) was measured twice weekly on 3 fully expanded leaves per tree located near the main trunk. Leaves were covered in mylar bags for a least 2 hours before excision and measurement using a Scholander pressure chamber (Model 610; PMS Instrument company, Albany OR, USA). Readings from the 3 leaves were averaged per tree and used in statistical analysis.

2.2.3 Soil moisture

Soil water was measured using dielectric sensors (GS3; Decagon Devices, Inc., Pullman, WA) one meter away from the tree trunk at depths of 20 cm and 80 cm. Sensor installations were replicated four times ($n = 4$) throughout the experimental plot. The manufacturer calibration equation for mineral soils was utilized to convert dielectric permittivity to volumetric water content (θ).

2.2.4 Sap flow index

Sap flow index (SFI) was measured using three needle sensors (East 30 Sensors; Pullman, WA). Needles were made of stainless steel and were 1.2 mm in diameter, 35 mm long and spaced 6 mm apart. The outermost needles contained three precision thermistors each located at 5 mm, 17.5 mm and 30 mm from the needle base. For this study the thermistors located at 30 mm were not used as they were judged to be into the non-conducting heartwood and the extra datalogger channels were needed to maximize sampling capacity. The innermost needle housed a 45 Ω nichrome wire heater which was excited with 12 V for 8 sec every half hour. Individual trees within the blocks were selected to accommodate sensor cable lengths with one tree instrumented per block. When installing sap flow sensors, a drill guide was used to ensure accurate spacing and prevent probe misalignment. Sensors were placed approximately 0.5 m from the soil surface, above the graft junction and below the lowest branches; care was taken to avoid knots and deformities (Fig. 2.2.1). Heat velocity was determined using the dual method approach (DMA) to resolve low and high rates of flow as suggested by Forster (2020). Briefly, the DMA utilizes the Péclet equation to transition between the heat ratio method (Bugress et al. 2001) and temperature maximum method (Cohen et al. 1981) based on whether conduction or convection is the dominant process of heat transfer. Thermal diffusivity was assumed to be 0.0023 cm^2/s based on previously reported values for apple (Forster, 2020). Measurements were taken every 30 min and averaged hourly and daily. A polynomial wounding correction was applied to measurements based on a 1.7 mm drill diameter (Burgess et al. 2001). Conversion of heat velocity to sap velocity was not attempted for reasons outlined in the discussion and average daily wound corrected heat pulse velocity measurements were treated as an index of sap flow and used in all statistical analyses.

2.2.5 Trunk circumferential variation

Tree trunk circumferential variation was measured using band dendrometers (D6; UMS, Munich, Germany) which work like a beam in bending. Maximum daily shrinkage was calculated by the difference in a 24-hour period between the maximum and minimum trunk circumference. Maximum daily trunk circumference was determined from the once a day from the maximum circumference measurement that occurred before solar noon. Daily circumferential growth (DCG) was calculated from the change in the maximum daily circumference from one day to the next. Circumferential growth patterns were analyzed by normalizing ending dendrometer voltages to final average scion circumferences. Dendrometers were installed approximately 0.5 m above the soil surface just above sap flow sensors on the North side of the tree (Fig. 2.2.1). A cable made of Invar steel, which has an expansion coefficient close to zero, was used to secure the dendrometer around the stem (Katerji et al. 1994). Teflon mesh was placed between the sensor apparatus and tree trunk which allowed the dendrometer to smoothly expand and contact diurnally while slowly expanding to accommodate trunk growth over the course of the season. Both the band dendrometer and sap flow sensors were shielded using a ridged metal frame which was insulated to minimize thermal loading.

2.2.6 Harvest and growth measurements

Fruit harvest was conducted on DOY 281, total fruit weight and number were determined per tree. At the end of the season final trunk circumferential measurements were taken 30 cm above the soil surface for all trees. Trunk cross sectional area (TCSA) was computed from these measurements. Stem elongation measurements were taken from three shoots per tree, measuring from the last years growth to the tip of the new shoot.

2.2.7 Environmental measurements

Environmental data were collected by a weather station maintained by the Utah Climate Center located approximately 0.25 km to the south west of the block. Sensors included in the weather station were a propeller blade and vane wind sensor (Model 05103, R.M. Young, Traverse City MI, USA), temperature/humidity probe (EE08, E+E Electronik, Engerwitzdorf, Austria), solar pyranometer (SP-230, Apogee Instruments, Logan UT, USA), and Tipping bucket rain gage (TE525, Texas Electronics, Dallas TX, USA). Alfalfa reference evapotranspiration was estimated using the ASCE standardized reference ET_r equation (Allen et al. 2005).

2.2.8 Statistics

Because of sensor cost four replicate blocks per treatment were instrumented with sap flow sensors (n =4) and three blocks per treatment were instrumented with band dendrometers (n = 3). With the exception of one replicate block where only sap flow sensors were used, the same trees were instrumented with both sap flow and band dendrometers and Ψ_{stem} were collected from the instrumented trees. When analyzing correlations to Ψ_{stem}, data were adjusted to reflect sample sizes of sap flow sensors and band dendrometers. Harvest, final trunk circumferential, and stem elongation data was analyzed from all blocks in the plot. Data were separated into “early” and “late” seasonal responses based on understanding of phenological stages and analysis of circumferential growth (Liu et al., 2012). Late season responses were judged to begin at day of the year 185 based on plateauing of AF circumferential growth which corresponded roughly with date of the end of leaf expansion and beginning of fruit development. Seasonal SFI and MDS were treated as response variables and environmental data as explanatory variables for correlation analysis. Stem water potential was treated as response variable for analysis of environmental drivers and an explanatory variable for correlations to MDS and SFI. Sap flow index, MDS and Ψ_{stem} were examined for correlations to environmental variables using linear regression. Sap flow index and MDS were also examined for correlations to Ψ_{stem} using linear regression. Differences between grafted scions over the course of the season in SFI, MDS and Ψ_{stem} were determined utilizing a linear mixed effects regression. Statistical analysis was conducted using R statistical software (R Foundation for Statistical Computing, Vienna, Austria) while correlations were determined in SigmaPlot 11.0 (Systat Software, Inc., San Jose, CA, USA).

2.3 Results

2.3.1 Environmental conditions

Daily averages for the most significant environmental variables (T_a, VPD, ET_r), and soil moisture readings at 20 cm and 80 cm below the soil surface are shown in Figure 2.4.1. Average daily air temperature was 21.9 °C and ranged from 7.1 to 31 °C. Vapor pressure deficit averaged 1.85 kPa daily over the season and ranged from 0.28 to 3.58 kPa. Average daily ET_r was 5.47 mm d^{-1} over the season and ranged between 2.03 and 8.38 mm d^{-1}. Rain fall occurred primarily in the beginning of the season and rainfall values totaled 97 mm over the course of the trial. There were 8 irrigation events over the course of the trial, accounting for approximately 185 mm of applied water. Soil VWC 20 cm below the surface of the soil averaged 0.21 m^3 m^{-3} and ranged from 0.09 to 0.34 m^3 m^{-3} while soil VWC 80 cm below the soil surface averaged 0.22 m^3 m^{-3} and ranged from 0.13 to 0.29 m^3 m^{-3}. Daily wind speed averaged 1.8 m s^{-1} over the course of the trial and solar radiation averaged 24.9 MJ m^{-2} d^{-1} ranging from 9.4 to 31.7 MJ m^{-2} d^{-1} (data not shown). There was a severe weather event that occurred on DOY 252 during which gusts of wind around 40 m s^{-1} were recorded, equivalent to wind speeds of that of a category 2 hurricane. As a result of this severe weather event, several trees (8) were blown over and a significant portion (>50%) of fruit was blown from the trees.

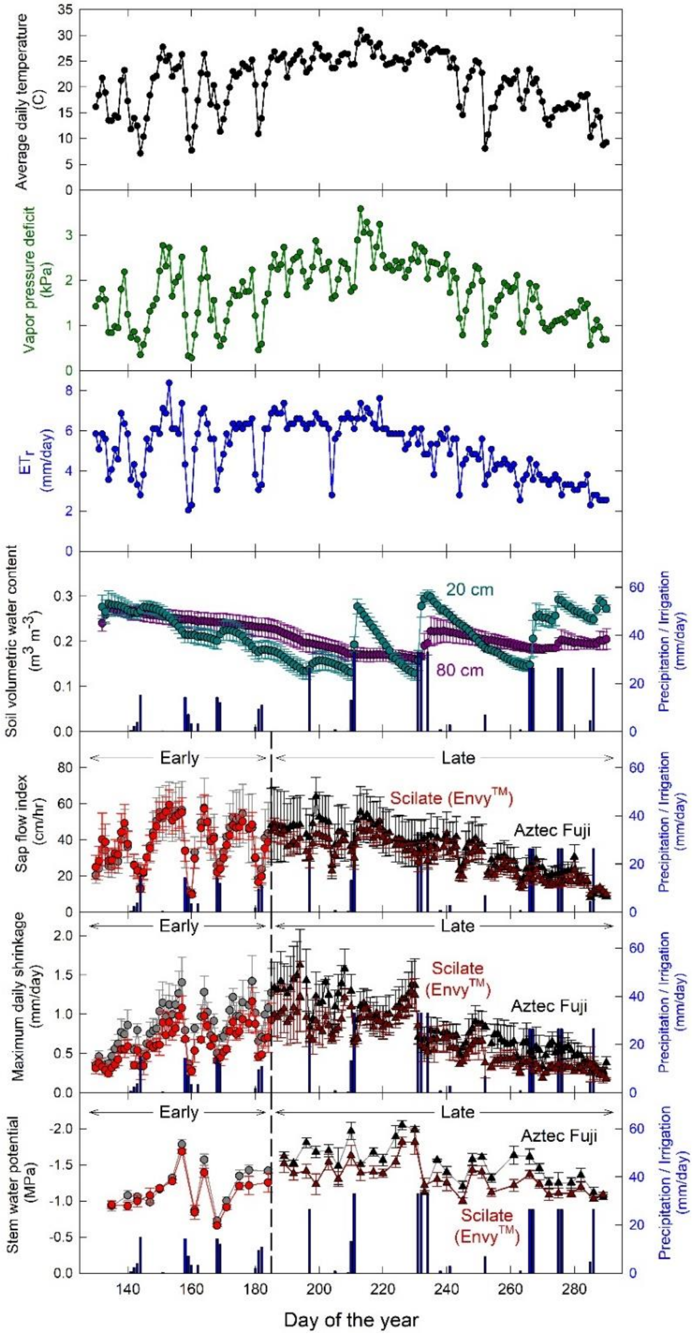

Fig. 2.3.1 Timeseries of four environmental parameters and three physiological responses in high density apple. Precipitation and irrigation are shown by bars with units on the left-hand axis. The vertical broken line indicates division into early and late seasonal responses of physiological responses.

2.3.2 Trunk circumferential variation

There was a significant difference ($P = 0.01$) in final trunk circumference between scions, with AF averaging 22.3 ± 0.7 cm and SE averaging 21.0 ± 0.8 cm (Table 2.3.2). Location within the orchard significantly impacted ($P < 0.01$) final trunk circumference. Seasonal circumferential growth, as measured by band dendrometers, was greater in SE ($P < 0.01$) averaging 2.0 ± 0.4 cm while AF averaged 0.9 ± 0.3 cm over the course of the growing season (Fig. 2.3.2). Differences in the pattern of circumferential growth could also be noted with AF rapidly putting on growth early in the season and then plateauing later season, while SE steadily put on growth until late in the season. Daily circumferential growth rate was significantly different ($P = 0.02$) between the two cultivars, with SE cultivars averaging higher daily growth rates over the course of the trial. Analysis of daily growth rate did not find significant correlations atmospheric with drivers R_n (AF, $r^2 = 0.05$; SE, $r^2 = 0.23$), T_a (AF, $r^2 = 0.02$; SE, $r^2 = 0.13$), VPD (AF, $r^2 = 0.03$; SE, $r^2 = 0.08$) or ET_r (AF, $r^2 = 0.02$; SE, $r^2 = 0.30$). Daily growth was also poorly correlated to soil moisture at the 20 cm level (AF, $r^2 = 0.01$; SE, $r^2 < 0.01$) and 80 cm level AF, $r^2 = 0.28$; SE, $r^2 = 0.12$) and no significant correlations were observed to stem water potential (AF, $r^2 = 0.10$; SE, $r^2 = 0.01$) (data not shown).

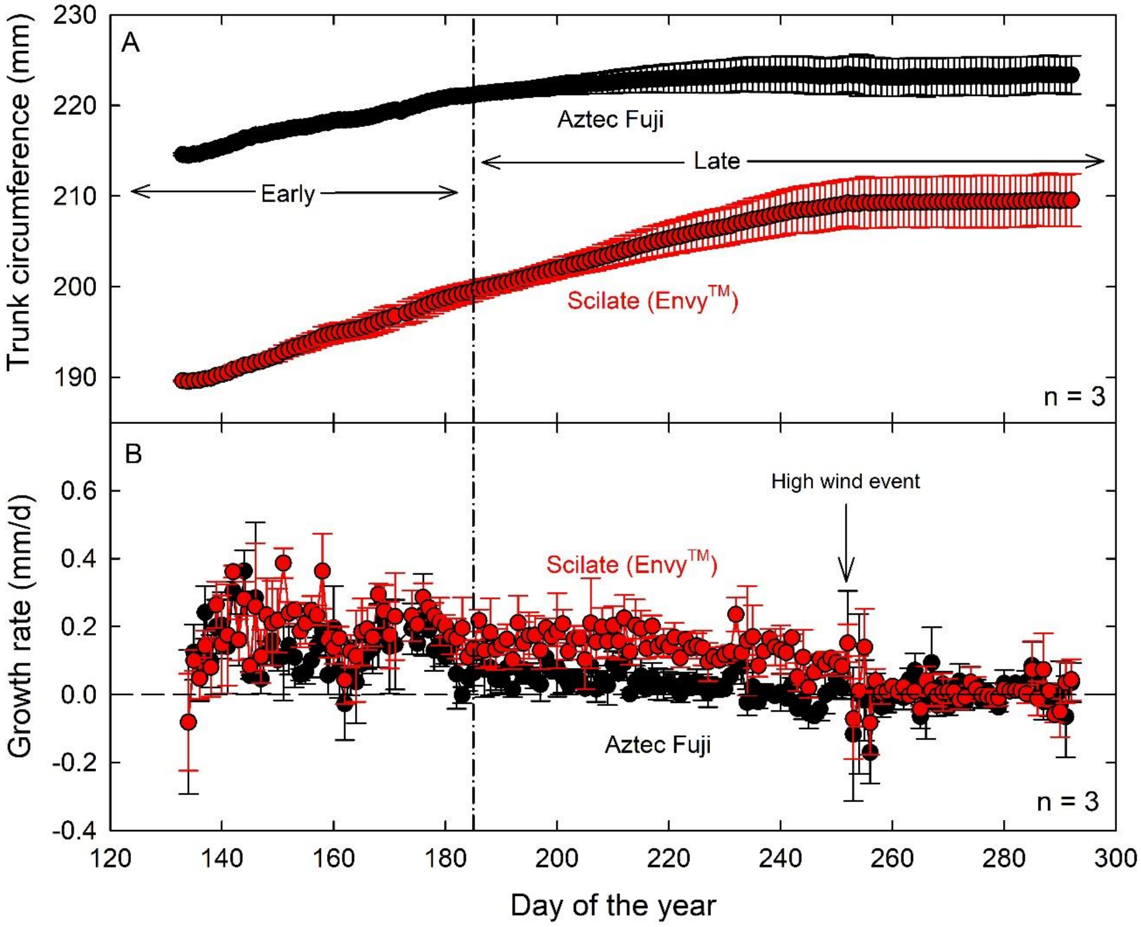

Fig. 2.3.2 Maximum daily circumference and daily growth of five year old 'Aztec Fuji' and 'Scilate (Envy™)' scions on Malling 9 rootstocks over the course of the growing season.

Measurements of MDS were not significantly different (P = 0.30) between scions over the course of the growing season, however data were still segregated by scion for analysis. Linear functions were used to fit all environmental variables while controlling for phenological stage (before and after DOY 185) (Fig. 2.3.3). Early in the season both AF and SE were most highly correlated to T_a (AF, $r^2 = 0.61$; SE $r^2 = 0.64$), followed by

VPD (AF, $r^2 = 0.53$; SE $r^2 = 0.56$), and ET_r (AF, $r^2 = 0.46$; SE $r^2 = 0.45$) (Table 2.4.1). Later in the season, scion correlation patterns to environmental drivers differed, with AF being most correlated to R_n ($r^2 = 0.71$), followed by ET_r ($r^2 = 0.69$) and then T_a ($r^2 = 0.45$) and VPD ($r^2 = 0.45$). In the SE scions the two most significant correlations were flipped compared to AF with ET_r ($r^2 = 0.75$) being the most highly correlated followed by R_n ($r^2 = 0.68$), and T_a ($r^2 = 0.45$) and VPD ($r^2 = 0.45$) being equal. Volumetric soil moisture was less correlated than atmospheric variables. Early in the season VWC at 80 cm depth was more correlated to MDS (AF $r^2 = 0.30$; SE $r^2 = 0.22$) than VWC at 20 cm (AF $r^2 = 0.17$; SE $r^2 = 0.19$). This pattern flipped later in the season with VWC at 20 cm being better correlated to MDS (AF $r^2 = 0.39$; SE $r^2 = 0.30$) than VWC at 80 cm (AF $r^2 = 0.02$; SE $r^2 = 0.08$). Prediction of Ψ_{stem} from MDS in both scions was best early in the season (AF $r^2 = 0.85$; SE $r^2 = 0.78$) though late season correlations remained high (AF, $r^2 = 0.67$; SE $r^2 = 0.70$) (Fig 2.4.5). When pooling data by scion and season, multiple linear regression utilizing R_n, T_a, VPD, and VWC at 20 and 80 cm accounted for 76% of variation in MDS.

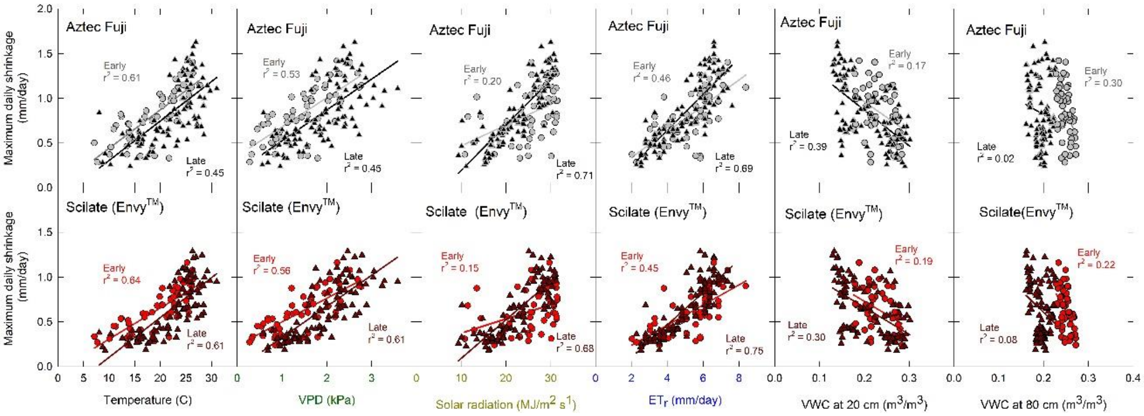

Fig. 2.3.3 Early and late season correlations of maximum daily trunk circumferential shrinkage in 'Aztec Fuji' and 'Scilate (Envy™)' scions grafted to Malling 9 rootstocks to the most significant environmental variables.

2.3.3 Stem water potential

There was no significant difference in Ψ_{stem} (P = 0.93) between the AF and SE scions with AF averaging -1.39 ± 0.07 MPa and SE averaging -1.23 ± 0.08 MPa over the course of the growing season (Fig. 2.3.4), but the responses of each scion to environmental drivers are shown for clarity. Responses to all environmental variables were examined using linear regressions (Fig. 2.3.4). For both AF and SE more negative stem water potentials were observed later in the season which was in line with an observed decline in soil water moisture (Fig. 2.3.1). Early season responses of Ψ_{stem} in both AF and SE showed strong correlations to VPD (AF, $r^2 = 0.76$; SE, $r^2 = 0.77$), T_a (AF, $r^2 = 0.70$; SE, $r^2 = 0.67$) and ET_r (AF, $r^2 = 0.70$; SE, $r^2 = 0.64$) (table 2.3.1). During the late season Ψ_{stem} responses showed medium correlations to almost all measured environmental variables, apart from wind speed. For AF scions the most significant correlations in declining order were VWC at 20 cm ($r^2 = 0.59$), ET_r ($r^2 = 0.46$), T_a ($r^2 = 0.42$), R_n ($r^2 = 0.39$), VPD ($r^2 = 0.31$), and VWC at 80 cm ($r^2 = 0.25$) (Table 2.3.1). In SE scions most significant correlations were: T_a ($r^2 = 0.46$), ET_r ($r^2 = 0.45$), VWC at 20 cm ($r^2 = 0.42$), VPD ($r^2 = 0.40$), R_n ($r^2 = 0.37$), and VWC at 80 cm ($r^2 = 0.25$). Because there was no significant difference between scion types for any of our physiological measurements, data were pooled and correlations of MDS and SFI to Ψ_{stem} were analyzed. Stem water potential was more correlated to MDS measurements than SFI across both scion types (Fig.2.3.6). Controlling for seasonality improved regression fits with early season coefficients of correlation higher for MDS measurements ($r^2 = 0.85$) than SFI ($r^2 = 0.69$). Late season correlations declined in both, though MDS remained well correlated ($r^2 = 0.71$), while SFI correlations declined significantly ($r^2 = 0.36$). When pooling data by scion and season, multiple linear regression utilizing R_n, T_a, VPD, and VWC at 20 and 80 cm accounted for 63% of the variation of SWP.

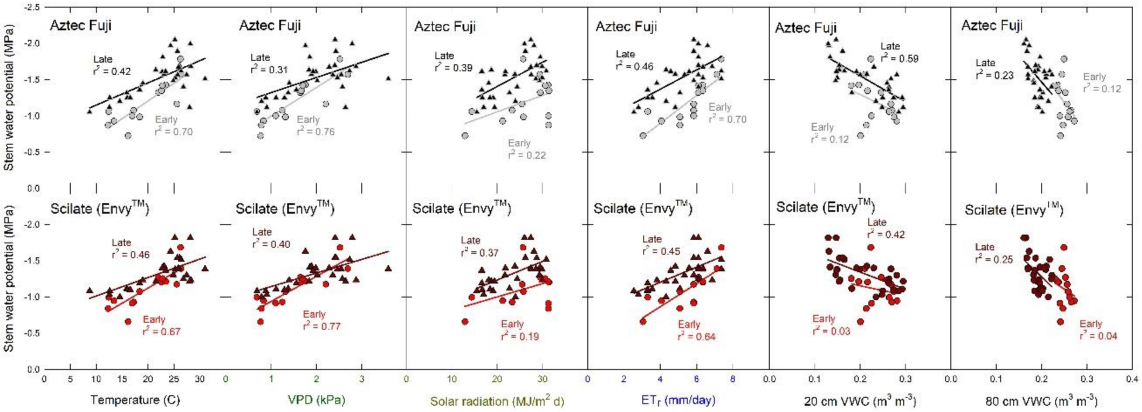

Fig. 2.3.4 Early and late season correlations of stem water potential in ‘Aztec Fuji’ and ‘Scilate (Envy™)’ scions grafted to Malling 9 rootstocks to the most significant environmental variables.

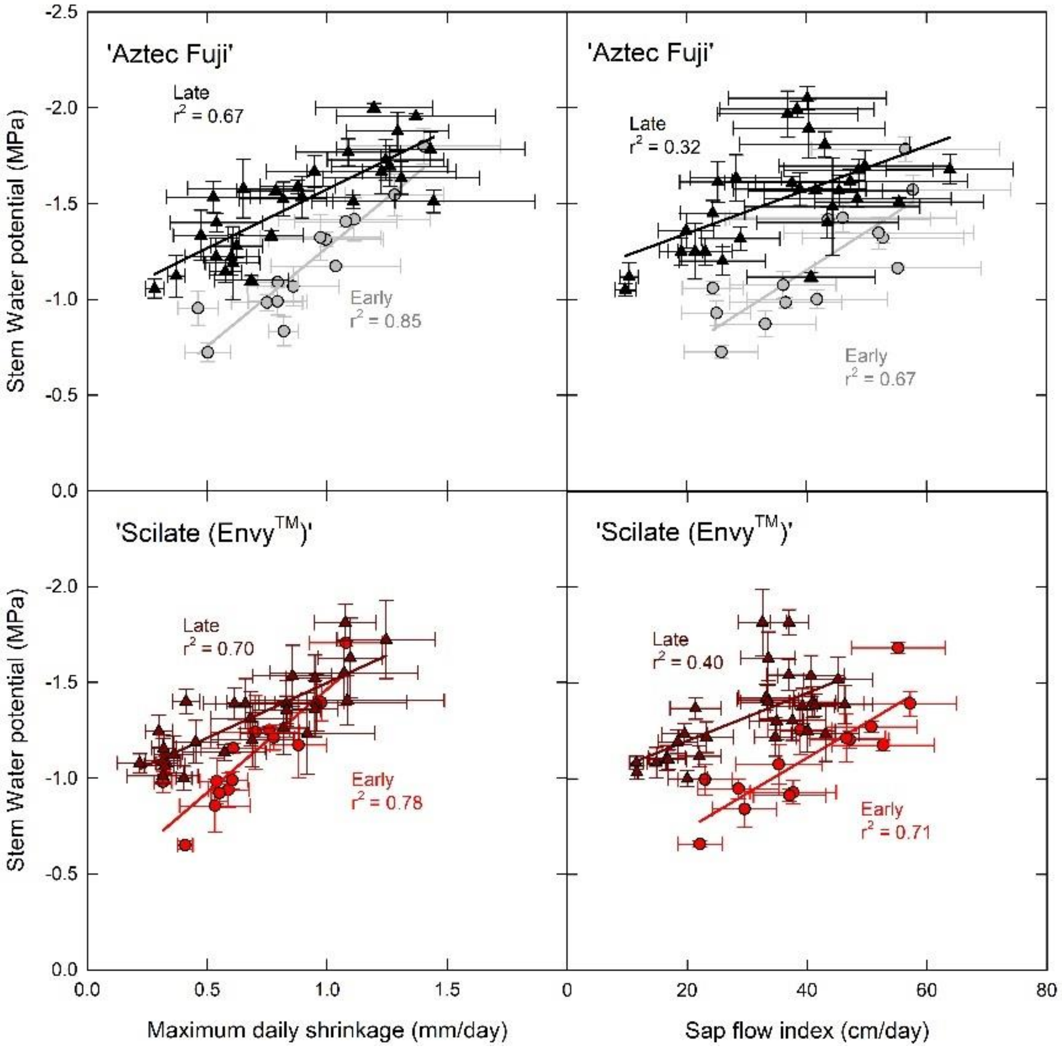

Fig. 2.3.5 Correlations of maximum daily trunk shrinkage and sap flow index to stem water potential in 'Aztec Fuji' and 'Scilate (Envy™)' on Malling 9 dwarfing rootstocks.

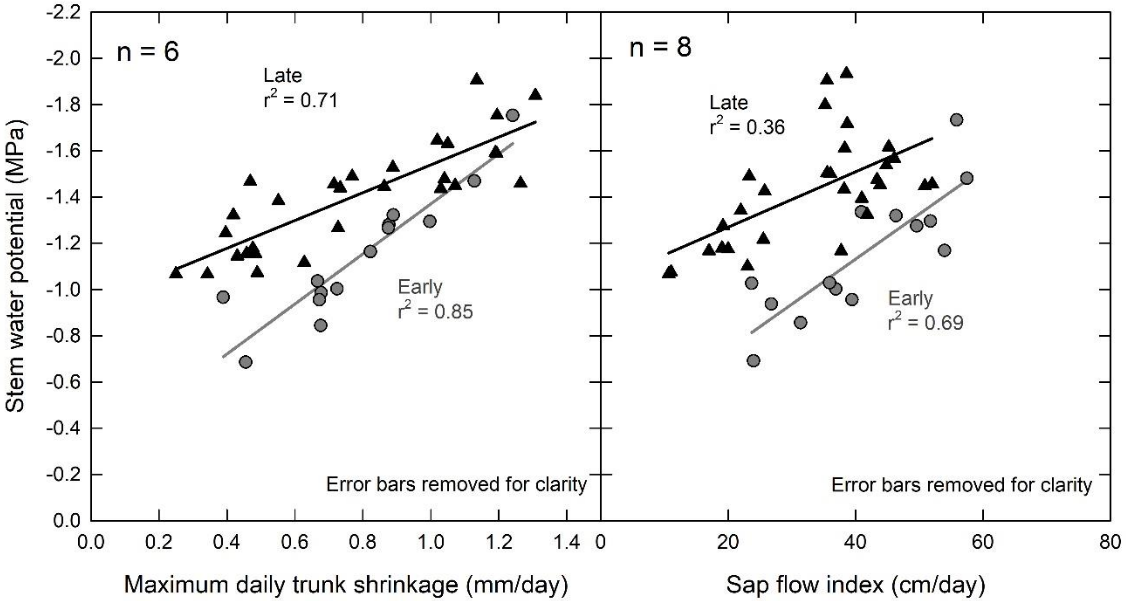

Fig. 2.3.6 Correlations of maximum daily trunk shrinkage and sap flow index to stem water potential measurements for pooled averages consisting of ‘Aztec Fuji’ and ‘Scilate (Envy™)’ on Malling 9 dwarfing rootstocks. Error bars removed for clarity.

2.3.4 Sap flow index

Sap flow index was not significantly different (P = 0.97) between the two grafted scions over the course of the season (Fig. 2.3.5). Early in the season both scions were highly correlated to T_a (AF, $r^2 = 0.84$; SE, $r^2 = 0.88$), VPD (AF, $r^2 = 0.77$; SE, $r^2 = 0.87$), and ET_r (AF, $r^2 = 0.76$; SE, $r^2 = 0.83$). Later in the season this trend remained the same with correlations to ET_r improving (AF, $r^2 = 0.88$; SE, $r^2 = 0.89$) followed by T_a (AF, $r^2 = 0.79$; SE, $r^2 = 0.80$), VPD (AF, $r^2 = 0.78$; SE, $r^2 = 0.82$), and R_n (AF, $r^2 = 0.74$; SE, $r^2 = 0.73$). There were no significant correlations to wind speed or VWC at either the 20 or 80 depth during either the early or late season. Sap flow index was a stronger predictor of Ψ_{Stem} early in the season for both AF ($r^2=0.67$) and SE ($r^2=0.71$) with correlations declining later in the season (AF, $r^2=0.32$; SE, $r^2=0.40$). When pooling data by scion and season, multiple linear regression utilizing R_n, T_a, VPD, and VWC at 20 and 80 cm accounted for 87% of variation in SFI readings.

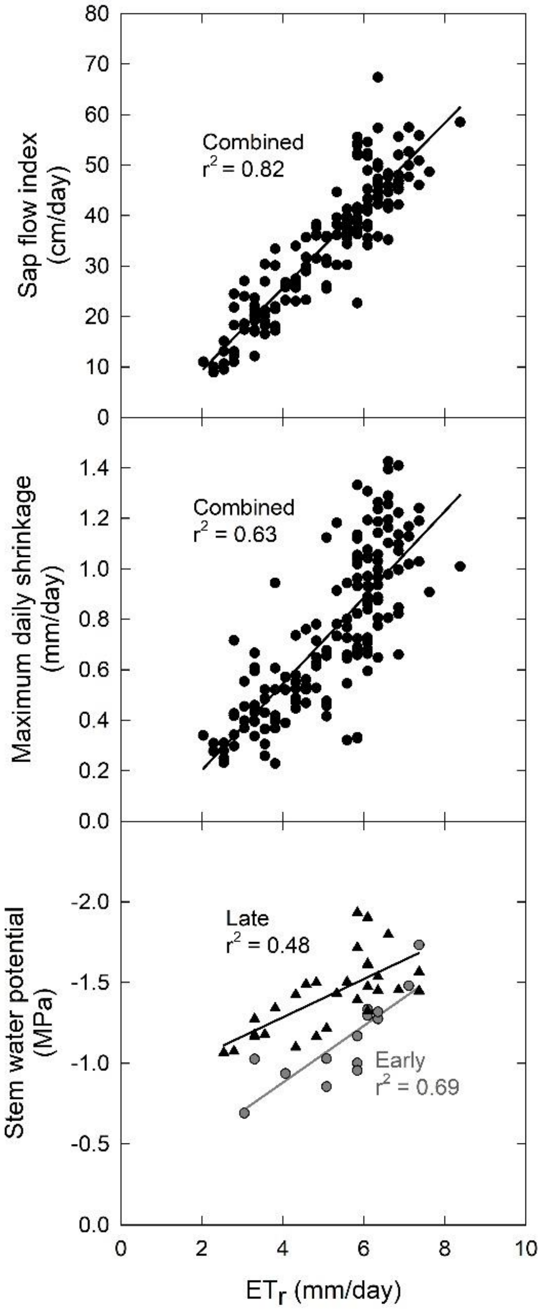

Fig. 2.3.7 Correlations of sap flow index, maximum daily shrinkage, and stem water potential to modeled evapotranspiration. Data are combined across rootstocks and over the growing season.

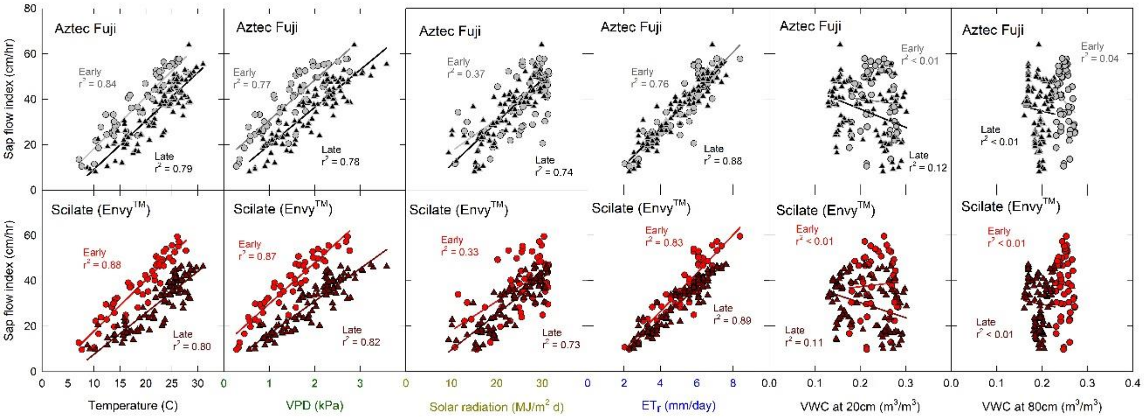

Fig. 2.3.8 Effect of environmental variables on early and late season sap flow index in ‘Aztec Fuji’ and ‘Scilate’ (Envy™) scions grafted to Malling 9 rootstocks.

Table 2.3.1
Coefficients of determination (r^2) between environmental inputs and physiological responses.

			Solar radiation	Temperature	VPD	ET$_r$	VWC at 20 cm	VWC at 80 cm
MDS	Early	Aztec Fuji	0.20	0.61	0.53	0.46	0.17	0.30
		Scilate (Envy™)	0.15	0.64	0.56	0.45	0.19	0.22
	Late	Aztec Fuji	0.71	0.45	0.45	0.69	0.39	0.02
		Scilate (Envy™)	0.68	0.61	0.61	0.75	0.30	0.08
SFI	Early	Aztec Fuji	0.37	0.84	0.77	0.76	< 0.01	0.04
		Scilate (Envy™)	0.33	0.88	0.87	0.83	< 0.01	< 0.01
	Late	Aztec Fuji	0.74	0.79	0.78	0.88	0.12	< 0.01
		Scilate (Envy™)	0.73	0.80	0.82	0.89	0.11	< 0.01
Ψ_{Stem}	Early	Aztec Fuji	0.22	0.70	0.76	0.70	0.12	0.12
		Scilate (Envy™)	0.19	0.67	0.77	0.64	0.03	0.04
	Late	Aztec Fuji	0.39	0.42	0.31	0.46	0.59	0.23
		Scilate (Envy™)	0.37	0.46	0.40	0.45	0.42	0.25

2.3.5 Measurement variability

Stem water potential had a combined seasonal coefficient of variation (CV; standard deviation divided by the mean) of 12.4% with average CV values being slightly lower for ‘Aztec Fuji’ (9.5%) than SE (11.2%). Maximum daily shrinkage had a combined CV of 31.9 % with ‘Aztec Fuji’ being slightly more variable (29.3%) than SE (25.8%). Sap flow indexes were the most variable, having an overall CV of 37.1% with AF being significantly (47.0%) more variable than SE (25.7 %).

Table 2.3.2
Coefficients of variation (%) of midday stem water potential (Ψs_{tem}), Maximum Daily Shrinkage (MDS) and a Sap Flow Index (SFI) measured on fruiting scions ‘Aztec Fuji’ and ‘Scilate’ both grafted to Malling 9 Selection NIC^{TM} 29 rootstocks. Values are averages of seasonal measures. These values indicate variability among replicate trees. Stem water potential had about half the variability of MDS and SFI.

Scion	Ψ_{stem}	MDS	SFI
Aztec Fuji	9.5 %	29.3 %	47.0 %
Scilate	11.2 %	25.8 %	25.7 %
Combined	12.4 %	31.9 %	37.1 %

2.3.6 Harvest data and growth

‘Aztec Fuji’ scions had significantly higher ($P > 0.01$) fruit harvest mass per tree averaging 22.2 ± 4.0 kg per tree while the SE averaged 11.7 ± 1.8 kg per tree (Table 2.4.2). Individual fruit mass was higher ($P = 0.02$) in SE averaging 227.6 ± 9.2 g per fruit compared to AF which average 192.9 ± 10.3 g per fruit. ‘Aztec Fuji’ had more ($P > 0.01$) fruit per tree, averaging 119 ± 15 compared to SE which averaged 52 ± 4 fruit per tree. Normalizing for trunk cross sectional area (TCSA) AF maintained higher numbers of fruit averaging 3.1 ± 0.6 fruits per cm^2 of TCSA while SE averaged 1.5 ± 0.2 fruits per cm^2 of TCSA. Stem elongation was nearly longer ($P = 0.07$) in AF averaging 33.7 ± 0.6 cm while SE averaged 31.0 ± 1.4 cm.

Table 2.3.3
Fruit harvest parameters and final trunk and stem measurements for ‘Aztec Fuji’ and ‘Scilate’ (Envy™) scions grafted to Malling 9 rootstocks

	Harvest Weight	Crop load	Crop load / TCSA	Fruit Size	Ending circumference	Trunk cross sectional area (TCSA)	Shoot elongation
Scion	kg fruit / tree	# / tree	# / cm^2 TCSA	g / fruit	cm	cm^2	cm
Fuji	22.2	119	3.1	192.9	22.3	40.1	33.7
Scilate	11.7	52	1.5	227.8	21.0	35.3	31
P value	< 0.01	< 0.01	< 0.01	< 0.01	0.03	0.03	0.07
Blocking effect P value	0.58	0.07	< 0.01	0.44	< 0.01	< 0.01	0.64

2.4 Discussion

2.4.1 Trunk circumferential variation and growth

Phenological stages of growth in orchard trees have been documented to affect responses of MDS to environmental drivers and Ψ_{stem} (Egaea et al. 2009; Mársal et al. 2002). Liu et al. (2012) delineated two seasonal stages of growth in apple (cv Golden Delicious) based on trunk diameter growth and leaf area index. In analyzing their data, they characterized the first stage of growth by rapid leaf area and trunk expansion during which time trees emerged from dormancy and anthesis occurred. The second stage was characterized by a plateauing of trunk growth and leaf area index coupled with rapid expansion and maturing of fruit. In analyzing maximum daily circumference in this study we observed a plateau of trunk growth starting around day 185 in the AF scion, however it was less pronounced than results seen by Liu et al. (2012) (Fig. 2.3.2). We observed only minor slowing of growth in the SE cultivar with circumference measurements continuing to increase until late in the growing season. While fruit size was larger in the SE scions overall crop load (fruit # / cm^2 TCSA) was lower (Table 2.3.3). Stem elongation was not statistically different at α of 0.05, which given smaller TCSA and lower crop load in the SE, suggests greater carbon allocation to vegetative growth. Continued circumferential growth later in the season in SE scions was likely due to lighter crop load.

When controlling for phenological stage of growth correlations of MDS to Ψ_{stem} were strongest early in the season and declined after DOY 185 when fruit development dominated tree responses. This was in line with previous studies using point dendrometers which documented declining correlation of MDS to Ψ_{stem} in plum and peach as the season progressed (Intrigliolo and Castel 2006; Marsal et al. 2002). In this study reduced correlation of MDS to Ψ_{stem} late in the season are hypothesized to be due to a combination of fruit load, and extended drought. The onset of fruit development increases osmotic loading of the phloem which impacts water storage dynamics of the tree (Ortuño et al. 2010). In well-watered trees increased sugar transportation for fruit production results in larger water potential gradients between the xylem and phloem resulting in greater swelling of cambium tissues during nighttime recharge of water (Wang et al. 1995). Because of this, crop load has been reported to increase MDS readings in relation to Ψ_{stem} under well-watered conditions (Intrigliolo and Castel, 2007). However, in this study the opposite trend was noted with MDS values decreasing for the same Ψ_{stem}. Additionally, AF had higher yield (kg/tree) and crop load at harvest but MDS values were not significantly higher than those of SE. Soil moisture content and Ψ_{stem} were on average lower throughout the plot after DOY 185 indicating greater water stress during fruiting. Limited soil water availability could have inhibited nighttime recharge of stem water and diurnal trunk expansion. In trees, stored water can account for up to 50% of transpirational demand (Köcher et al 2013). More negative osmotic potentials in the cambium tissues could have acted as a competitive sink for stored water, limiting trunk contraction due to transpirational loss. The combination of greater osmotic loading and limited soil water availability could in this way depress MDS responses.

In examining the relationship of MDS to Ψ_{stem} previous studies have reported that MDS values increase until a species-specific threshold Ψ_{stem} after which MDS values decline (Ortuño et al. 2010). This parabolic response of MDS to Ψ_{stem} has been attributed to depletion of water reservoirs in the phloem and surrounding xylem tissues as well as stomatal and osmotic regulation (Remorini and Massai, 2003; Garnier and Berger 1986). In this study this pattern was not observed with MDS values increasing linearly with more negative Ψ_{stem}. Doltra (2003) reported Ψ_{stem} values after which MDS began to decline in potted apple trees as -2.5 MPa. Minimum Ψ_{stem} values in this study reached ~ -2.0 MPa at which time leaf curling, tip burn and low soil VWC were observed. This was judged to be significant waster stress, beyond what would be tolerated in commercial production and may be an indication that these scion/rootstock combinations may not follow this same pattern of MDS to Ψ_{stem} response.

In rapidly growing young trees, researchers have previously identified daily growth as a better indicator of drought stress than MDS (Nortes et al. 2005). In this study we did not observe significant correlations of daily growth to environmental parameters or Ψ_{stem} (data not shown). Trees were at the 6th leaf at the time of the study which is considered to be mature and past the stage of initial rapid growth in which daily growth is the most sensitive parameter for water stress.

2.4.2 Sap flow index

Previous studies have noted sap flow correlations to environmental drivers vary with phenological stage (Chen et al. 2014; Tie et al., 2017) Daily sap flow rates presented by Liu et al. (2012) for early and late season responses showed correlations of sap flow to T_a, VPD, and ET_r improving later in the season. Data presented here for R_n and ET_r follow the same pattern of improved correlations early in the season, but correlations to T_a and VPD showed no significant improvement (Fig. 2.3.7). Findings by Liu et al. (2012) may be due to the inclusion of data in early spring before trees had fully leafed out, producing many daily readings with low sap flow and driving down regression fits. Tie et al. (2017) controlled for this problem by normalizing to leaf area index which significantly improved coefficients of determination throughout the season. In the study presented here, data collection was initiated after trees had leafed out, which improved early season correlations and more closely matched data from Tie et al. (2017). Previous studies have identified VPD, R_a, T_a, soil moisture, and leaf area index as predominant drivers of sap flow in a range of trees and environmental conditions (Arneth et al., 1996;

Dragoni et al. 2005; Ford et al., 2004; Mobe et al., 2020;). In this study T_a, VPD, and ET_r were most strongly correlated to SFI while R_a was only well correlated later in the season and θ was not significantly correlated to SFI at any time. Shifts in seasonal SFI response were most pronounced in correlations to Ta, VPD and Ψ_{stem} with SFI readings decreasing later in the season. Lowered SFI readings past DOY 185 were most likely due to reduced soil moisture availability. In addition, later in the season older leaves make up a larger portion of the tree canopy and are less responsive to environmental drivers due to greater internal shading and reduced stomatal conductance compared to recently matured leaves (Flore and Lakso 1989; Constable and Rawson 1980). This could have reduced transpiration rates when normalized on a leaf area basis and played a role in depressing SFI response to environmental drivers.

This contrasted with correlations to ET_r which were high during both the early and late season and showed significant overlap. High correlations of SFI to ET_r was unexpected given the published literature which points to divergence in orchard ET from reference ET (Dragoni et al 2005; Jarvis 1985). Reference ET models utilize idealized values for canopy characteristics that reflect a continuous, short, dense, and homogenous crop with a relatively large boundary layer. Because of these assumed canopy characteristics and boundary layer effects reference ET models have been found to be more strongly correlated to incoming solar radiation than bulk atmospheric conditions (Jarvis, 1985). The tall, dispersed nature of tree canopies as well as the large amount of self-shading that occurs results in bulk atmospheric conditions, best quantified by VPD, having a greater impact on orchard ET values (Dragoni et al. 2005). We hypothesize that in high density orchard plantings with interconnected canopies, aerodynamic resistance values are such that boundary layer effects decouple orchard ET to a greater extent from bulk air properties, especially later in the season as leaf area indexes reach a maximum. Additionally, highly managed fruit tree orchards utilize pruning and training techniques that maximize canopy radiation capture for maximum fruit production.

Previous research has shown linear correlations of sap flow readings to Ψ_{stem} under non-limiting soil water conditions (Ortuño et al. 2006). Both early and late seasonal responses followed this same pattern of increasing sap flow with decreasing Ψ_{stem}. Correlations of SFI to Ψ_{stem} were stronger in the early season while late season correlations decreased especially in the AF scions. Declining correlations later in the season could be a function of stomatal regulation however fruiting deciduous orchards have been shown to have higher stomatal conductance, transpiration and carbon assimilation than de-fruited trees of the same age (Naor et al., 2008). Similar to depressed correlations of MDS, SFI correlations to Ψ_{stem} declined due to limited soil water availability combined with solute loading, leaf age and crop load.

Several studies have reported reductions of peak sap velocities. Researchers have also noted an inward radial shift of peak velocities toward the heartwood (Cermak and Nadezhdina, 1998; Ford et al., 2004). Based on these findings Nadezhdina et al. (2007) suggested that analysis of the shape of the sap wood profile might be a reliable indicator for irrigation scheduling. However, studies in olive, apple and Asian pear did not show significant variation in sap velocity profiles under a range of soil water availability and atmospheric demand (Fernández et al. 2008a). Our analysis looking at the ratio of outer to inner SFI found a moderate correlation to Ψ_{stem} when pooling scions ($r^2 = 0.50$) (Appendix A). It may be possible that the proposed technique may not be applicable for diffuse porous species such as apple but could work for ring porous species which due to their bimodal radial distribution of vessel diameters produce steep gradients in sap velocity between the inner and outer sapwood (Bush et al. 2010; Tyree and Zimmermann 2002).

2.4.3 Stem water potential

Previous studies have documented linear correlations of Ψ_{stem} to T_a and modeled ET over the course of the entire season (Fereres and Goldhamer, 2003). Data presented in this study shows a similar response when not corrected for phenological stage. A saturating response of season long Ψ_{stem} to VPD has previously been reported in apple (De Swaef et al., 2009) where a linear relationship has been described in olive (Moriana et al., 2012), prune (Fereres and Goldhammer, 2003) and plum (Intrigliolo and Castel, 2006). De Swaef et al. (2009) speculated that the saturating response seen in apples may be due to restricted root volumes. In dwarfing rootstocks, like the ones used in this study, lower rootzone volumes have been associated with reduced drought tolerance (Tworkoski et al., 2016). Late season responses to VPD in this study showed lower slope angles which would suggest a more saturating response. When Ψ_{stem} is pooled for scions and analyzed seasonally a polynomial function produces a greater fit ($r^2 = 0.67$) than a simple linear one ($r^2 = 0.55$; Data not shown). Overall Ψ_{stem} appeared to be less impacted by seasonal variation than measures of SFI and MDS, with division of readings by seasonality improving early season correlations and depressing correlations later in the season.

2.4.4 Relative sap flow index

Accurate calibration of absolute sap flow from heat pulse techniques necessitates the measure of sapwood density, sapwood moisture content, area of conducting tissue, correction of needle misalignment, and thermal diffusivity of the sapwood (Taylor et al. 2013). These technically involved calibrations present significant hurdles to adoption as an irrigation tool for commercial orchards where necessary expertise and equipment may not be available. Even in research settings accurate calibration for absolute sap flow is challenging with a review of published studies finding an average error rate of 34% with most measurements underestimating tree water usage (Forster, 2017).

Determinations of sap flow rely on measured changes in temperature and time elapsed to calculate the velocity of a pulse of heat as it is carried through the trunk (Burgess et al. 2001; Cohen 1981; Swanson and Whitfield 1981). Thermal accounting of conductive and convective properties of the trunk and sap are then employed to derive sap velocity from heat velocity and estimates of conducting tissue area are subsequently used to estimate sap flow. Thermal properties of the measured tree are generally derived either through tree coring before the start of the trial, or through destructive harvesting of the tree after the trial. These properties are then implemented as constants throughout the measurement campaign. In this study we focused solely on measurements of heat velocity without attempting to derive accurate measures of sap flow. By removing the extensive technical calibrations and instead focusing on the underlying pattern of response we remove barriers to use and simplify application. The use of relative measures of sap flow is not without precedent, several researchers have used either indexes (Nadezhdina, 1999), normalized values (Ballester et al. 2013; Burgess and Dawson 2008) or relative measures (Doronila and Forster, 2015) of sap flow in tree water use analysis. We argue that that the underlying pattern of sap flow informs tree responses to water stress and absolute measurements are not necessary to understand these patterns. SFI values presented here follow trends seen in the literature of calibrated sap flow as detailed above.

2.4.5 Measurement variability

High tree-to-tree measurement variability has been cited as a reason for limited adoption of sap flow sensors and dendrometers as irrigation tools in commercial orchards (Ortuño et al. 2010; Fernández 2017). In analyzing coefficients of variation (CV = Standard deviation/mean reading) for this trial we found that MDS and SFI were more variable than Ψ_{stem} (table 2.3.2). Naor and Cohen (2003) noted the same pattern in apple with Ψ_{stem} having the least tree to tree variability, followed by MDS and SFI. The study authors attributed high variability of MDS to variability in vasculature area and tree hydraulic conductance. Variability in SFI was speculated by the authors to be due to tree to tree differences in canopy size and thus rates of transpiration. Plant water status on the other hand is a more holistic measure of plant response that incorporates many different crop characteristics and physiological responses (Naor et al. 2006). The higher degree of variability in MDS and SFI is also partially explained by the high degree of environmental correlation of these measures. When pooling data by scion and season, multiple linear regression utilizing R_n, T_a, VPD, and VWC at 20 and 80 cm accounted for 63% of the variation of SWP, 76% in MDS and 87% of SFI. This analysis was confirmed by comparing correlations of SFI, MDS, and Ψ_{stem} to ET_r, which is a robust model for environmental demand (Fig 2.3.7). Stem water potential continued to show seasonal response segregation to a greater extent than SFI and MDS. This is due to lowered average soil moisture availability later in the season which depressed average stem water potentials. Previous studies have compared the signal intensity (SI) of MDS, sap flow and Ψ_{stem} by contrasting readings from well-watered trees to those of deficit irrigated or drought stressed trees. While utilizing SI reduced the amount of environmental variability, physiological parameters followed the same pattern seen in this study with Ψ_{stem} having the lowest variability, followed by MDS and SFI (Fernández and Cuevas 2010). Greater variability necessitates more instrumentation to reduce sampling errors, this in turn drives up the cost of initial investment of any sensor-controlled irrigation system. These cost however, should be measured against the amount of labor and time needed to collect Ψ_{stem} measurements and the ability of automated measurements to more comprehensively capture seasonal records.

2.5 Conclusions

Controlling for phenological stage of growth in apple improved correlations of MDS and SFI to Ψ_{stem}. Readings from both sensor types were more highly correlated to Ψ_{stem} early in the season and declined as the season progressed, likely due to the effects of crop loading, limited soil water availability and leaf age. Both MDS and SFI were more highly correlated to atmospheric drivers than volumetric soil moisture, while SWP later in the season correlated to atmospheric drivers and VWC. Sap flow index was highly correlated to ET_r and showed little phenological response. This response was unexpected given the divergence of single tree responses and traditional orchard ET from reference ET. In high density orchard plantings overlapping canopies and pruning and training practices increase radiation capture and influence aerodynamic resistance such that ET_r is more highly correlated than individual measures of environmental drivers which is often the case in traditional, low planting density orchards. Orchard density needs to be considered when implementing irrigation scheduling based on either MDS or SFI measurements.

In this study, SFI, consisting of wound corrected heat velocity, showed responses to environmental drivers and water stress similar to previously published studies that used calibrated sap flow. Given the relative difficulty of accurately calibrating heat pulse sensors to derive sap flow, relative values or indexes provide reliable indicators of sap flow responses to environmental conditions and water stress. However, irrigation scheduling based on sap flow faces challenges not only in deriving actionable readings but also from the costs of infrastructure. Wounding effects that occur because of the intrusion of sensor needles necessitate the repositioning of sap flow sensors every one to two seasons. These wounding effects also make it very difficult to remove sensors without causing serious damage. Irrigation systems based on sap flow sensors would in effect require purchasing new sensors on a seasonal basis driving up costs for any irrigation system.

Band dendrometers worked well in this study to capture diurnal shrinkage and seasonal growth rate. It is unclear as to whether band dendrometers provide significant advantages to point dendrometers, which have traditionally been used in irrigation studies. One potential advantage over some styles of point dendrometers that require invasive installations to secure them in place is that band dendrometers can quickly and non-invasively be installed or removed without lasting impact to the tree. Maximum daily shrinkage measurements were well correlated to stem water potential while trunk growth rates were not well correlated in this study. In semiarid regions the use of MDS readings could be used to replace labor intensive Ψ_{stem} measurements, even with changes in readings caused by phenological stages.

2.6 Literature Cited

Adams, S. W. 2016. The effects of rootstock, scion, grafting method and plant growth regulators on flexural strength and hydraulic resistance of apple. Master Thesis, Digital commons USU, Logan, UT, USA. https://digitalcommons.usu.edu/cgi/viewcontent.cgi?article=6102&context=etd

Adams, S., Lordan, J., Fazio, G., Bugbee, B., Francescatto, P., Robinson, T.L., Black, B., 2018. Effect of scion and graft type on transpiration, hydraulic resistance and xylem hormone profile of apples grafted on Geneva ® 41 and M.9-NIC™29 rootstocks. Scientia Horticulturae 227, 213–222, https://doi.org/10.1016/j.scienta.2017.09.052

Allen, R.G., Pereira, L.S., Raes, D., Smith, M., 1998. Crop evapotranspiration-Guidelines for computing crop water requirements-FAO Irrigation and drainage paper 56. FAO, Rome.

Allen, R.G., Walter, I.A., Elliott, R.L., Howell, T.A., Itenfisu, D., Jensen, M.E. and Snyder, R.L. eds., 2005. The ASCE standardized reference evapotranspiration equation. American Society of Civil Engineers. https://doi.org/10.1061%2F9780784408056.ch0

Annandale, J., Stockle, C., 1994. Fluctuation of crop evapotranspiration coefficients with weather: a sensitivity analysis. Irrigation Science 15, 1–7. https://doi.org/10.1007/bf00187789

Arneth, A., Kelliher, F., Bauer, G., Hollinger, D., Byers, J., Hunt, J., McSeveny, T., Ziegler, W., Vygodskaya, N., Milukova, I., 1996. Environmental regulation of xylem sap flow and total conductance of *Larix gmelinii* trees in eastern Siberia. Tree Physiology 16, 247–255. https://doi.org/10.1093/treephys/16.1-2.247

Ballester, C., Castel, J., Testi, L., Intrigliolo, D.S., Castel, J.R., 2013. Can heat-pulse sap flow measurements be used as continuous water stress indicators of citrus trees? Irrigation Science 31, 1053–1063. https://doi.org/10.1007/s00271-012-0386-5

Bonany, J., Camps, F., 1998. Effects of different irrigation levels on apple fruit quality. Presented at the II Workshop on Pome Fruit 466, 47–52. https://doi.org/10.17660/actahortic.1998.466.8

Burgess, S.S., Adams, M.A., Turner, N.C., Beverly, C.R., Ong, C.K., Khan, A.A., Bleby, T.M., 2001. An improved heat pulse method to measure low and reverse rates of sap flow in woody plants. Tree physiology 21, 589–598. https://doi.org/10.1093/treephys/21.9.589

Burgess, S.S.O., Dawson, T.E., 2008. Using branch and basal trunk sap flow measurements to estimate whole-plant water capacitance: a caution. Plant and Soil 305, 5–13. https://doi.org/10.1007/s11104-007-9378-2

Bush, S.E., Hultine, K.R., Sperry, J.S., Ehleringer, J.R., 2010. Calibration of thermal dissipation sap flow probes for ring- and diffuse-porous trees. Tree Physiology 30, 1545–1554. https://doi.org/10.1093/treephys/tpq096

Chen, D., Wang, Y., Liu, S., Wei, X., Wang, X., 2014. Response of relative sap flow to meteorological factors under different soil moisture conditions in rainfed jujube (*Ziziphus jujuba* Mill.) plantations in semiarid Northwest China. Agricultural Water Management 136, 23–33. https://doi.org/10.1016/j.agwat.2014.01.001

Cohen, Y., Fuchs, M., Green, G.C., 1981. Improvement of the heat pulse method for determining sap flow in trees. Plant, Cell & Environment 4, 391–397. https://doi.org/10.1111/j.1365-3040.1981.tb02117.x

Conejero, W., Alarcón, J.J., García-Orellana, Y., Nicolás, E., Torrecillas, A., 2007. Evaluation of sap flow and trunk diameter sensors for irrigation scheduling in early maturing peach trees. Tree physiology 27, 1753–1759. https://doi.org/10.31428/10317/2100

Constable, G., Rawson, H., 1980. Effect of leaf position, expansion and age on photosynthesis, transpiration and water use efficiency of cotton. Functional Plant Biology 7, 89–100. https://doi.org/10.1071/pp9800089

Corell, M., Girón, I.F., Galindo, A., Torrecillas, A., Torres-Sánchez, R., Pérez-Pastor, A., Moreno, F., Moriana, A., 2014. Using band dendrometers in irrigation scheduling: influence of the location inside the tree and comparison with point dendrometer. Agricultural Water Management 142, 29–37. https://doi.org/10.1016/j.agwat.2014.04.005

Daudet, F. A., Améglio, T., Cochard, H., Archilla, O., Lacointe, A. ,2005. Experimental analysis of the role of water and carbon in tree stem diameter variations. Journal of Experimental Botany, 56, 135-144. http://dx.doi.org/10.1093/jxb/eri026.

De Swaef, T., Steppe, K., Lemeur, R., 2009. Determining reference values for stem water potential and maximum daily trunk shrinkage in young apple trees based on plant responses to water deficit. Agricultural Water Management 96, 541–550. https://doi.org/10.1016/j.agwat.2008.09.013

Doltra, J., 2003. (Spanish) Funcionamiento hı´drico del manzano adulto y utilizacio´n de indicadores biolo´ gicos para la gestio´n del agua. Doctoral dissertation, Universitat de Lleida, Spain, 171 pp.

Doltra, J., Oncins, J.A., Bonany, J., Cohen, M., 2007. Evaluation of plant-based water status indicators in mature apple trees under field conditions. Irrig. Sci. 25, 351–359. https://doi.org/10.1007/s00271-006-0051-y

Dragoni, D., Lakso, A.N., Piccioni, R.M., 2005. Transpiration of apple trees in a humid climate using heat pulse sap flow gauges calibrated with whole-canopy gas exchange chambers. Agricultural and Forest Meteorology 130, 85–94. https://doi.org/10.1016/j.agrformet.2005.02.003

Dzikiti, S., Volschenk, T., Midgley, S.J.E., Lötze, E., Taylor, N.J., Gush, M.B., Ntshidi, Z., Zirebwa, S.F., Doko, Q., Schmeisser, M., Jarmain, C., Steyn, W.J., Pienaar, H.H., 2018. Estimating the water requirements of high yielding and young apple orchards in the winter rainfall areas of South Africa using a dual source evapotranspiration model. Agricultural Water Management 208, 152–162. https://doi.org/10.1016/j.agwat.2018.06.017

Egea, G., Pagán, E., Baille, A., Domingo, R., Nortes, P.A., Pérez-Pastor, A., 2009. Usefulness of establishing trunk diameter based reference lines for irrigation scheduling in almond trees. Irrigation Science 27, 431–441. https://doi.org/10.1007/s00271-009-0157-0

Fallahi, E., Colt, W.M., Fallahi, B., Chun, I.J., 2002. The Importance of Apple Rootstocks on Tree Growth, Yield, Fruit Quality, Leaf Nutrition, and Photosynthesis with an Emphasis on ‘Fuji’. HortTechnology 12, 38–44. https://doi.org/10.21273/horttech.12.1.38

Fereres, E., Goldhamer, D., 2003. Suitability of stem diameter variations and water potential as indicators for irrigation scheduling of almond trees. The Journal of Horticultural Science and Biotechnology 78, 139–144. https://doi.org/10.1080/14620316.2003.11511596

Fernández, J.E., 2014. Plant-based sensing to monitor water stress: Applicability to commercial orchards. Agricultural water management 142, 99–109. https://doi.org/10.1016/j.agwat.2014.04.017

Fernández, J.E., Cuevas, M.V., 2010. Irrigation scheduling from stem diameter variations: a review. Agricultural and Forest Meteorology 150, 135–151. https://doi.org/10.1016/j.agrformet.2009.11.006

Fernández, J.E., Green, S.R., Caspari, H.W., Diaz-Espejo, A., Cuevas, M.V., 2008 A. The use of sap flow measurements for scheduling irrigation in olive, apple and Asian pear trees and in grapevines. Plant and Soil, 305, 91-104. https://doi.org/10.1007/s11104-007-9348-8

Fernández, J.E., Romero, R., Montaño, J.C., Diaz-Espejo, A., Muriel, J.L., Cuevas, M.V., Moreno, F., Girón, I.F., Palomo, M.J., 2008 B. Design and testing of an automatic irrigation controller for fruit tree orchards, based on sap flow measurements. Australian Journal of Agricultural Research 59, 589–598. https://doi.org/10.1071/ar07312

Flore, J.A., Lakso, A.N., 1989. Environmental and Physiological Regulation of Photosynthesis in Fruit Crops. Horticultural Reviews, Volume 11, 111-158. https://doi.org/10.1002/9781118060841.ch4

Ford, C.R., Goranson, C.E., Mitchell, R.J., Will, R.E., Teskey, R.O., 2004. Diurnal and seasonal variability in the radial distribution of sap flow: predicting total stem flow in *Pinus taeda* trees. Tree Physiology 24, 951–960. https://doi.org/10.1093/treephys/24.9.951

Forster, A.M., 2017. How Reliable Are Heat Pulse Velocity Methods for Estimating Tree Transpiration? Forests 8, 350. https://doi.org/10.3390/f8090350

Forster, M.A., 2020. The importance of conduction versus convection in heat pulse sap flow methods. Tree Physiology, 40, 683-694. https://doi.org/10.1093/treephys/tpaa009

Garnier, E. and Berger, A., 1986. Effect of water stress on stem diameter changes of peach trees growing in the field. Journal of Applied Ecology, pp.193-209. http://dx.doi.org/10.2307/2403091

Ginestar, C., Castel, J., 1995. Use of stem dendrometers as indicators of water stress in drip-irrigated citrus trees. Presented at the II International Symposium On Sensors in Horticulture 421, 209–222. https://doi.org/10.17660/actahortic.1998.421.22

Goldhamer, D., Fereres, E., 2001. Irrigation scheduling protocols using continuously recorded trunk diameter measurements. Irrigation Science 20, 115–125. https://doi.org/10.1007/s002710000034

Gonçalves, B., Moutinho-Pereira, J., Santos, A., Silva, A.P., Bacelar, E., Correia, C., Rosa, E., 2006. Scion–rootstock interaction affects the physiology and fruit quality of sweet cherry. Tree Physiology 26, 93–104. https://doi.org/10.1093/treephys/26.1.93

Intrigliolo, D.S., Castel, J.R., 2006. Usefulness of diurnal trunk shrinkage as a water stress indicator in plum trees. Tree Physiology 26, 303–311. http://doi.org/10.1093/treephys/26.3.303

Intrigliolo, D.S., Castel, J.R., 2007. Crop load affects maximum daily trunk shrinkage of plum trees. Tree Physiology 27, 89–96. http://doi.org/10.1093/treephys/27.1.89

Jarvis, P.G., 1985. Coupling of transpiration to the atmosphere in horticultural crops: the omega factor. Acta Horticulturae 171, 187–205. https://doi.org/10.1093/jxb/erh213

Jones, H.G., 2004. Irrigation scheduling: advantages and pitfalls of plant-based methods. Journal of Experimental Botany, 55, 2427–2436. https://doi.org/10.1093/jxb/erh213

Katerji, N., Tardieu, F., Bethenod, O., Quetin, P., 1994. Behavior of maize stem diameter during drying cycles: comparison of two methods for detecting water stress.

Crop science 34, 165–169. https://doi.org/10.2135/cropsci1994.0011183x003400010029x

Köcher, P., Horna, V., Leuschner, C., 2013. Stem water storage in five coexisting temperate broad-leaved tree species: significance, temporal dynamics and dependence on tree functional traits. Tree Physiology 33, 817–832. https://doi.org/10.1093/treephys/tpt055

Liu, C., Du, T., Li, F., Kang, S., Li, S., Tong, L., 2012. Trunk sap flow characteristics during two growth stages of apple tree and its relationships with affecting factors in an arid region of northwest China. Agricultural Water Management 104, 193–202. https://doi.org/10.1016/j.agwat.2011.12.014

Mobe, N., Dzikiti, S., Volschenk, T., Zirebwa, S., Ntshidi, Z., Midgley, S., Steyn, W., Lötze, E., Mpandeli, S., Mazvimavi, D., 2020. Using sap flow data to assess variations in water use and water status of apple orchards of varying age groups in the Western Cape Province of South Africa. Water SA 46, 213–224. https://doi.org/10.17159/wsa/2020.v46.i2.8236

Moriana, A., Pérez-López, D., Prieto, M.H., Ramírez-Santa-Pau, M., Pérez-Rodriguez, J.M., 2012. Midday stem water potential as a useful tool for estimating irrigation requirements in olive trees. Agricultural Water Management 112, 43–54. https://doi.org/10.1016/j.agwat.2012.06.003

Mpelasoka, B.S., Behboudian, M., Dixon, J., Neal, S.M., Caspari, H., 2000. Improvement of fruit quality and storage potential of ‘Braeburn’apple through deficit irrigation. The Journal of Horticultural Science and Biotechnology 75, 615–621. https://doi.org/10.1080/14620316.2000.11511296

Naor, A., Cohen, S., 2003. Sensitivity and variability of maximum trunk shrinkage, midday stem water potential, and transpiration rate in response to withholding irrigation from field-grown apple trees. HortScience 38, 547–551.

Naor, A., Gal, Y., Peres, M., 2006. The inherent variability of water stress indicators in apple, nectarine and pear orchards, and the validity of a leaf-selection procedure for water potential measurements. Irrigation science 24, 129–135. https://doi.org/10.1007/s00271-005-0016-6

Naor, A., Klein, I., Doron, I., 1995. Stem water potential and apple size. Journal of the American Society for Horticultural Science 120, 577–582. https://doi.org/10.21273/jashs.120.4.577

Naor, A., Naschitz, S., Peres, M., Gal, Y., 2008. Responses of apple fruit size to tree water status and crop load. Tree Physiology 28, 1255–1261. https://doi.org/10.1093/treephys/28.8.1255

Nortes, P., Pérez-Pastor, A., Egea, G., Conejero, W., Domingo, R., 2005. Comparison of changes in stem diameter and water potential values for detecting water stress in young almond trees. Agricultural Water Management 77, 296–307. https://doi.org/10.1016/j.agwat.2004.09.034

Oguntunde, P.G., van de Giesen, N., Savenije, H.H., 2007. Measurement and modelling of transpiration of a rain-fed citrus orchard under subhumid tropical conditions. Agricultural Water Management 87, 200–208. https://doi.org/10.1016/j.agwat.2006.06.019

Ortuño, M.F., García-Orellana, Y., Conejero, W., Ruiz-Sánchez, M.C., Mounzer, O., Alarcón, J.J., Torrecillas, A., 2006. Relationships Between Climatic Variables and Sap Flow, Stem Water Potential and Maximum Daily Trunk Shrinkage in Lemon Trees. Plant and Soil 279, 229–242. https://doi.org/10.1007/s11104-005-1302-z

Ortuño, M.F., Conejero, W., Moreno, F., Moriana, A., Intrigliolo, D.S., Biel, C., Mellisho, C.D., Pérez-Pastor, A., Domingo, R., Ruiz-Sánchez, M.C., Casadesus, J., Bonany, J., Torrecillas, A., 2010. Could trunk diameter sensors be used in woody crops for irrigation scheduling? A review of current knowledge and future perspectives. Agricultural Water Management 97, 1–11. https://doi.org/10.1016/j.agwat.2009.09.008

Pardossi, A., Incrocci, L., Incrocci, G., Malorgio, F., Battista, P., Bacci, L., Rapi, B., Marzialetti, P., Hemming, J., Balendonck, J., 2009. Root zone sensors for irrigation management in intensive agriculture. Sensors, 9, 2809–2835. https://doi.org/10.3390/s90402809

Remorini, D., Massai, R., 2003. Comparison of water status indicators for young peach trees. Irrigation Science 22, 39–46. https://doi.org/10.1007/s00271-003-0068-4

Robinson, T., 2008. The evolution towards more competitive apple orchard systems in the USA. Acta Horticulturae, 772, 491–500. https://doi.org/10.17660/actahortic.2008.772.81

Robinson, T., Hoying, S., Reginato, G., 2011. The tall spindle planting system: Principles and performance. Acta Horticulturae, 903, 571–579. https://doi.org/10.17660/actahortic.2011.903.79

Swanson, R., Whitfield, D., 1981. A numerical analysis of heat pulse velocity theory and practice. Journal of experimental botany 32, 221–239. https://doi.org/10.1093/jxb/32.1.221

Taylor, N., Ibraimo, N., Annandale, J., Everson, C., Vahrmeijer, J., Gush, M.B., 2013. Are sap flow measurements useful for determining water use of fruit orchards, when absolute values are important? Presented at the IX International Workshop on Sap Flow 991, 77–83. https://doi.org/10.17660/actahortic.2013.991.9

Tie, Q., Hu, H., Tian, F., Guan, H., Lin, H., 2017. Environmental and physiological controls on sap flow in a subhumid mountainous catchment in North China.

Agricultural and Forest Meteorology 240–241, 46–57. https://doi.org/10.1016/j.agrformet.2017.03.018

Tognetti, R., d’Andria, R., Morelli, G., Calandrelli, D., Fragnito, F., 2004. Irrigation effects on daily and seasonal variations of trunk sap flow and leaf water relations in olive trees. Plant and Soil 263, 249–264. https://doi.org/10.1023/B:PLSO.0000047738.96931.91

Tworkoski, T., Fazio, G., Glenn, D.M., 2016. Apple rootstock resistance to drought. Scientia Horticulturae 204, 70–78. https://doi.org/10.1016/j.scienta.2016.01.047

Tyree, M.T., Zimmermann, M.H., 2002. Xylem structure and the ascent of sap. Springer Series in Wood Science. Ed. T.E. Timell. Springer, New York.

Villalobos, F., Testi, L., Moreno-Perez, M., 2009. Evaporation and canopy conductance of citrus orchards. Agricultural Water Management 96, 565–573. https://doi.org/10.1016/j.agwat.2008.09.016

Wang, Z., Stutte, G.W., 1992. The role of carbohydrates in active osmotic adjustment in apple under water stress. Journal of the American Society for Horticultural Science 117, 816–823. https://doi.org/10.21273/jashs.117.5.816

Wang, Z., Quebedeaux, B., Stutte, G., 1995. Osmotic adjustment: effect of water stress on carbohydrates in leaves, stems and roots of apple. Functional Plant Biology 22, 747–754. https://doi.org/10.1071/pp9950747

White, A., Food Research Institute of New Zealand Ltd., 2008. Apple tree variety named 'Scilate'. U.S. Patent Application 12/072,658.

CHAPTER 3

NAVAJO PEACH TREES HAVE GREATER PHYSIOLOGICAL RESILIENCE TO WATER STRESS THAN LOVELL PEACH TREES: PRECISION WATER STRESS USING AUTOMATED WEIGHING LYSIMETERS[1]

Abstract

Native American tribes have been cultivating peaches (*Prunus persica* (L.) Batsch) since their introduction to North America in the 1600's. In the American Southwest, peach orchards derived from centuries of seed selections have been maintained in relative isolation from commercial cultivars. These Native American peach selections may be better adapted to the arid climate of the Intermountain West. We compared physiological robustness during water stress of seedling peaches from a 60-year-old orchard maintained by Navajo farmers in southwestern Utah to the commercial peach rootstock Lovell. Six replicate trees of each rootstock were subjected to eight cycles of controlled drought on an automated lysimeter system, which continuously monitored transpiration rate. Trees were selected for uniform size and transpiration rate at the start of the study. During the drought cycles, individual trees were watered when their transpiration rate decreased below 250 grams of water per day, approximately 20 % of their well-watered daily transpiration rate. After the first cycle of drought, the transpiration rate of the Navajo was greater than the Lovell trees, so they more rapidly

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depleted their root-zone water and experienced greater water stress. In spite of greater stress, the Navajo selection had greater leaf area and dry mass at harvest. Because the root-system was confined, these results indicate that the Navajo selection may have greater resilience when experiencing drought, independent of the depth and distribution of the root system. However, this study was not able to determine whether physiological resilience during drought was due to canopy or root characteristics. Field studies are needed to determine if root distribution or depth also contribute to drought tolerance on the Navajo selection.

3.1 Introduction

Irrigated agricultural crops account for 70% of water consumption in the United States and water consumption can be as high as 90% in some Western states (Schaible and Aillery, 2017). Agricultural water scarcity is expected to increase as a result of increased population growth and climate change (Strzepek and Boehlert, 2010). Tree fruits such as peaches, apples, cherries and apricots are high value crops but have some of the highest water requirements (Fereres and Evans, 2006). In commercial orchard production, a fruiting scion is nearly always grafted onto a rootstock cultivar. Breeding and selection of rootstock cultivars frequently targets tree size and precocity, pest and disease resistance and adaptability to different soils. Less effort has been devoted to the selection of rootstocks that might confer tolerance of drought (Ernst et al., 2012). Selection of rootstocks adapted to drought would benefit arid fruit growing regions.

Native American populations in Southwestern North America, including the Navajo, Hopi, and Zuni tribes, have been cultivating peaches for over 400 years (Benavidez, 1996). Native American cultivation practices differ dramatically from European production. Rather than relying on grafting to combine desirable stock and scions, seeds of desirable trees are collected and directly planted to form new orchards (Jett, 1977). Orchards receive no formal irrigation after initial establishment, and often receive as little as 280 mm of annual precipitation (Singletary et. al, 2014). Orchards were commonly planted below canyon rims, which historically receive precipitation runoff from mesa cliffs, demonstrated by Navajo, Hopi, and Zuni historic orchard sites (Singletary, et.al, 2014; Ferguson, 1996; Jett, 1979). The seed selection of peaches from orchards in these remote locations over hundreds of years resulted in land-race type populations, which may have greater drought tolerance than commercial rootstock cultivars. Using these as rootstocks could improve drought-tolerance of modern orchards requiring less irrigation.

Weighing lysimeters provide a reliable method of applying drought stress because the transpiration rate of an entire tree can be determined over short intervals, summed over a day and integrated over the study (Ben-Gal et al., 2010). Studies in lysimeters require that the plants are grown in a limited root-zone, which eliminates the variable of root distribution and facilitates studies of physiological adaptation to stress. Several studies have compared rootstocks in containers in a greenhouse environment. A study of drought effects on apple rootstocks in a greenhouse withheld water and used single-leaf measurements of water potential and stomatal conductance to assess the magnitude of water stress (Tworkoski et al., 2016). This is a valuable study but there are significant challenges in extrapolating from short-term measurements on single leaves to whole plants (Jones, 2004).

A common technique in drought stress studies in containers is to maintain constant frequency of watering but reduce the volume of water applied. This leads to less negative water potential at the top of the container than the bottom because the hydraulic conductivity of soil declines exponentially with decreasing water content. This problem is even more significant in coarse textured media (Hillel, 1998). Atkinson et al. (1999) studied drought in apple rootstocks in 14-L containers with a compost media; Chahal (2018) imposed water stress for Amaranthus in 9-L containers with a silt loam soil. Both studies reduced the volume of water applied without changing the frequency of watering. Chahal (2018) carefully measured water potential or volumetric content but only in the top 30 % of the container. Differences in root-zone water potential between the top and bottom of the container make it difficult to know the magnitude of water stress of the plant. Changing the frequency of watering rather than the volume of water provides a better method of regulating water stress because it allows uniform wetting of the root-zone.

Both Tworkoski et al. (2016) and Atkinson et al. (1999) used potting soil as a medium, which is well aerated but is a poor substrate for drought studies because it has a rapid decrease in water potential over a narrow range of volumetric water content

(Handrek, et al, 2005; Bunt, 1988). Weighing lysimeters facilitate the use of soil because overwatering and waterlogging can be avoided. The use of soil allows for a gradual decrease in water potential that better approximates field conditions. Lysimeters not only indicate volumetric water content of the entire container, they provide a real-time measurement of water stress via the hourly transpiration rate.

Our objective was to use weighing lysimeters for precision water stress to compare resilience during drought of a Navajo peach selection to the commercial seed-propagated rootstock cultivar Lovell.

3.2 Materials and Methods

Seeds from a Navajo peach land-race population were collected from an orchard near Navajo Mountain, UT that has been maintained by native Navajo residents for at least 60 years. Peaches from the Navajo population are predominately white free-stone but can be yellow flesh. Irrigation is occasional and thinning is not practiced, potentially contributing to reduce fruit size to that of commercial cultivars. The trees are shrub like due to no pruning practices. The seed selection was a subsample of all peach trees within the orchard. Efforts for further characterize these populations are ongoing (Wytsalucy, MS Thesis). The peach cultivar Lovell was used as a control standard because it is a widely-used seed-propagated rootstock. Lovell rootstock seeds were provided by a commercial source (Sierra Gold Nurseries, Yuba City, CA). Both selections were planted in 4-L containers filled with an 80% peat, 20% perlite media and grown for 2 months with supplemental lighting in the Utah State University Research Greenhouses. Plants were watered with a complete nutrient solution at 100 ppm N fertigation (Peters Excel 21N-2.2P-16.6K, Everris, Dublin, OH). When the root system reached the bottom of the containers, six trees from each seed source were selected for uniformity and transplanted into 22-L containers with a 90% silty clay loam, 10% peat mixture. Soil was a Battle Creek silty clay loam series obtained from an agricultural field located in Cove, UT (41°57’48”N 111°48’26”W). Silty clay loam was used to achieve a longer, more gradual release of water in the container. Because of the addition of peat and careful soil placement in the container, the bulk density in the containers was approximately 1.2 kg m^{-3}. Soil mixture had an initial electrical conductivity of 1.97 mS cm^{-1} and was irrigated with water which had approximately 0.35 mS cm^{-1} so salt stress was judged to be minimal. To ensure ample nutrients, the soil was amended with 8 g L^{-1} of controlled release fertilizer (Polyon 16N-2.6P-9.1K, 5-6 month release, Pursell Industries, Sylacauga, AL). Soil mixture after 30 days of growth the trees were established in the larger containers. Each tree was visually equal in size at the start of the trial. Before the start of the first trial Navajo trees had an average transpiration rate 4.3% higher than Lovell (593 vs. 565 g d^{-1}). During this pre-trial period differences were not statistically significant and the uniform transpiration rate indicated the relative uniformity of trees. Peak transpiration rates, generally observed two to three days after the last irrigation event, increased to approximately 1000 g d^{-1} at the start of the trial.

Fig. 3.2.1. Navajo and Lovell peach seedlings on the lysimeter system at the start of trial one (A) and trial two (B).

Each container and its tree was then placed on a load cell platform in a 12-container weighing lysimeter system (Fig. 3.3-1). This greenhouse lysimeter system has been described in detail by Chard et al. (2016). Each container had two, 2-L h^{-1} drip emitters at the surface, which were used to slowly add water until leaching was observed from drain tubes at the bottom. Before filling, porous ceramic cylinders (1.5 cm diameter x 6.5 cm length; effective pore size ~ 1.7 μm) were installed at the bottom of each container by drilling a hole through the side wall and fixing the cups in place. After irrigation, when leaching was observed to stop, a vacuum (-0.05 MPa) was connected to the porous ceramic cylinders and run overnight. This removed approximately 250 ml of additional water from each container. The container surface was then covered by 5 cm of perlite to minimize surface evaporation. The combined mass of each tree and container was then determined (~22 to 27 kg per tree) and programmed into the measurement and control software as the near field-capacity baseline-mass.

Transpiration rates were determined every 30-minutes from the change in container mass, summed daily and a cumulative transpiration total was calculated at midnight. Trees were irrigated when the daily cumulative transpiration decreased to less than a programmed threshold of 250 g per tree per d. This change represented an approximate 80% decline in daily transpiration from maximum, or peak, daily transpiration totals after irrigation. This threshold was selected because it was associated with visual wilting of the leaves. The transpiration rate of each tree was independently measured and automatically irrigated using a datalogger-based controller. After each drought cycle, containers were drip-irrigated back up to the near field-capacity baseline-mass. To allow daily measurements of transpiration, plants were irrigated only at night. Irrigation cycled on for 15 seconds each minute to minimize ponding and allow for slow percolation of water into the soil. This slow irrigation (up to 6 h) preserved the soil structure and air-filled porosity during the trial. Each tree was subjected to four dry down and irrigation cycles, followed by a two-week well-watered recovery period, and then four additional dry down and irrigation cycles, for a total of eight drought and six recovery events.

Internal circulation fans in the greenhouse minimized variation of temperature and humidity. During the first trial, the average day/night temperature was 28.9/20.3 °C and was 27.8/18.2°C in the second trial. Vapor pressure deficit averaged 3.2 kPa for the first trial and 2.5 kPa for the second trial. The CO_2 concentration was ambient (~ 400 ppm) for both trials. Supplemental lighting was provided by four, 1000-W high pressure sodium fixtures arranged to provide a uniform photosynthetic photon flux density (PPFD) at the canopy surface. Trees were arranged in a complete randomized design to minimize any potential differences in lighting intensity. The integrated daily photon flux density (measured with multiple replicate full spectrum quantum sensors; Apogee Instruments, model SQ-500, Logan, UT) averaged 45.5 mol m^{-2} d^{-1} for the first trial and 41.6 mol m^{-2} d^{-1} for the second trial. These values are about 85 % of full summer sunlight under regional field conditions.

Trees were destructively harvested at the end of the second trial. Trunk diameter at 3 cm above the soil surface was measured and cross-sectional area was calculated. Chlorophyll was measured on 10 recently-developed, fully expanded leaves per tree using a hand-held chlorophyll meter (Apogee Instruments, model MC-100). Leaves were removed, counted and the area was measured (LI-COR, model LI-3100C area meter, Lincoln, NE). Leaf and wood tissue dry mass were measured after drying for three days at 80°C. Root balls were removed from their containers and shaken to remove bulk soil, weighed, visually evaluated and photographed.

The six replicate trees were arranged in a completely randomized design with seed sources treated as fixed effects. Cumulative transpiration and number of dry down days were analyzed utilizing two-way repeated measures multivariate analysis of variance (MANOVA). All other variables were analyzed using two-way analysis of variance (ANOVA). Trees dried down to threshold transpiration rates over variable number of days, which resulted in irrigation frequency differing for each tree. Cumulative transpiration was normalized within the graphs so that the start of each drought cycle began with the first day after an irrigation event for all trees. Total dry down days were averaged, and the graph normalized to reflect that average. All data were analyzed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria).

3.3 Results and Discussion

The time between irrigation intervals was shorter for the larger trees, but individual trees were watered when transpiration decreased to less than 250 g per tree per day. In practice transpiration rates always declined below this threshold with minimum daily transpiration rates averaging 220 g per tree per day for the first trial and 205 g per tree per day for the second trial. Comparing these minimum values to peak daily transpiration rates observed after the last irrigation event (initially around 1000 g per tree per d), the minimum transpiration rates represent 22.5% of peak daily transpiration for the first trial and 16% for the second trial. As trees approached lower threshold transpiration rates, there was visible wilting of the leaves throughout the canopy and during the subsequent recovery periods leaf necrosis and abscission were observed in all trees. Girona et al. (2002) used a similar reduction to 20% of maximum tree evapotranspiration and reported ~80% reduction in stem water potential by the end of the dry down period.

Osmotic adjustment of *Prunus* species has been reported to occur after prolonged, gradual dry downs, most commonly observed under field conditions or carefully controlled greenhouse studies (Arndt et al., 2000; Jimenez et al., 2013). Through this mechanism leaf turgor can be maintained under stress, though species vary greatly in their capacity for osmotic adjustment (Abrams, 1994). Mellisho et al. (2011) reported that *P. persica* (L.) Batsch cvar Flordastar grafted to *P. persica* × *P. amygdalus* GF-677 rootstock did not have sufficient osmotic adjustment to overcome the turgor loss point in leaves. This study utilized eight cyclic periods of 7 to 9 days of withholding irrigation and it is uncertain whether osmotic adjustment occurred in either rootstock or to what extent it may have contributed to resilience.

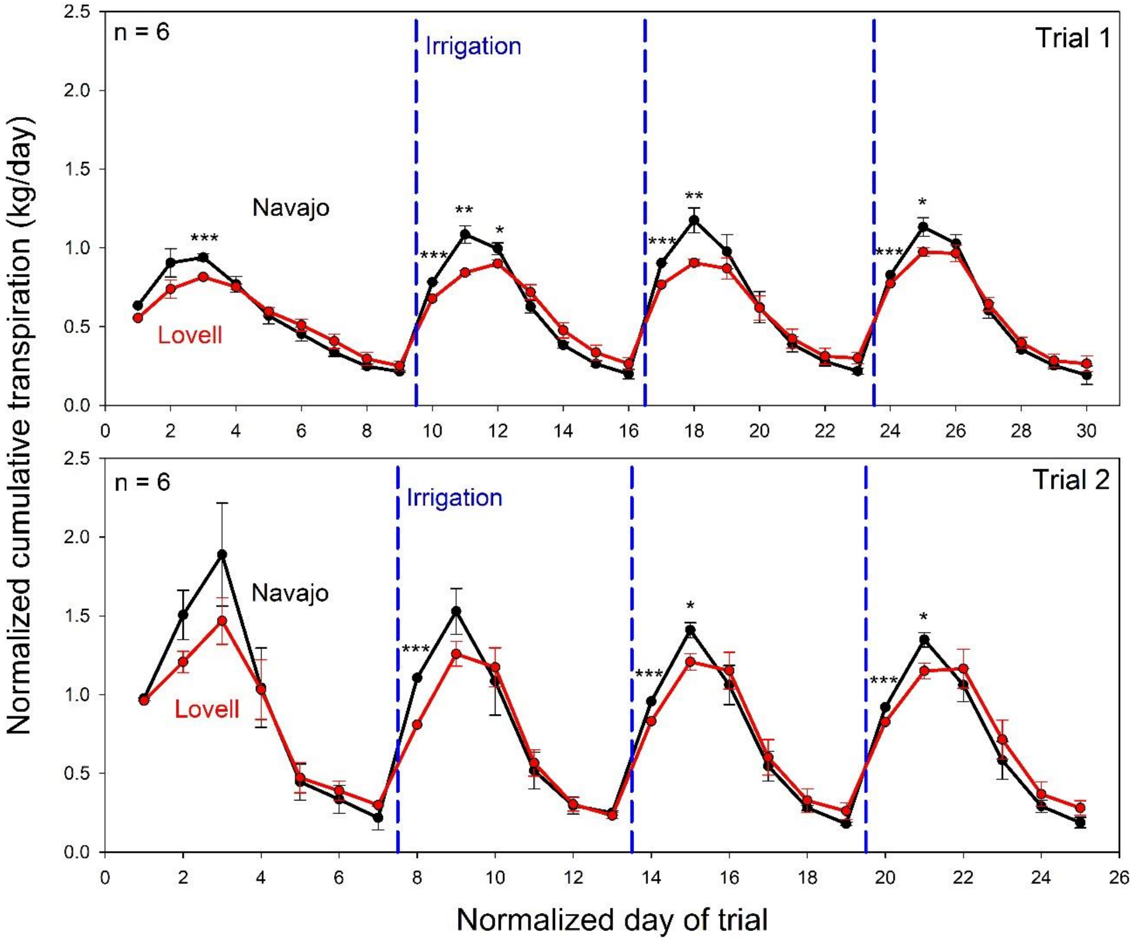


Fig. 3.3.1. Cumulative transpiration for Navajo (black line) and Lovell (red line) peach selections. Data were normalized to the first day of the trial.

In spite of this drought stress, both genotypes recovered to their pre-drought-stress peak transpiration rates within three days of re-watering (Fig. 3.4.1). For the entire first trial and the last two drought events of the second trial, Navajo had higher peak transpiration rates. As a result, the Navajo selection experienced greater drought stress as a percentage of peak daily transpiration in the first trial (*P* = 0.02). The Navajo also tended to have greater drought stress in the second trail, but the difference was not statistically significant (Fig. 3.4-2).

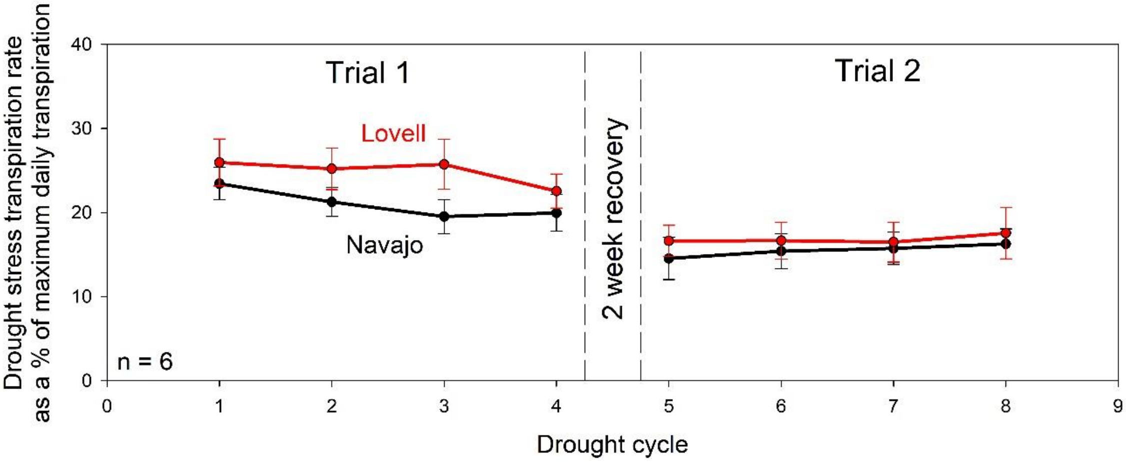


Fig. 3.3.2. Maximum drought stress as a percentage of peak transpiration rate for Navajo (black line) and Lovell (red line) peach selections.

At harvest, the Navajo selection had 16 % higher leaf dry mass per tree (*P* = 0.05), 32 % greater leaf number (*P* = 0.01) and 30 % greater leaf area (*P* = 0.05) (Table 1). Because the trees were of equal size at the start of the trials, these differences reflect increased growth of the Navajo selection during the trial, despite slightly greater drought stress. Transpiration rate is highly correlated with CO_2 uptake, photosynthesis and growth of trees (Welander and Ottosson, 2000; Breda and Granier, 1996; Adams et al., 2018; Obojes et al., 2018), so reduced transpiration rates are a good indicator of reduced whole-tree photosynthesis and dry mass gain. Higher measurements of leaf biomass at harvest confirm that the differences in transpiration rate during the trial were associated with growth differences. Rieger and Duemmel (1991) reported that shoot characteristics in six cultivated peach species were more correlated with drought adaption than root characteristics. There was no significant difference between specific leaf area of the Navajo and Lovell rootstocks (13.3 and 12.4 m^2 kg^{-1} respectively), which suggests that leaf contribution to resilience during water stress may be minimal. However, because these were ungrafted rootstocks, adaption of the shoot may have contributed to the resilience of the tree to water stress. Further studies with each rootstock grafted to a common scion could help differentiate rootstock from scion effects.

Table 3.3.1. Destructive harvest parameters for the two peach selections. Both selections were drought stressed eight times approximately 20% of their peak transpiration rates.

Cultivar	Trunk & stem dry mass (g)	Trunk diameter (cm)	Total leaf area (m^2)	Leaf dry mass (g)	Number of leaves per tree	Root ball mass (g)
Navajo	106	1.57	1.27	95.8	847	1625
Lovell	97	1.55	0.98	79.1	568	1197
Ratio	1.10	1.01	1.29	1.21	1.49	1.36
Significance	ns	ns	0.05	0.05	0.01	ns

There were no statistically significant differences in trunk cross sectional area or trunk dry weight at harvest (Table 3.4-1). The root systems of both genotypes were vigorous and healthy (Fig. 4). Although the Navajo selection tended to have higher root-ball fresh mass at harvest, the difference was not statistically significant (Table 3.4-1).

Leaf chlorophyll concentration was slightly higher in Lovell (data not shown), but the difference was not visually apparent and the high chlorophyll concentration in both cultivars indicated ample nutrition. Visually, the Navajo selection had a slightly more horizontal branch orientation (Fig. 3.4-3).

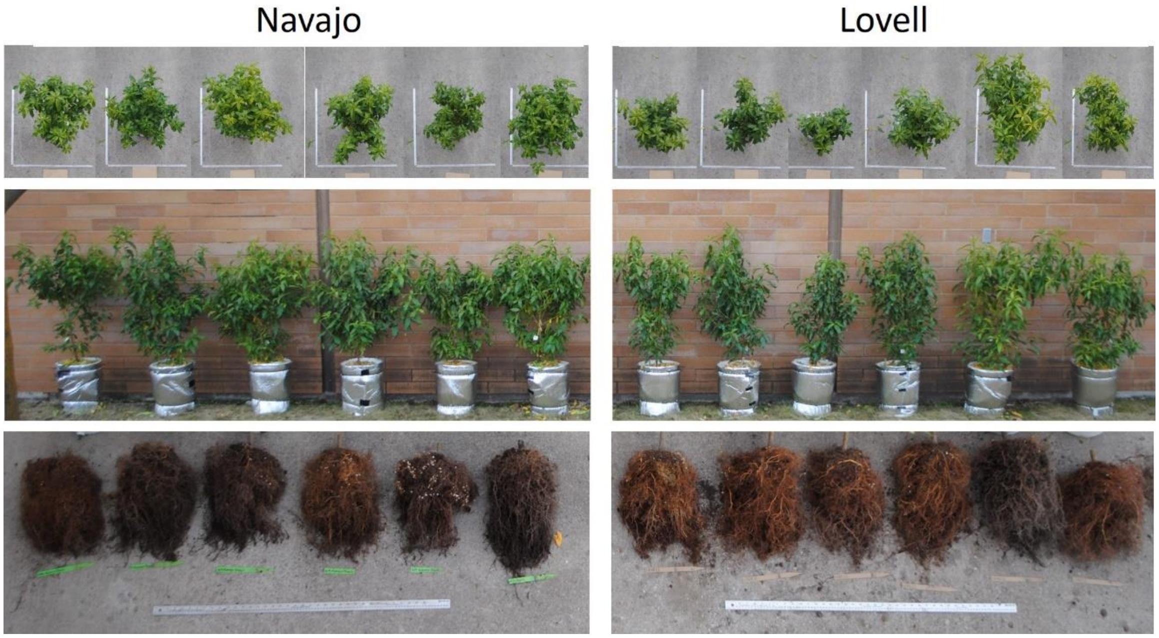


Fig. 3.3.3. Navajo and Lovell peach trees at the end of trial two.

3.4 Conclusions

This study indicates that the Navajo selection is physiologically more robust under drought conditions than Lovell rootstock when root volume is held constant. Commercial peach rootstocks in the field can differ in root distribution (Black et al., 2010). Additional work is needed to determine how root distribution compares between the Navajo selection and commercial rootstocks, as well as how the differences observed here translate into differences in field-based drought response. The historic peach land-races developed by traditional Native American farmers in the desert Southwest may provide useful traits to commercial breeders in the development of rootstocks and scion cultivars adapted to arid environments.

3.5 Literature Cited

Abrams, M. D. 1994. Genotypic and phenotypic variation as stress adaptations in temperate tree species: a review of several case studies. Tree Physiol., 14: 833-842.

Adams, S., J. Lordan, G. Fazio, B. Bugbee, P. Francescatto, T.L. Robinson and B. Black. 2018. Effect of scion and graft type on transpiration, hydraulic resistance and xylem hormone profile of apples grafted on Geneva®41 and M.9-NIC™29 rootstocks. Scientia Hort. 227:213–222.

Arndt, S. K., W. Wanek, S. C. Clifford, & M. Popp. 2000. Contrasting adaptations to drought stress in field-grown *Ziziphus mauritiana* and *Prunus persica* trees: water relations, osmotic adjustment and carbon isotope composition. Functional Plant Biol., 27: 985-996.

Atkinson, C.J., M. Policarpo, A.D. Webster, and A.M. Kuden. 1999. Drought tolerance of apple rootstocks: production and partitioning of dry matter. Plant and Soil, 206:223-235.

Benavides, F.A. 1996. A harvest of Reluctant Souls: The Memorial of Fray Alonzo de Benavides, 1630.Translated by B. H. Morrow. Univ. Press of CO.

Ben-Gal, A., D. Kool, N. Agam, G.E. van Halsema, U. Yermiyahu, A. Yafe, E. Presnov, R. Erel, A. Majdop, I. Zipori, E. Segal, S. Ruger, U. Zimmermann, Y. Cohen, V. Alchanatis, and A. Dag. 2010. Whole-tree water balance and indicators for short-term drought stress in non-bearing ‘Barnea’ olives. Agr. Water Mgt. 98:124–133.

Black, B.L., D. Drost, T. Lindstrom, J. Reeve, J. Gunnell, and G.L. Reighard. 2010. A comparison of root distribution patterns among Prunus rootstocks. J. of the Amer. Pomol. Soc. 64:52-62.

Bréda, N., and A. Granier. 1996. Intra- and interannual variations of transpiration, leaf area index and radial growth of a sessile oak stand (*Quercus petraea*). Ann. Des Sciences For. 53:521–536.

Bunt, A.C., 1988. Media and Mixes for Container-Grown Plants, second ed. Unwin Hyman Ltd., London.

Chahal, P.S., S. Irmak, M. Jugulam, and A.J. Jhala. 2018. Evaluating Effect of Degree of Water Stress on Growth and Fecundity of Palmer amaranth (*Amaranthus palmeri*) Using Soil Moisture Sensors. Weed Science, 66:738-745.

Chard, J., M. van Iersel, and B. Bugbee. 2016. Mini-lysimeters to monitor transpiration and control drought stress: system design and unique applications. https://www.semanticscholar.org

Ernst, T., S.D. Rowley, B.L. Black and T.R. Roper. 2012. Reviewing potential local fruit markets: A Utah case study. J. Amer. Pom. Soc. 66:16-22.

Ferguson, T.J. 1996. Historic Architecture & Society. University of Arizona Press. Tucson, AZ.

Fereres, E. and R. Evans, 2006. Irrigation of fruit trees and vines: an introduction. Irrig. Sci, 24:55-57.

Girona, J., M. Mata, E. Fereres, D.A. Goldhamer, & M. Cohen. 2002. Evapotranspiration and soil water dynamics of peach trees under water deficits. Agr. Water Mgt. 54:107-122.

Handrek, K.A., N.D. Black. 2005. Growing media for ornamental plants and turf. UNSW Press.

Hillel, D. 1998. Environmental Soil Physics. p. 208. Academic Press, San Diego.

Jiménez, S., J. Dridi, D. Gutiérrez, D. Moret, J. J. Irigoyen, M. A. Moreno, & Y. Gogorcena, Y. 2013. Physiological, biochemical and molecular responses in four *Prunus* rootstocks submitted to drought stress. Tree physiol., 33:1061-1075.

Jett, S.C. 1977. History of Fruit Tree Raising among the Navajo. Agricultural History, 51:681-701.

Jett, S.C. 1979. Peach Cultivation and Use among the Canyon de Chelly Navajo. Econ. Bot., 33:298-310.

Jones, H.G. 2004. Irrigation scheduling: advantages and pitfalls of plant-based methods. J. of Expt. Bot. 55:2427–2436.

Mellisho, C. D., Z. N. Cruz, W. Conejero, M. F. Ortuño, & P. Rodríguez. 2011. Mechanisms for drought resistance in early maturing cvar Flordastar peach trees. The J. of Agr. Science, 149:609-616.

Obojes, N., A. Meurer, C. Newesely, E. Tasser, W. Oberhuber, S. Mayr, and U. Tappeiner. 2018. Water stress limits transpiration and growth of European larch up to the lower subalpine belt in an inner-alpine dry valley. New Phytol. 220:460–475.

Rieger, M., & M. J. Duemmel. 1992. Comparison of drought resistance among *Prunus* species from divergent habitats. Tree physiol., 11:369-380.

Schaible, G.D., and M.P. Aillery. 2017. Challenges for US Irrigated Agriculture in the Face of Emerging Demands and Climate Change p. 44–79. In: J. Ziolkowska and J.M. Peterson. (eds.) Competition for Water Resources. Elviser.

Singletary, L., S. Emm, M. Loma'omvaya, J. Clark, M. Livingston, M.K. Johnson, and R. Oden, 2014. People of the Land: Sustaining Agriculture on the Hopi Reservation. Univ. of NV. Coop. Ext. Reno, NV.

Strzepek, K., and B. Boehlert, 2010. Competition for water for the food system. Phil. Trans. of the Royal Soc. - B: Biol. Sciences, 365:2927-2940.

Tworkoski, T., G. Fazio, and D.M. Glenn. 2016. Apple rootstock resistance to drought. Scientia Hort., 204:70-78.

Welander, N. T., and B. Ottosson. 2000. The influence of low light, drought and fertilization on transpiration and growth in young seedlings of *Quercus robur* L. For. Ecol. and Mgt., 127:139-151.

CHAPTER 4

RESPONSE OF KRYMSK® 5 AND 6 ROOTSTOCKS TO ACUTE WATER STRESS USING AUTOMATED WEIGHING LYSIMETERS

Abstract

Establishment of new cherry orchards are increasingly moving to high density plantings and dwarfing rootstocks to maximize production. There is limited detailed characterization of the ability of dwarfing rootstocks to adjust to and recover from drought stress. In this study ungrafted Krymsk® 5 and 6 rootstocks were subjected to repeated acute drought on a 16-cell automated lysimeter system. Transpiration rates were reduced to roughly 25% of their peak values before irrigation was automatically and independently triggered for each individual tree as they cycled through drought and recovery. The trial was run twice, once with trees shipped directly from the nursery and then with trees which were over wintered on site. During the second trial, leaf level gas exchange measurements were taken during peak drought stress and two to three days after rewatering when transpiration rates had recovered. There were significant interactions between trials and rootstock responses. Despite these interactions, several patterns could be observed in the data. Krymsk® 6 cultivars reached transpiration thresholds faster during the first trial and exhibited higher leaf level transpiration and stomatal conductance during the second trial suggesting that these rootstocks may confer more vigorous growth but less stomatal regulation. Krymsk® 5 rootstocks took significantly longer to dry down in the first trial and had lower leaf level transpiration and

stomatal conductance in the second trial. In both trials Krymsk® 5 had a lower percent root mass than Krymsk® 6. The more conservative rate of water use seen by Krymsk® 5 could be a function of greater stomatal control or a lower canopy to root ratio which thereby limited transpiration rates.

4.1 Introduction

Sweet (*Prunus avium* L.) and tart (*Prunus cerasus* L.) cherry are significant economic crops in many regions of the world with the top five producers being Turkey, the U.S., China, Iran, and Chile (Milošević et al. 2020; Lang 2017). Commercial orchard production of sweet cherry has shifted to the use of dwarfing rootstocks and high-density plantings to maximize yields per hectare (Morandi et al. 2018; Robinson, 2006). Dwarfing rootstocks increase precocity and reduce costs associated with pruning and harvesting while shortening return on investment times compared to full sized trees (Cline, 2019; Forner-Giner et al. 2014). In the field, vigorous rootstocks may be less susceptible to drought because large rootzone volumes allow greater access to soil moisture (Tworkoski and Fazio 2015). However, studies indicate that dwarfing rootstocks can confer drought tolerance through greater stomatal regulation, increased ABA production, and reducing transpiration, vegetative growth, hydraulic conductance and leaf and stem water potentials (Atkinson et al. 2000; Hajagos and Végvári 2013; Martínez-Ballesta et al. 2010). This is of interest due to increasing competition for water resources from a growing population and greater uncertainty in precipitation patterns due to climate change. Tolerance of water stress is also desirable to facilitate implementation of deficit irrigation to improve orchard management and fruit quality (Naor, 2006).

The degree to which dwarfing rootstocks can withstand drought can vary significantly within the same commercial series and between dwarfing lines. The Krymsk® series rootstocks were selected by Dr. Guennadi Eramin of the Krymsk® Experimental breeding station in the Krasnodar Krai region in Russia near the Black Sea. (Eremin, 2003). Krymsk® 5 (K5) (*Prunus fruticosa* × *Prunus serrulata* var. lannesiana) is a semi-dwarfing rootstock which performs well under a range of soil types and has been reported to tolerate heat and cold stress well. Krymsk® 6 (K6) (*Prunus cerasus* × (*Prunus cerasus* × *Prunus maackii*)) was originally reported to be more dwarfing than K5 but has subsequently been observed to have significant environmental interactions impacting height in relation to K5 (Roper et al., 2019). Krymsk® 6 has been reported to be tolerant to water stress and adapted to cold and heat stress. Both cultivars are sensitive to prune dwarf virus and Prunus necrotic ringspot virus (Long et al., 2014). Despite being widely planted there is limited quantitative information as to how resilient Krymsk® series rootstocks are to drought.

Assessment of water stress in orchard trees can be accomplished through direct monitoring of physiological parameters or indirect methods such as soil moisture sensing. The most common physiological parameters which have been researched for irrigation scheduling include stem and leaf water potentials, canopy temperature, stomatal conductance, sap flow, and trunk diameter variation (Jones, 2004). However, when focusing on any one method, there is the possibility of losing an understanding of the whole plant response to water stress (Ben-Gal et al., 2010). Weighing lysimeters incorporate whole tree responses and results can be interpreted directly without scaling or extensive data processing (Beeson, 2011). Lysimeters also avoid localized sensor placement which can increase error from individual measurements and are unaffected by growth rate.

Our objective was to investigate the response of K5 and K6 rootstocks to acute severe drought and their ability to recover using a weighing lysimeter.

4.2 Materials and Methods

Ungrafted K5 and K6 rootstocks grown in Ellepot™ containers were obtained from a commercial nursery (Sierra Gold Nurseries, Yuba City, CA) in the fall. Trees for the first trial were transplanted into 20L containers filled with an 80% silty clay loam 20% peat media amended with 4.15g/L slow release fertilizer (Polyon 18-6-12, 5-6 Mo.) and allowed to establish for 3 months. Trees used in the second trial were transplanted into 3 L containers filled with the same media, amended with the same amount of fertilizer and allowed to overwinter outdoors. The following spring trees used in the second trial were transplanted into 20 L containers after leafing out and allowed to establish for 1.5 months. For both trial establishment periods, watering was carried out as needed (generally every 2-3 days) to maintain trees under well-watered conditions. In both trials, once trees were judged to have been established in the 20 L containers eight trees per cultivar were selected for uniformity and transferred onto a 16-cell weighing lysimeter system (Fig. 4.3-1). This greenhouse system has previously been described by Chard et al. (2016) and will be briefly reviewed here.

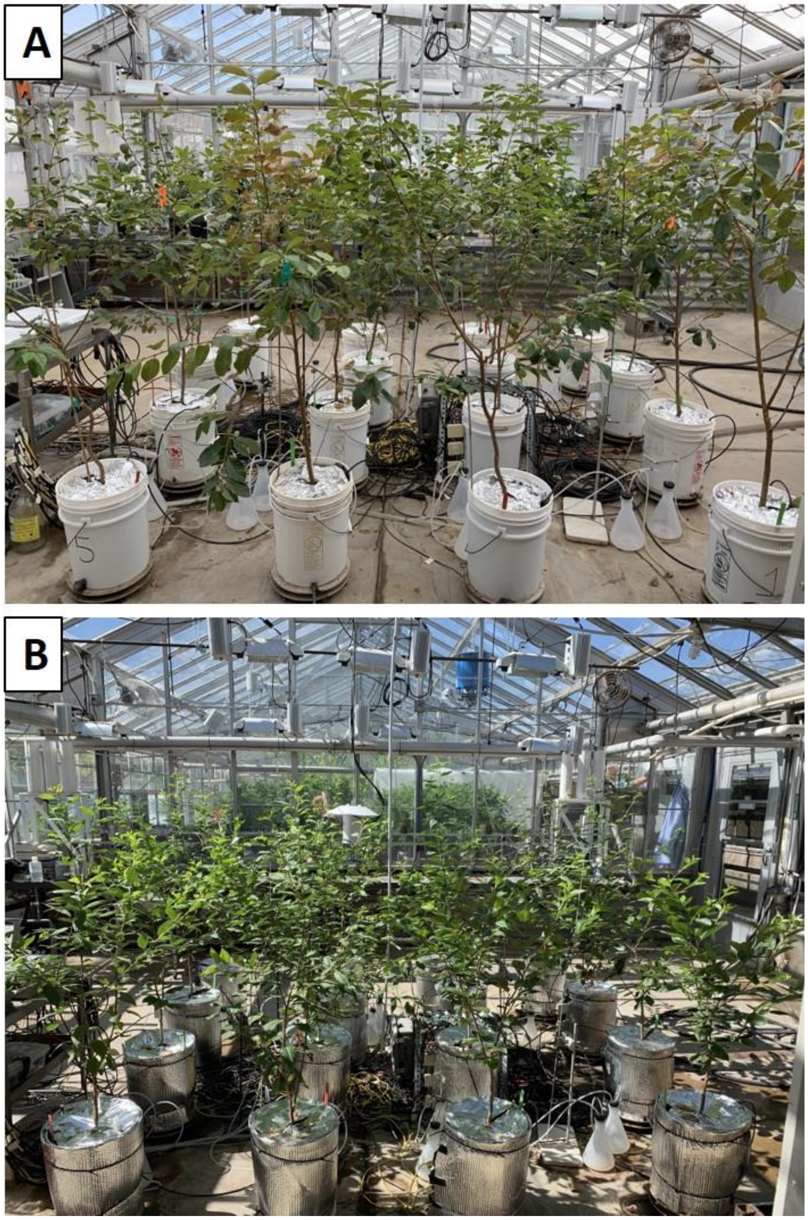


Fig. 4.2.1. A 16-cell lysimeter system used to assess transpiration rates for Krymsk® 5 and 6 rootstocks for the first (A) and second (B) drought trials.

Each load cell platform consisted of a 35 kg beam load cell anchored between two aluminum plates. Each container on the load cell was insulated to minimize thermal changes in the root zone and the soil surface was covered with aluminum foil to prevent evaporation. Load cells were connected to a data logger which continuously logged weight. Transpiration was calculated from changes in weight over the course of twenty-four hours as there was no drainage from the bottom of the containers and minimal evaporation from the soil surface. Trees were brought up to field capacity and the individual weights were programmed into the data logger as the upper threshold. Transpiration rates were allowed to decline to roughly 25% of peak values before irrigation was triggered during the first trial. This was increased to roughly 30% of peak transpiration rates for the second trial because of leaf burn and defoliation were observed during the winter trial. A relay driver was used to control 16 solenoid valves that were activated when individual load cells fell below a pre-programmed lower threshold.

During the second trial, gas exchange measurements were made with a portable photosynthesis measurement system (Li-Cor 6800, Li-Cor, Lincoln NB, USA) the day of peak drought stress, and two days after irrigation when recovery transpiration rates peaked. Chamber conditions were set to the following parameters: fan speed of 5000 rpm, flow rate of 600 μmol s^{-1}, CO_2 concentration of 400 μmol mol^{-1}, leaf temperature of 25°C, leaf vapor pressure of 1.5 kPa and irradiance of 700 μmol m^{-2} s^{-1} with 10% blue and 90% red. Three trees per treatment were measured with three leaves sampled per tree.

After clamping on a leaf, the system was given 10 min to allow readings to come to equilibrium before taking a running average of gas exchange parameters for 20 min.

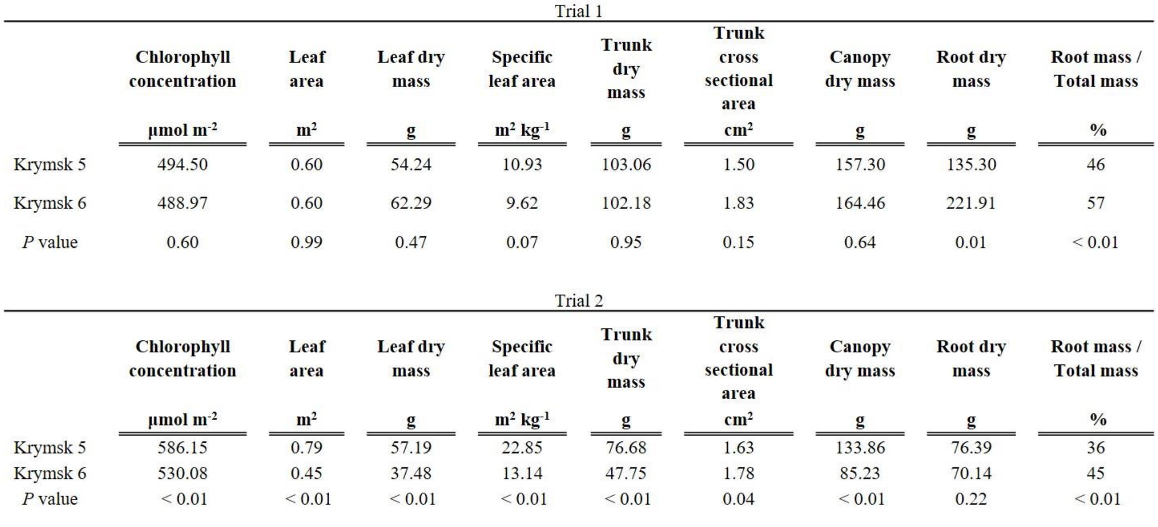
Trees were destructively harvested at the end of each trial. Chlorophyll was measured on 10 recently developed, fully expanded leaves per tree using a hand-held chlorophyll meter (Apogee Instruments, model MC-100). Trunk diameter at 3 cm above the soil surface was measured and trunk cross-sectional area was calculated. Leaves were removed and weighed and area (LI-COR, model LI-3100C area meter, Lincoln, NE) of leaves representing 10% of leaf fresh weight was determined, and whole canopy leaf area extrapolated. Leaf and woody tissue dry mass were measured after drying for three days at 80°C. Root balls were washed to remove all bound soil with care taken to try and retain as many fine roots as possible. Roots were oven dried for three days at 80°C before dry mass taken.

Both trials were completely randomized on the lysimeter system with rootstock cultivar treated as a fixed effect. Each rootstock cultivar had eight replicate trees. Cumulative transpiration, number of dry down days, and gas exchange parameters were analyzed utilizing two-way repeated measures multivariate analysis of variance (MANOVA). All other variables were analyzed using a two-way analysis of variance (ANOVA). All data were analyzed using R statistical software (R Foundation for Statistical Computing, Vienna, Austria).

4.3 Results

Analysis of destructive harvest parameters for the first trial showed K6 had significantly more dry root mass (P = 0.01) and a lower canopy to root ratio (P < 0.01) than K5 rootstocks (Table 4.4-1). All other parameters showed no statistical differences between rootstocks. This contrasted with the second trial in which K5 rootstocks had significantly higher average values for almost all destructive harvest parameters measured. The exceptions to this pattern were trunk cross-sectional area, which was lower than K6, and root dry mass, which had a higher average in K5 but was not significantly different than the K6 (P = 0.22) (Table 4.4-1).

Table 4.3.1. Destructive harvest parameters for Krymsk® 5 and 6 for two trials.



Trial 1

	Chlorophyll concentration	**Leaf area**	**Leaf dry mass**	**Specific leaf area**	**Trunk dry mass**	**Trunk cross sectional area**	**Canopy dry mass**	**Root dry mass**	**Root mass / Total mass**
	µmol m^{-2}	**m^2**	**g**	**m^2 kg^{-1}**	**g**	**cm^2**	**g**	**g**	**%**
Krymsk 5	494.50	0.60	54.24	10.93	103.06	1.50	157.30	135.30	46
Krymsk 6	488.97	0.60	62.29	9.62	102.18	1.83	164.46	221.91	57
P value	0.60	0.99	0.47	0.07	0.95	0.15	0.64	0.01	< 0.01

Trial 2

	Chlorophyll concentration	**Leaf area**	**Leaf dry mass**	**Specific leaf area**	**Trunk dry mass**	**Trunk cross sectional area**	**Canopy dry mass**	**Root dry mass**	**Root mass / Total mass**
	µmol m^{-2}	**m^2**	**g**	**m^2 kg^{-1}**	**g**	**cm^2**	**g**	**g**	**%**
Krymsk 5	586.15	0.79	57.19	22.85	76.68	1.63	133.86	76.39	36
Krymsk 6	530.08	0.45	37.48	13.14	47.75	1.78	85.23	70.14	45
P value	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	0.04	< 0.01	0.22	< 0.01

Average days to reach minimum transpiration thresholds were significantly different (P = 0.02) during the first trial with K5 averaging 12 days and K6 averaging 8.8 days across all trees over three dry down events. During the second trial there was no significant difference between rootstocks in the number of days to dry down to the minimum threshold between treatments with both rootstocks taking approximately 6 days.

Prior to the first dry down for the first trial there was a significant difference (P = 0.02) in transpiration rates with peak rates for K5 and K6 at approximately 0.9 kg/day and 1.2 kg/day respectively. For the second trial pre-dry down transpiration rates were not significantly different (P = 0.13) with K5 transpiration peaking at 1.5 kg/day and K6 peaking at 1.7 kg/day. For both the first and second trials transpiration rates in both rootstocks did not recover to their pre-droughted levels after the first drought event (Fig. 2). There were no significant differences between recovery (peak) transpiration rates between rootstocks following the second and third drought events in either the first (P = 0.22 and P = 0.15) or second (P = 0.15 and P = 0.48). However, during the second trial after the first drought event K5 recovered to a greater extent (P = 0.04) than K6 on the day before peak transpiration rates were recorded. Additionally, K5 rootstocks had a nearly significant peak recovery (P = 0.10) after the first dry down period.

Gas exchange measurements taken throughout the second trial indicated that the K6 rootstock had higher rates of transpiration (P = <0.01), stomatal conductance (P = <0.01), and carbon assimilation (P = 0.01) (Fig. 3). There were no differences between rootstocks in water use efficiency over the course of the trial (P = 0.67) however water use efficiency increased significantly in the K6 rootstocks (P = 0.01) while there was a less significant increase in K5 (P = 0.10).

4.4 Discussion

There were significant interactions between all measured parameters and trial, complicating analysis of the data (Fig. 4.5-1).

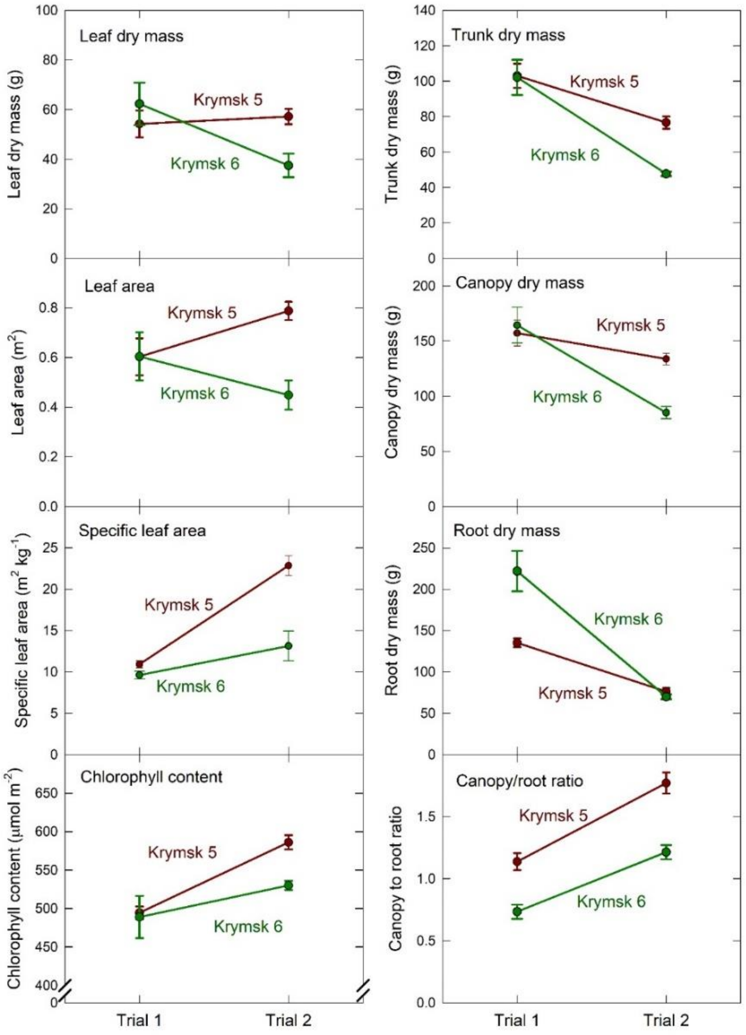


Fig. 4.4.1. Average destructive harvest parameters for rootstocks Krymsk® 5 (red) and Krymsk® 6 (green) over the course of two trials

There were several factors that may have accounted for the differing results between trials. High spider mite pressure was noted during periods of the first trial despite regular sprays. This affected both treatments but may have depressed physiological responses and was an added source of stress. Differences in seasonality and overwintering of trees used in the second trail may have also played a role. Trees for the first trial were received in the late fall, transplanted and allowed to establish for three months before being placed on the lysimeter system. Because it was already late in the season it may be possible that these trees had already begun downregulation of their metabolism in preparation for winter dormancy. Artificial extension of their growing season in a greenhouse setting may have resulted in more muted physiological responses as can be seen in lowered transpiration rates for the first trial (Fig. 4.5.2). Trees for the first trial had a higher canopy and root weights when compared to the second trial, however, this was a result of being given three months to establish before start of the trial, partially due to their relatively slow growth rate. For the second trial, vigorous growth was noted in both rootstocks coming out of the over wintering period and were placed on the lysimeter system after one and a half months of establishment. The shorter establishment time was also deemed beneficial to the study as smaller trees were better accommodated by the lysimeter system. Despite both treatments having what appeared to be roughly equal canopy volumes before the start of the second trial, the K6 rootstocks had significantly higher whole canopy transpiration rates, as measured by the lysimeter system (data not shown). This was confirmed by leaf level gas exchange measurements taken during the trial in which K6 was seen to have higher rates of transpiration overall (Fig. 4.5.3). To equalize transpiration rates the K6 rootstocks were heavily pruned before the start of the second trial.

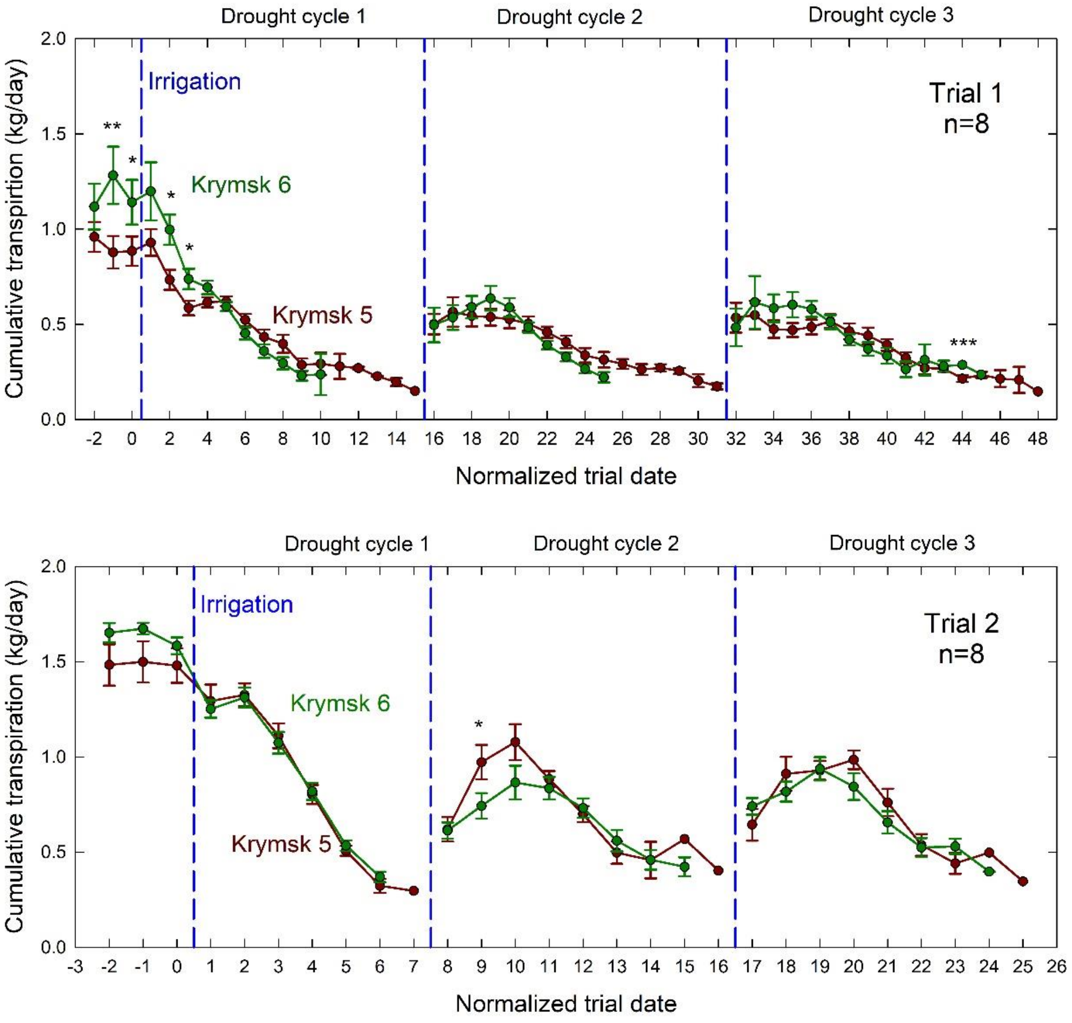


Fig. 4.4.2. Average daily transpiration rates for Krymsk® 5 (red) and Krymsk® 6 (green) rootstocks. during the first trial (top) and second trial (bottom).

Despite these limitations there are several general patterns that can be observed from both trials. Over the course of the first trial K6 had significantly shorter (P = 0.02) average days to minimum transpiration thresholds. During the second trail, while the difference in average days to minimum transpiration was not significant (P = 0.75), K6 trees showed a pattern of using available water more quickly than K5. This observation was corroborated by higher leaf level transpiration rates and stomatal conductance in the K6 measured by gas exchange throughout the second trial. Canopy effects can play a significant role in plant water use strategy and adaptation to drought stress through increased stomatal regulation and decreased leaf area (dos Santos et al. 2017; Rieger and Dummel, 1992). This requires careful consideration when drawing conclusions about physiological changes in

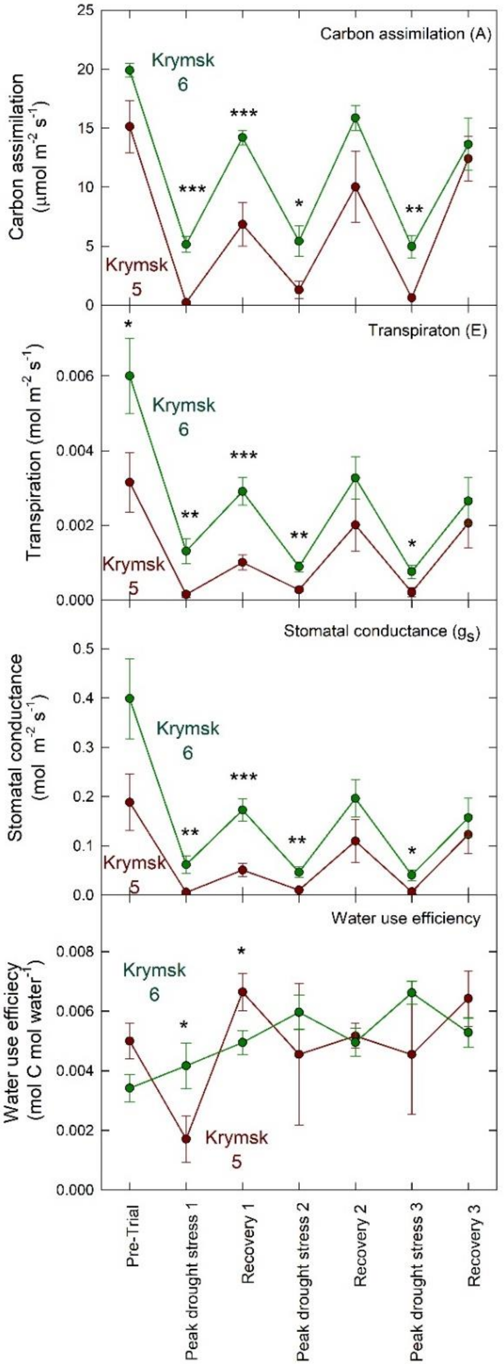


Fig. 4.4.3. Leaf level gas exchange parameters for rootstocks Krymsk® 5 (red) and 6 (green) during the second drought stress trial.

grafted trees that might be inferred from studies done on ungrafted rootstocks. In *Prunus* rootstocks however, more vigorous cultivars have been shown to increase stem water potential, CO_2 assimilation rate, stomatal conductance, intercellular CO_2 concentration and photochemical efficiency in grafted scions (Gonçalves et al. 2005). Rootstock growth potential has also been positively correlated to cytokinin levels in xylem sap which in turn impacts shoot vigor (Sorce et al., 2002). These changes to whole plant physiology conferred by rootstocks suggest that responses observed here may provide a good indication of responses in grafted trees. Furthermore, research conducted in the Netherlands found ‘Kordia’ scions grafted to K6 rootstocks produced significantly more fruit per tree than those grafted to K5 which is in line with findings of this study (Mass et al., 2012).

In examining water use strategies, consideration of how parent species differ between cultivars is useful. Krymsk® 6 is a hybrid between the domesticated *P. cerasus* and another hybrid consisting of *P. cerasus* × *P. maackii*. Krymsk® 5 by contrast is a hybrid between the relatively wild species of *P. fruticosa* and the ornamental *P. serrulata*. While *P. cerasus* is thought to originated through a natural cross between *P. avium* and *P. fruticosa*, it has been used in domesticated settings since 300 BCE making it more responsive to intensive management (Serradilla et al. 2016). *Prunus fruticosa* is a relatively short, wild shrub (0.5 - 1m) which when crossed with other *Prunus* species confers dwarfing qualities in addition to having good drought and frost tolerance (Barać et al. 2017). *Prunus serrulata*, while cultivated in landscaped settings, has not been the focus of agronomic breeding and may be less developed for vigorous growth (Kato et al., 2014). During the first study, there was no significant differences in leaf area (P = 0.99) or dry mass (P = 0.47) but, as stated previously, K5 took significantly longer to reach irrigation thresholds. During the second trial, time to reach irrigation thresholds was not significant between K5 and K6 but K5 rootstocks had a significantly larger canopy with greater leaf area (P = <0.01) and leaf dry mass (P = <0.01) (Table 4.4.1). In both trials K5 had lower percent root mass as a function of total dry weight and lowered measured transpiration rates during the second trial. This pattern of more conservative water use in K5 could be a function of either greater stomatal control or a smaller root system which in turn limited transpiration rates. More conservative water usage by K5 rootstocks may be due to their relatively less domesticated linage as prolonged severe drought is more common in wild land settings. Krymsk® 6 rootstocks, with a predominance of *P. cesarus* genetics may be more adapted to high input agricultural settings in which resources are generally abundant and trees are bred for vigor.

Drought events in the field often occur gradually whereas the imposition of individual drought events in this study occurred over the course of 6 to 9 days. Osmotic adjustment is dependent on the rate of stress development with more progressive water stress allowing for greater up regulation of osmotic potential in plant tissues (Jones and Rawson 1979; Wang et al. 1995). Significantly reduced leaf osmotic potentials (Ψ_π) have been observed in peach scions grafted to *Prunus* rootstocks after 16 days and one month of progressive drought when compared to well-watered controls (Jiménez et al., 2013;

Mellisho et al., 2011). This contrasts with findings in ungrafted *P. persica* where, following rapid imposition of drought over the course of 8 days, stressed trees showed no significant differences in Ψ_π when compared to well-watered controls (Escobar-Gutiérrez et al. 1998). The relatively rapid onset of individual drought events used in this study may have limited the initial degree of osmotic adjustment. However, the repeated cycling of drought and rewatering may have allowed for osmotic adjustment over the course of the trials which lasted 48 days for the first trial and 25 days in the second trial.

4.5 Conclusions

Both K5 and K6 did not return to pre-drought transpiration rates following reductions in total daily water transpired of ~75%. Krymsk® 6 utilized available water faster in the first trial and had higher leaf level transpiration rates and stomatal conductance as measured by a portable gas exchange system. Krymsk® 5 had a lower root mass fraction as a function of the total mass in both trials and lower leaf level transpiration rates. More conservative water use by K5 seen here could be a function of greater stomatal control or the result of a smaller root system from which to draw water thereby resulting in lowered transpiration rates. Both cultivars appeared to be able to recover from repeated, acute drought though transpiration rates were depressed following drought. Further studies are needed to determine if these patterns seen here in ungrafted rootstocks are consistent when rootstocks are grown with a common grafted scion.

4.6 Literature cited

Atkinson, C.J., J. Policarpo, A.D. Webster, and G. Kingswell. 2000. Drought tolerance of clonal Malus determined from measurements of stomatal conductance and leaf water potential. Tree Physiol. 20:557-563.

Barać, G., V. Ognjanov, D.O Vidaković, D. Dorić, M. Ljubojević, J. Dulić, M. Miodragović, and K. Gašić. 2017. Genetic diversity and population structure of European ground cherry (*Prunus fruticosa* Pall.) using SSR markers. Scientia Hort. 224:374-383.

Beeson Jr, R.C. 2011. Weighing lysimeter systems for quantifying water use and studies of controlled water stress for crops grown in low bulk density substrates. Agr. Water Mgt. 98:967-976.

Chard, J., M. van Iersel, and B. Bugbee. 2016. Mini-lysimeters to monitor transpiration and control drought stress: system design and unique applications. https://www.semanticscholar.org

Chalmers D.J., P.D. Mitchell, L. Van Heek. 1981. Control of peach tree growth and productivity by regulated water supply, tree density and summer pruning. J. Am. Soc. Hort. Sci. 106:307–312

Cline, J.A. 2019. Planting density and size-controlling rootstocks influence the performance of Montmorency tart cherry (*Prunus cerasus* L.). Can. J. of Plant Sci., 100:16-28.

dos Santos, I.C., A.A.F. de Almeida, C.P. Pirovani, M.G.C. Costa, B.S. Bellete, L. Freschi, W. Soares Filho, M.A. Coelho Filho, and A. da Silva Gesteira. 2017. Differential accumulation of flavonoids and phytohormones resulting from the canopy/rootstock interaction of citrus plants subjected to dehydration/rehydration. Plant Physiol. and Biochem. 119:147-158.

Eremin, G., 2003. *Prunus* plant named 'VSL-*2*'. U.S. Patent Application 09/880,953.

Eremin, G., Varieties International LLC, 2005. Prunus plant named ‘LC-52’. U.S. Patent Application 09/880,950.

Eremin, G.V., V.N. Podorozhniy, and O.V. Eremina. 2017. Use of genetic diversity of the genus *Prunus* L. In selection of clonal rootstocks for stone fruit crops and features of their reproduction. Proc. of the Latvian Acad. of Sci. 71:173-177.

Escobar-Gutiérrez, A.J., B. Zipperlin, F. Carbonne, A. Moing, and J.P. Gaudillére. 1998. Photosynthesis, carbon partitioning and metabolite content during drought stress in peach seedlings. Func. Plant Biol. 25:197-205.

Forner-Giner, M.A., J. Rodriguez-Gamir, B. Martínez-Alcántara, A. Quinones, D.J. Iglesias, E. Primo-Millo, and J. Forner. 2014. Performance of Navel orange trees grafted onto two new dwarfing rootstocks (Forner-Alcaide 517 and Forner-Alcaide 418). Scientia Hort. 179:376-387.

Goldhamer, D.A., M. Viveros, and M. Salinas. 2006. Regulated deficit irrigation in almonds: effects of variations in applied water and stress timing on yield and yield components. Irr. Sci. 24:101-114.

Gonçalves, B., J. Moutinho-Pereira, A. Santos, A.P. Silva, E. Bacelar, C. Correia, and E. Rosa. 2006. Scion–rootstock interaction affects the physiology and fruit quality of sweet cherry. Tree Physiol. 26:93-104.

Hajagos, A. and G. Végvári. 2013. Investigation of tissue structure and xylem anatomy of eight rootstocks of sweet cherry (*Prunus avium* L.). Trees, 27:53-60.

Jiménez, S., J. Dridi, D. Gutiérrez, D. Moret, J. J. Irigoyen, M. A. Moreno, & Y. Gogorcena, Y. 2013. Physiological, biochemical and molecular responses in four Prunus rootstocks submitted to drought stress. Tree physiol. 33:1061-1075.

Johnson, R.S., and D.F. Handley. 2000. Using water stress to control vegetative growth and productivity of temperate fruit trees. HortScience. 35:1048–1050.

Jones, H.G. 2007. Monitoring plant and soil water status: established and novel methods revisited and their relevance to studies of drought tolerance. J. of expt. Bot. 58:119-130.

Jones, M.M. and H.M. Rawson. 1979. Influence of rate of development of leaf water deficits upon photosynthesis, leaf conductance, water use efficiency, and osmotic potential in sorghum. Physiol. Plantarum. 45:103-111.

Kato, S., A. Matsumoto, K. Yoshimura, T. Katsuki, K. Iwamoto, T. Kawahara, Y. Mukai, Y. Tsuda, S. Ishio, K. Nakamura, and K. Moriwaki. 2014. Origins of Japanese flowering cherry (*Prunus* subgenus *Cerasus*) cultivars revealed using nuclear SSR markers. Tree Genet. & Genom. 10:477-487.

Ladjal, M., N. Deloche, R. Huc, and M. Ducrey. 2007. Effects of soil and air drought on growth, plant water status and leaf gas exchange in three Mediterranean cedar species: *Cedrus atlantica, C. brevifolia* and *C. libani*. Trees. 21:201-213.

Lang, G.A., 2017. The cherry industries in the USA: current trends and future perspectives. In: VIII Intl. Cherry Symp. 1235:119-132.

Long, E. L. and C. Kaiser, C. 2010. Sweet Cherry Rootstocks. Pacific Northwest Ext. Publ. Oregon State Univ., OR., USA. 619, 1–8. Corvallis, OR, USA. 619:1–8

Long, L. E., L. J. Brewer, and C. Kaiser. 2014. Cherry rootstocks for the modern orchard." OSU Extension Service. Oregon State Univ.: Pacific Northwest Ext. Publ. Feb. 2014. http://extension. oregonstate.edu/wasco/sites/default/files/cherryrootstocksmodern-long. pdf.

Long, L.E., C. Kaiser, and L.J. Brewer. 2013. Sweet cherry (*Prunus avium*) cultivar, rootstock and training system interactions in Oregon, USA. In: VII Intl. Cherry Symp. 1161:331-338.

Maas FM, J. Balkhoven-Baart, P.A.H. van der Steeg. 2014. Evaluation of Krymsk® 5 (VSL-2) and Krymsk® 6 (LC-52) as rootstocks for sweet cherry ‘Kordia’. Acta Hort. 1058:531–536.

Martínez-Ballesta, M.C., C. Alcaraz-López, B. Muries, C. Mota-Cadenas, and M. Carvajal. 2010. Physiological aspects of rootstock–scion interactions. Scientia Hort. 127:112-118.

Massonnet, C., E. Costes, S. Rambal, E. Dreyer, and J.L. Regnard. 2007. Stomatal regulation of photosynthesis in apple leaves: evidence for different water-use strategies between two cultivars. Ann. of Bot. 100:1347-1356.

Mellisho, C.D., Z.N. Cruz, W. Conejero, M.F. Ortuño, and P. Rodríguez. 2011. Mechanisms for drought resistance in early maturing c. var. Flordastar peach trees. The J. of Agr. Sci. 149:609-616.

Milošević, T., N. Milošević, and J. Mladenović. 2020. Combining fruit quality and main antioxidant attributes in the sour cherry: The role of new clonal rootstock. Scientia Hort. 265:109-236.

Morandi, B., L. Manfrini, S. Lugli, A. Tugnoli, A. Micheli, A. Boini, G. Perulli, K. Bresilla, and L. Corelli Grappadelli. 2018. Physiological responses to rootstocks vigor in cherry: why dwarfing is efficient? In: XXX Intl. Hort. Congr. IHC2018: Intl. Symp. on Cultivars, Rootstocks and Mgt. Systems 1281:487-492.

Nadezhdina, N., J. Cermak. 1997. Automatic control unit for irrigation systems based on sensing the plant water status. Anais do Instituto Superior de Agronomia 46:149–157.

Naor, A., 2006. Irrigation scheduling and evaluation of tree water status in deciduous orchards. Hort. Rev. 32:111–166.

Rieger, M. and M.J. Duemmel. 1992. Comparison of drought resistance among *Prunus* species from divergent habitats. Tree physiol. 11:369-380.

Robinson, T., 2008. The evolution towards more competitive apple orchard systems in the USA. Acta Hort. 772:491-500

Roper, T., B. Black, M. Stasiak, R. Marini, J. Cline, T. Robinson, G. Lang, L. Anderson, R. Anderson, J. Freer, G. Greene and R. Perry. 2019. Performance of ‘Montmorency’ Sour Cherry (*Prunus cerasus* L.) on Size-Controlling Rootstocks at Six NC140 Trial Locations in North America. J. of the Amer. Pomol. Soc. 73:168-177.

Sorce, C., L. Mariotti, R. Lorenzi, and R. Massai. 2007. Hormonal factors involved in the control of vigour of grafted peach [*Prunus persica* (L.) Batsch] trees and hybrid rootstocks. Adv. in Hort. Sci.:68-74.

Tworkoski, T. and G. Fazio. 2015. Effects of size-controlling apple rootstocks on growth, abscisic acid, and hydraulic conductivity of scion of different vigor. Intl. J. of Fruit Sci. 15:369-381.

Wang, Z., B. Quebedeaux, and G.W. Stutte. 1995. Osmotic adjustment: effect of water stress on carbohydrates in leaves, stems and roots of apple. Func. Plant Bio. 22:747-754.

CHAPTER 5

SUMMARY AND CONCLUSIONS

5.1 Research summary

New fruit orchard plantings are shifting to the use of dwarfing rootstocks, high density plantings, and open canopy training systems to maximize production (Robinson, 2006). Dwarfing rootstocks have smaller rootzone volumes necessitating careful irrigation management (Tworkoski and Fazio 2015). At the same time deficit irrigation has been shown to improve fruit post-harvest quality, control vegetative growth and maximize water use efficiency in orchard crops (Fereres and Evans, 2006). Current methods of irrigation scheduling, which rely on evaporative modeling or measurements of mid-day stem water potential (Ψ_{stem}), are either not precise enough or are too labor intensive to be effective for deficit irrigation scheduling. Greater research is also needed to identify dwarfing rootstocks which are well suited to growing conditions common to the Intermountain West and are resilient to drought. The research summarized in this dissertation was conducted to improve understanding automated measures of plant water status and rootstock drought tolerance.

In the first study of this dissertation we examined automated measures of sap flow and trunk circumferential growth and diurnal variation in relation to environmental drivers and response to drought. Both sap flow index (SFI) and maximum daily trunk shrinkage (MDS) showed greater day to day variation due to environmental drivers than Ψ_{stem}. This was due in part to greater correlations of MDS and SFI to environmental drivers. Additionally, both MDS and SFI showed seasonal variation as trees shifted from vegetative growth to fruit maturation. Seasonal variability for MDS however was less than that of SFI in which seasonal responses to T_a and VPD were particularly pronounced. Correlations of both MDS and SFI to Ψ_{stem} were improved by controlling for phenological stage of growth. Maximum daily shrinkage showed the strongest overall correlations to Ψ_{stem}. Overall, the use of dendrometers seemed the most promising for future research due to their lower variability, minimal invasiveness and ability to be automated.

The second study presented in this dissertation compared peach selections from a Navajo orchard to the commercial seed propagated rootstock Lovell. Genetic lines used by the Navajo peoples have been relatively isolated since their introduction in the 1600's creating a land-race type population that may be better adapted to drought (Wytslauncy et al., 2020). Navajo peach selections had higher recovery transpiration after drought and higher canopy dry mass at the end of the study. This was a strong indication that Navajo trees are better adapted to drought and may be of use to rootstock breeders.

The third study presented here examined cherry rootstocks Krymsk® 5 and 6 for drought tolerance. The Krymsk® series were originally developed in the U.S.S.R. and are widely used as dwarfing rootstocks for cherry production. Using a portable gas exchange system measured leaf level transpiration and stomatal conductance were found to be higher in Krymsk® 6. Leaf level measurements corresponded with faster days to wilt during drought compared to Krymsk® 5 and more vigorous growth during well-watered conditions. Krymsk® 5 showed more conservative water use, with lower transpiration rates and longer days to wilt. Krymsk® 5 also had a smaller percent root mass as a function of total biomass which may have contributed to its greater water use efficiency.

This research provides insight into multiple approaches to manage water more efficiently in orchard production. Automated physiological indicators of water stress that were identified here strengthen future research and provide the basis for future commercial systems. Screening of new and commonly used rootstocks inform both breeders and growers in their selections for new cultivar developments and orchard plantings.

5.2 Future research

Research on both plant-based methods to schedule orchard irrigation and characterization of dwarfing rootstocks goes back decades. Sap flow sensors and dendrometers have previously been evaluated in irrigation scheduling and seen successful implementations (Goldhamer and Fereres, 2001; Fernández et al. 2008a). Sap flow as a mechanism to schedule irrigation faces several hurtles. Accurate calibration of sensors is technically challenging and prone to error (Forster, 2017). Indexes of sap flow may be useful to examine changes in response patterns of sap flow but are not well correlated to Ψ_{stem}, which researchers widely agree is the best indicator of plant water stress (Jones, 2004). Large shifts in response patterns that occur seasonally, and the high degree of tree to tree variability in measurements and response patterns further limit use (Dragoni et al., 2005). A workable automated irrigation system based on sap flow sensors would require extensive instrumentation to account for within orchard variability caused by microclimates and soil heterogeneity (Cueavas et al., 2013). Sap flow sensors are frequently damaged when attempting to remove them, and wounding response around the sensor means that sensors need to be re-positioned every season. The necessity of widespread instrumentation coupled with yearly sensor replacement means that an automated sap flow-based irrigation system would likely be expensive to establish and maintain (Fernandez, 2017). Nevertheless, researchers have had success in utilizing sap flow sensors in scheduling irrigation through comparing rates of sap flow from well-watered controls to plants under automated irrigation scheduling (Fernández et al., 2008b). Future research advances could focus on ways to standardize sap flow measurements to limit the need for reference controls.

Dendrometers provide a better option for automating irrigation in commercial orchards. Dendrometer measurements have been shown to be well correlated to Ψ_{stem} readings in multiple fruit tree species, providing a usable mechanism by which to schedule irrigation (Goldhamer et al. 1999). Point dendrometers have been widely used in orchard research and are inexpensive but can require invasive installation to anchor sensor in place. Recent technological advances have made band dendrometers more precise and have the added advantage of being able to be non-invasively installed and re-used over multiple seasons (Drew and Downs 2009). Band dendrometers also take into account the entire circumferential change of the tree making them less prone to positioning errors (Corell et al. 2014).. Differences in absolute values of trunk diameter or circumferential variation and the extent of instrumentation required to accurately gauge orchard wide water needs present challenges to the use of dendrometers as irrigation scheduling tools (Naor and Cohen, 2003). Future research could focus on ways to normalized measurements of trunk circumferential variation so that more universal recommendations can be made. The development of cheaper and more robust sensors could help reduce costs and make more comprehensive instrumentation attainable. Other researchers have suggested the pairing of remote sensing or drone imaging data with dendrometer measurements to categorize and instrument orchards by zones (Fereres and Goldhamer, 2003).

The development of new rootstocks is an ongoing process and rootstocks specifically adapted to semi-arid climates with calcareous soils would be a huge benefit to growers throughout the world. Follow up research is needed to determine how selections of Navajo peaches perform in the field and potential functionality as rootstocks. Because the variety is propagated by seed, work is needed to identify promising lines and develop clonal vegetative propagation to improve uniformity. Further characterization of these populations is being undertaken at Utah State University (Wytsalucy, 2019).

Further characterization of Krymsk® and other dwarfing rootstocks could benefit grower and improve recommendations. Rootstocks are often screened in coordinated planting trials spanning a range of growing environments (Roper et al., 2019). These trials are essential but can be costly and difficult to coordinate. Intensive screening in greenhouse studies can provide a rapid and less expensive way of determining performance and elucidate mechanisms of rootstock response. Crucial to these studies however is the comparison of ungrafted and grafted performance as physiological responses are dictated by both root shoot interactions, hormonal regulation and genetic interactions (Gregory et al., 2013).

5.3 Literature cited

Corell, M., Girón, I.F., Galindo, A., Torrecillas, A., Torres-Sánchez, R., Pérez-Pastor, A., Moreno, F. and Moriana, A., 2014. Using band dendrometers in irrigation scheduling: influence of the location inside the tree and comparison with point dendrometer. Agricultural Water Management, 142:29-37.

Cuevas, M.V., Martín-Palomo, M.J., Diaz-Espejo, A., Torres-Ruiz, J.M., Rodriguez-Dominguez, C.M., Perez-Martin, A., Pino-Mejías, R. and Fernández, J.E., 2013. Assessing water stress in a hedgerow olive orchard from sap flow and trunk diameter measurements. Irrigation Science, 31:729-746.

Dragoni, D., Lakso, A.N. and Piccioni, R.M., 2005. Transpiration of apple trees in a humid climate using heat pulse sap flow gauges calibrated with whole-canopy gas exchange chambers. Agricultural and Forest Meteorology, 130:85-94.

Drew, D.M. and Downes, G.M., 2009. The use of precision dendrometers in research on daily stem size and wood property variation: a review. Dendrochronologia, 27:159-172.

Fereres, E., Goldhamer, D., 2003. Suitability of stem diameter variations and water potential as indicators for irrigation scheduling of almond trees. The Journal of Horticultural Science and Biotechnology 78, 139–144.

Fereres, E., and Evans, R.G. 2006. Irrigation of fruit trees and vines: an introduction. Irrigation Science, 24:55–57.

Fernández, J.E., Green, S.R., Caspari, H.W., Diaz-Espejo, A. and Cuevas, M.V., 2008a. The use of sap flow measurements for scheduling irrigation in olive, apple and Asian pear trees and in grapevines. Plant and Soil, 305:91-104.

Fernández, J.E., Romero, R., Montaño, J.C., Diaz-Espejo, A., Muriel, J.L., Cuevas, M.V., Moreno, F., Girón, I.F., and Palomo, M.J. 2008b. Design and testing of an automatic irrigation controller for fruit tree orchards, based on sap flow measurements. Australian Journal of Agricultural Research 59:589–598.

Fernández, J.E., 2017. Plant-based methods for irrigation scheduling of woody crops. Horticulturae, 3:35.

Forster, A.M., 2017. How Reliable Are Heat Pulse Velocity Methods for Estimating Tree Transpiration? Forests 8:350

Goldhamer, D.A., Fereres, E., Mata, M., Girona, J., Cohen, M., 1999. Sensitivity of continuous and discrete plant and soil water status monitoring in peach trees subjected to deficit irrigation. Journal of the American Society for Horticultural Science 124:437–444.

Goldhamer, D.A. and Fereres, E., 2001. Irrigation scheduling protocols using continuously recorded trunk diameter measurements. Irrigation Science, 20:115-125.

Gregory, P.J., Atkinson, C.J., Bengough, A.G., Else, M.A., Fernández-Fernández, F., Harrison, R.J., Schmidt, S., 2013. Contributions of roots and rootstocks to sustainable, intensified crop production. Journal of Experimental Botany 64:1209–1222.

Jones, H.G. 2004. Irrigation scheduling: advantages and pitfalls of plant-based methods. Journal of Experimental Botany, 55:2427–2436.

Naor, A., Cohen, S., 2003. Sensitivity and variability of maximum trunk shrinkage, midday stem water potential, and transpiration rate in response to withholding irrigation from field-grown apple trees. HortScience 38:547–551.

Robinson, T., 2006, August. The evolution towards more competitive apple orchard systems in the USA. Acta Horticulturea 772:491-500

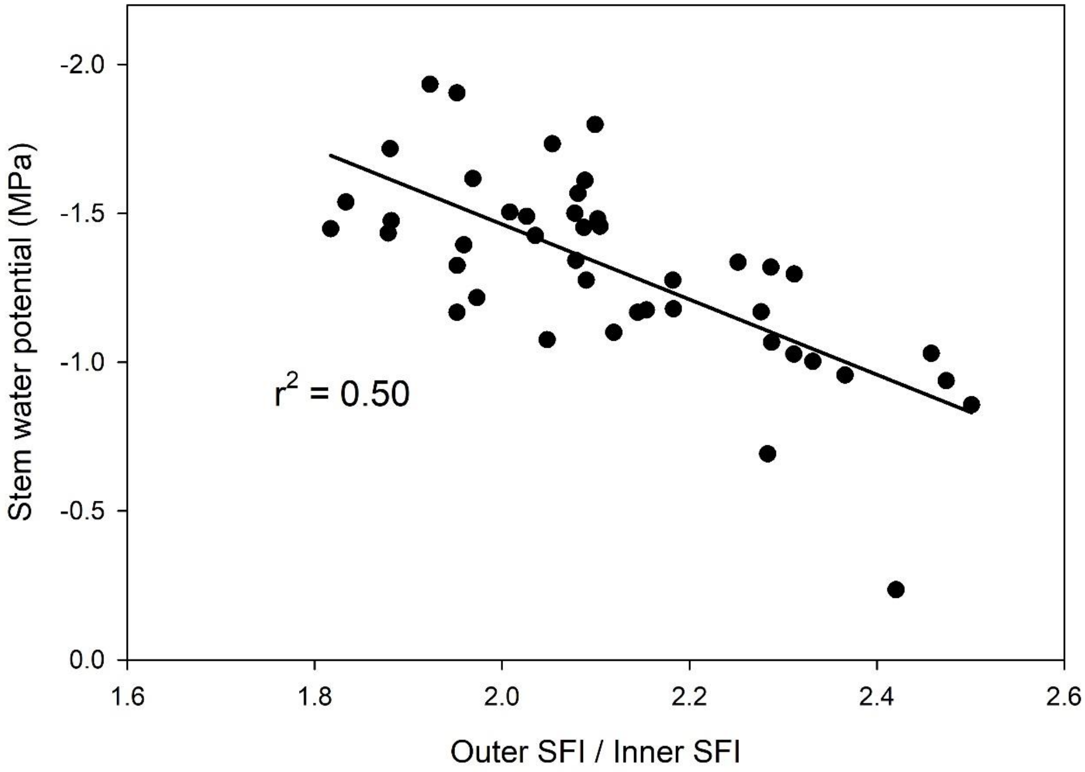
Roper, T., B. Black, M. Stasiak, R. Marini, J. Cline, T. Robinson, G. Lang, L. Anderson, R. Anderson, J. Freer, G. Greene and R. Perry. 2019. Performance of ‘Montmorency’ Sour Cherry (*Prunus cerasus* L.) on Size-Controlling Rootstocks at Six NC140 Trial Locations in North America. Journal of the American Pomological Society. 73(3):168-177.

Tworkoski, T. and 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, 15:369-381.

Wytsalucy, R.C., 2019. Explorations and Collaborations on Two Under-Recognized Native American Food Crops: Southwest Peach (*Prunus Persica*) and Navajo Spinach (*Cleome Serrulata*). Master’s thesis, Utah State University. https://digitalcommons.usu.edu/etd/7612/

Wytsalucy, R.C., K. Gasic, M. Robbins, G. Cardon, D. Drost, and B. Black. 2020. Genetic analysis of peaches grown by Native Americans in the North American Southwest. Journal of the American Pomological Society. 74(2):76-83.

APPENDIX A SUPPLIMENTAL GRAPHS



Correlation of outer sap flow index to inner sap flow index to stem water potential from data pooled from ‘Aztec Fuji’ and ‘Scilate (Envy™)’ scions on Malling 9 rootstocks.

CURRICULUM VITAE

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Education:

Utah State University **Logan, Utah**
PhD candidate in Crop Physiology Jan. 2017 - Present
Dissertation: "Fruit tree responses to water stress: sap flow, trunk circumferential variation and rootstock evaluation."
GPA: 3.85

University of Georgia **Athens, Georgia**
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Thesis: "Soil moisture senor based automated irrigation use in specialty crop production and root disease suppression"
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University of Vermont **Burlington, Vermont**
Bachelor of Science Botany Sept. 2002 - May 2007
GPA: 2.92

Research Experience:

Utah State University **Logan, Utah**
Graduate research assistant Jan. 2017 – Present

- Research, testing and data analysis related to the development and use of experimental sap flux sensors
- Operation and maintenance of 16 cell weighing lysimeter system
- Supervision and mentoring of undergraduate researchers
- Presentation of scientific data and analysis at conferences

University of Georgia **Athens, Georgia**
Graduate research assistant Jan. 2014 – Aug. 2016

- Installed research trials utilizing soil moisture sensor based automated irrigation in commercial nurseries and greenhouses
- Developed research trials to test specific hypotheses
- Statistical analysis, reviewed scientific literature and created of articles for submission to professional journals

Advanced Services Inc. **Gilroy, California**
Cross category technician for Syngenta Flowers Sept. 2012 – Nov. 2013

- Worked as part of a product research and development technical team to coordinate and carry out seeding, transplanting, growing, scouting, and data collection of floricultural research crops
- Provided dynamic feedback on ongoing research trials to principal investigators

- Shared observations and knowledge of trials with key stakeholders during meetings and tours

Conrad Fafard inc. **Suffolk County, New York**
Fafard research and development intern Jan. 2012 – Aug. 2012

- Worked as a field technician as part of the Fafard research and development team to trial soilless media
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Teaching Experience:

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University of Georgia **Athens, Georgia**
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Publications:

- Wheeler, W.D., P.A. Thomas, M. van Iersel, M. Chappell. 2020 Implementation of soil moisture sensor based automated irrigation in woody ornamental production. Journal of Environmental Horticulture, 38(1):1-7
- Wheeler, W.D., R. Wytsalucy, B. Black, G. Cardon, and B. Bugbee. 2019. Navajo peach trees have greater physiological resilience to water stress than Lovell peach trees: Precision water stress using automated weighing lysimeters. HortScience 54(5): 799-803.
- Wheeler, W.D., P.A. Thomas, M. van Iersel, and M. Chappell. 2018. Implementation of soil moisture sensor based automated irrigation in commercial floriculture production. HortTechnology 28(6): 719-727.
- Wheeler, W.D., J. Williams-Woodward, P.A. Thomas, M. van Iersel, and M.R. Chappell. 2017. Impact of Substrate Volumetric Water on *Pythium aphanidermatum* Infection in *Petunia*× *hybrida*: A Case Study on the Use of Automated Irrigation in Phytopathology Studies. Plant Health Progress, 18(2): 120-125.

Conference Presentations:

- Wheeler, W.D., B. Bugbee. 7/2019. Determination of tree water status based on the ratio of relative sap velocity measurements to modeled evaporative demand. The American Society of Horticultural Science Annual Conference. Las Vegas, NV.
- Wheeler, W.D., B. Black, B. Bugbee. 4/2019. Precision drought stress using weighting lysimeters. Committee on Controlled Environment Technology and Use. Montréal, Quebec, CA.
- Wheeler, W.D. 12/2018. Automated monitoring of tree hydration. USU Department of Plants, Soils & Climate seminar series. Logan, UT.
- Wheeler, W.D., B. Black, B. Bugbee. 05/2018. Orchard tree hydration monitoring using sap flow sensors and evapotranspirative modeling. USU Kaysville Fruit and Vegetable Field Day. Kaysville, UT.
- Wheeler, W.D., B. Black, B. Bugbee. 04/2018. Evaluation of Tree Water Status Based on the Ratio of Sap Flow to Evaporative Demand. Committee on Controlled Environment Technology and Use. Raleigh, NC.
- Wheeler, W.D. 03/2018. Measurement of tree water status based on the ratio of sap velocity to evaporative demand. Spring Runoff Conference. Logan, UT.
- Wheeler, W.D. 01/2018. Automated irrigation for greenhouse and nursery production. Utah Green Industry Conference & Trade Show. Sandy, UT.
- Wheeler, W.D., B. Black, B. Bugbee. 11/2017. Analysis of drought tolerance of cherry rootstocks using automated weighing lysimeters. Regional Rootstock Improvement Committee. Wenatchee, WA.
- Wheeler, W.D., B. Black, B. Bugbee. 04/2017. Analysis of drought tolerance among cherry rootstocks using automated weighing lysimeters in controlled environments. Committee on Controlled Environment Technology and Use. Pacific Grove, CA.
- Wheeler, W.D., J. Williams-Woodward, P.A. Thomas, M. van Iersel, M. Chappell. 06/2016. Sensor based automated irrigation impacts *Pythium aphanidermatum* infection in *Petunia* × *hybrida* ‘Dreams Red’. The American Society of Horticultural Science Annual Conference. Atlanta, GA.
- Wheeler, W.D., J. Williams-Woodward, P.A. Thomas, M. van Iersel, M. Chappell. 06/2015. Root disease incidence and severity in nursery crops with soil moisture sensor-based automated irrigation. The American Society of Horticultural Science Annual Conference. New Orleans, LA.
- Wheeler, W.D., P.A. Thomas, M. van Iersel, J. Williams-Woodward, M. Chappell, 04/2015. Benefits of soil moisture sensor based automated irrigation in commercial greenhouse and nursery production. The Southern Regional American Society of Horticultural Science Annual Conference. Atlanta, GA.

Honors and Awards:

- Utah Extension Water Initiative Grant. 8/2018. $68942. Measurement of fruit tree water status based on the ratio of sap velocity to evaporative demand.
- Dr. Royce S. and Pearl Bringhurst Scholarship. 04/2018. $895 USU Plant, Soils and Climate Graduate Student Assistantship.
- Bertrand D. Tanner/Campbell Scientific Graduate Fellowship 04/2018. $5000 USU Plant, Soils and Climate Graduate Student Assistantship.
- Apogee Instruments - Campbell Scientific Graduate Fellowship 04/2018. $1000 USU Plant, Soils and Climate Graduate Student Assistantship.
- Dr. Royce S. and Pearl Bringhurst Scholarship 04/2017. $1200 USU Plant, Soils and Climate Graduate Student Assistantship.
- International Fruit Tree Association research grant. 03/2017. $5000. Cherry rootstock evaluation of drought resistance and recovery to improve recommendations for selection and irrigation management to producers in arid regions.
- 3rd place, Norman F. Childers M.S. Graduate student paper competition. 04/2015. Annual Meeting of the Southern Regional American Society of Horticultural Science.