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# Water Use in Jujube (Ziziphus jujuba) with Applications in Irrigation Timing and Quantity

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# WATER USE IN JUJUBE (*ZIZIPHUS JUJUBA*) WITH APPLICATIONS IN IRRIGATION TIMING AND QUANTIY

by

Preston S. Colver

A thesis submitted in partial fulfillment
of the requirements for the degree

of

MASTER OF SCIENCE

in

Plant Science

Approved:

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UTAH STATE UNIVERSITY
Logan, Utah

2020

# ABSTRACT

## Water Use in Jujube (*Ziziphus jujuba*) with Applications in Irrigation Timing and Quantity

by

Preston S. Colver, Master of Science

Utah State University, 2020

Major Professors: Dr. Larry Rupp and Dr. Roger Kjelgren
Department: Plants, Soils and Climate

Jujube (*Ziziphus jujuba*) is a major fruit crop in China where it has been a favored cash crop and successfully used to address erosion problems in the Loess Plateau region of western China. Further use of jujube in forestry projects and improved agricultural efficiency are very promising. This study sought to repeat a water-use study in two climates: a hot, semi-arid climate in Yangling, Shaanxi, China and a dry-summer, continental climate in Logan, Utah, USA. The study examined the physiological stress responses of the jujube tree to drought stress with the intent of measuring physiological indicators of drought stress and characterizing its water-use strategy. The aim was to inform the creation of an irrigation scheduling tool for jujube that could be used by smallholder farmers in China and growers in the arid US interested in a promising new fruit crop. Three treatment groups were formed: control (irrigating 110% of actual evapotranspiration [$ET_A$] daily), moderate stress (60% of $ET_A$ daily) and severe stress (30% of $ET_A$ daily). Drought stress treatments were applied intermittently throughout a time-series study. Measurements of water use, stomatal conductance, leaf temperature and leaf water potential were analyzed. The study in Yangling was fraught with difficulties both in the cooperative process between Utah State University and Northwest Agriculture and Forestry University and in the instrumentation required for data collection. That study yielded no data that contributed to scientific discussion, but commentary and insights are given as to the value of failed research in the academic process. The study in Logan was completed successfully and found that jujube's responses to the drought stress treatments revealed a recovery phenomenon wherein trees that had been subjected to drought stress then shifted back to well watered conditions began to use more water than the control group. Variations in leaf water potential measurements support this recovery phenomenon. These findings contribute to the suggestion of jujube using an anisohydric drought response strategy. There is a concern for using jujube in agricultural applications where every drop of water must be carefully rationed because anisohydric plants do not reduce water consumption during drought conditions.

(95 pages)

# PUBLIC ABSTRACT

## Water Use in Jujube (*Ziziphus jujuba*) with Applications in Irrigation Timing and Quantity

Preston S. Colver

Jujube (*Ziziphus jujuba*) is a major fruit crop in China where it has been a favored cash crop and successfully used to address erosion problems in the Loess Plateau region of western China. Further use of jujube in forestry projects and improved agricultural efficiency are very promising. This study sought to repeat a water-use study in two climates: a hot, semi-arid climate in Yangling, Shaanxi, China and a dry-summer, continental climate in Logan, Utah, USA. The study took physiological measurements on the trees with the aim of characterizing the way that jujube uses water. This would help to create an irrigation scheduling tool for the jujube that could be used by smallholder farmers in China and growers in the arid US interested in a promising new fruit crop. Three treatments were applied: (1) would water the trees generously, (2) would restrict irrigation to produce moderate drought stress, and (3) would restrict irrigation heavily to produce severe stress. The physiological measurements included how much water was being used by the trees, the rate at which the water was being transpired by the leaves, the surface temperature of the leaves, and the internal water pressure of the trees. The study in Yangling nearly failed. That study yielded no data that contributed to scientific discussion, but commentary and insights are given as to the value of failed research in the academic process. The study in Logan was completed successfully and found that jujube's responses to the drought stress treatments revealed an interesting phenomenon in the time after the drought treatments ended and were receiving ample water. These findings contribute to the suggestion that jujube maintains normal water usage during drought stress. Because of this, there is a concern for using jujube in agricultural applications where water must be used carefully.

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# INTRODUCTION

## A Problem of Climate and Agriculture in the Loess Plateau

The Loess Plateau in north-central China represents about 7% of the nation's land area (Zhao et al., 2009) – about the size of Texas – and a similar percentage of the nation's population – about 100 million people. The region is arid to semi-arid and is prone to seasonal droughts with a majority of annual precipitation falling between June and September (Zhao et al., 2009). More than 70% of farmland in north-western China is dryland agriculture (Deng et al., 2004). This region faces a number of environmental factors that threaten to destabilize the economy and way of life for the people.

Global climate change is influencing droughts in vulnerable areas of China (Cao et al., 2011). Understanding the factors of climate change, and their impact on agriculture, is important in mitigating their impact on human lives (Wu et al., 2010).

Measures of the Palmer Drought Severity Index show a clear trend of increasing drought severity in agricultural areas of China with water supplies becoming a critical issue in those areas (Wu et al., 2010). One study established a warming trend in Northern China; temperatures have increased by as much as $1.5^{o}$ to $2.0^{o}$ C in the last 30 years (Gillies et al., 2012). In China's arid and semi-arid regions, water scarcity is limiting the growth and survival of local vegetation (Cao et al., 2011).

Erosion by wind and water increases land degradation in the area. With soils that have been called 'the most erodible in the world,' the Loess Plateau has experienced an increase of soil erosion over the last 30 years (Normile, 2007).

Desertification is characterized by land losing vegetation and becoming degraded due to the effects of drought and erosion. Loss of vegetation reinforces this degradation. The rate of desertification in China has steadily increased since the 1950s (Wang et al., 2010).

All of these factors contribute to a need in the Loess Plateau for better water resource management and for improved agricultural output to help maintain stability in the region. Currently, a majority of China’s population is supported by irrigated agriculture (Wu et al., 2010); however, China is on the verge of losing food security (Long et al., 2010), and population migration has been documented as a result of deforestation and drought in the region (Huang and Su, 2009). As crop yields are reduced by drought (Zhao et al., 2009), more of the population normally supported by semi-subsistence farming are migrating to coastal China.

The soft, silty soils in combination with the extreme slope of the hills contribute heavily to measured soil and water loss as a result of rainfall runoff in the Loess Plateau region (Zhao et al., 2009). These factors also make conventional irrigation infeasible. Historically, open soil channels were the predominant method of irrigation in China; however, this type of irrigation has been shown to be extremely inefficient, losing 50-70% of the deliverable water (Wu et al., 2010). Since 1990, advancement and increased use of irrigation technology have had a positive influence on the ratio of the agricultural irrigation water quantity to the effective irrigation area (Wu et al., 2010). China will not be able to maintain food security and address water supply issues without using irrigation technology (Deng et al., 2004; Wu et al., 2010).

## Reforestation Projects Address Desertification and Erosion

One course of action employed to address some of the needs in this region is large-scale plantings of woody perennials to stabilize slopes and reduce erosion. China has invested heavily in reforestation and afforestation projects that aim to reduce the effects of desertification (Cao et al., 2011; World Bank, 2006). In the last fifteen years, China has invested more than US $100 billion in forestry programs and they include more than 76 million hectares of afforestation (Cao et al., 2011). While some authorities claim that these forestry programs are succeeding (Liu et al., 2008; Wang et al., 2010), others have suggested that success of the forestry programs is marginal at best because of poor implementation, management, and species selection (Cao et al., 2011). For example, some trees in forestry projects in the Loess Plateau have been stunted by lack of water (McVicar et al., 2010; Zhao et al., 2009). With the growing tally of failing forestry projects, there is a call for proposed solutions to be ecologically suitable for the area being replanted (Normile, 2007; Lamb et al., 2005).

## Jujube: A Valuable Crop and Sustainable Solution

Jujube (*Ziziphus jujuba*) is a small tree or large bush with native distribution extending throughout arid parts of southeastern Europe to China (Outlaw et al., 2002) including the Loess Plateau. Jujube shows promise in both forestry, to control erosion, and agricultural applications in this region. In one example, jujube was planted on hillsides of the Loess Plateau in an effort to stabilize the soil. Though initially chosen for its drought tolerance and sustainability, it was discovered that irrigation and cultural techniques could improve jujube fruit production up to fifteen times (Wu et al. 2010). For millennia, the fruit has been cultivated heavily in China and is both culturally significant and valuable as a food source (Outlaw et al., 2002). Thus, expanding the cultivation of jujube trees on hillsides of the Loess Plateau has the potential to contribute to ecological stability through soil stabilization and, further, has the potential to be an improvement to small-holder livelihood through fruit production.

Northwest Agriculture and Forestry University (NWAFU) in Yangling, Shaanxi, China has devised a system for irrigating jujube in which runoff from slopes is pumped to reservoirs on hilltops and then water from the reservoir is used to supply a micro-irrigation system. Small irrigation emitters slowly saturate soils around jujubes on the steep slopes and, contrary to most other methods, water rarely flows away from the target. These micro-irrigation systems are being utilized to reduce erosion and so brings previously unused land into production of a high-value crop, which further opens an opportunity for subsistence farmers to increase income.

Requisite amounts and optimal timing for irrigating jujube are unknown. How much water is needed just to keep a jujube plant of a particular size alive? How much water is needed to help a jujube plant maximize yield? The answer to these questions resides in the tree’s natural patterns of water usage, or “water use strategy.” Climatic conditions also have a direct effect on the daily water needs of any individual plant. Understanding the daily water needs of jujube would greatly facilitate the creation of an irrigation schedule that minimizes the wasting of water while maximizing yield and responding to changing environmental conditions.  To this end, conducting research to develop an understanding of jujube water use, and subsequently creating recommendations for implementation, are the primary objectives of this project.

## Measuring Water Use in Trees

Measuring water use in trees is achieved using weighing lysimeters, leaf porometers, infra-red thermometers and pressure chambers. Lysimeters directly measure evapotranspiration from a containerized plant. Stomatal conductance, leaf temperature and leaf water potential are measured by leaf porometers, infra-red thermometers and pressure chambers respectively. These four measurements can be interpreted to produce a picture of a plant's real-time water status.

Weighing lysimeters have been in use for decades, and have been established as a reliable way to directly measure water use in woody plants (Beeson, 2011). Measurements from lysimeters do not require interpretation, transformation or scaling. Not only can lysimeter data be related to climate conditions over periods of weeks or months, but can also be paired with any hour-by-hour weather data. Such a relationship gives a very clear and in-depth picture of water use.

Assessing plant water status through direct measures of plant physiological parameters has been related to plant water status extensively (Acevedo-Opazo et al., 2008). Measurements made at intervals throughout diurnal cycles provide baselines from which water use strategies and water stress levels can be derived (Idso et al., 1981; Schultz, 2003).

## Project Objectives

The key objectives of this project were: First, to measure physiological indicators of drought stress for jujube to establish a baseline for determining real-time water status in jujube; and, second, to characterize the water use strategy of *Ziziphus jujuba* in terms of isohydric vs. anisohydric as summarized by Domec and Johnson (2012). Identifying drought stress indicators and characterizing water use has the potential to inform management decisions of when to irrigate and how much irrigation to apply. These objectives point to an additional outcome of the project, which is to inform the creation of an irrigation scheduling tool. Such a tool would apply our findings to reforestation projects as well as to everyday jujube farming in the area of the Loess Plateau and beyond.

## A Gap between Research and Solutions

These research outcomes may give insights into the physiological workings of the jujube, but they are also applicable to socio-economic problems of jujube farmers in China. The results are intended to empower decision-makers with information upon which they can act. Research on jujube and the problems of the Loess Plateau has already been conducted in China. A significant gap remains, however, between the research being done and the implementation of sustainable and successful solutions. In particular, this gap critically impacts Chinese smallholder farmers who are economically and politically disconnected from these solutions.

Because of this difficulty in implementing solutions, a partner study in social science was developed by Dr. Zhao Ma of the USU College of Natural Resources and her doctoral candidate, Mr. Morey Burnham. Their study took a closer look at smallholder farmers in the Loess Plateau region and the factors that influence their decisions about climate change adaptation. The results of their research point the way for researchers to better direct the results of their studies to be applicable, and for policymakers to better implement recommendations that result from research on subject (Burnham and Ma 2016).

# LITERATURE REVIEW

## Physiological Control of Water in Plants

Physiological mechanisms for regulating water use in plants are well understood and are generally consistent from species to species. Evaporative demand from vapor pressure deficit gradients is the primary driving force for plant water use. However, the primary necessity is for the plant to maintain a favorable energy balance between itself and its environment such that the plant temperature does not exceed thresholds that damage the function or fitness of the plant. While atmospheric vapor pressure deficit is the main driving force for transpiration, stomatal aperture is the plant's primary mechanism for controlling transpiration. Chemical signals regulate stomatal opening and closing in a way that allows the plant to be cooled by evaporative action, but that generally avoids failures such as cavitation of the water column (Monteith, 1973). Variations in stomatal activity from one species to another are characterized by differences in stomatal sensitivity to dry air that is manifested in contrasting strategies: isohydric and anisohydric.

Ansiohydric plants tend to keep their stomata open continuously during drought stress, and as water supplies become increasingly depleted, the leaf water potential of the plants becomes more negative. This behavior allows anisohydric plants to maintain productive growth and development during mild and moderate drought stress (Sade et al., 2012), and is also associated with greater success in most drought-prone environments (Sade et al., 2012; Voelker et al., 2018). In the face of severe drought, however, the behavior is said to be risky because they are operating with narrower safety margins and higher mortality rates have been observed (Sade et al., 2012; McDowell et al., 2008).

Isohydric plants conserve water supplies by reducing stomatal conductance and maintaining plant water potential throughout the day. Schultz (2003) summarized isohydric behavior in this way: “[isohydric plants] modify their growth and physiology to conserve current resources and to control their demand for future resources.” This behavior is often said to be “pessimistic” (Jones 1980) meaning that the plant rations its water resources carefully during drought conditions.  As stomata are closed, leaf temperature rises, depending on leaf size, to maintain energy balance, and limiting gas exchange also prejudices photosynthetic output. If leaf temperatures go too high, the leaf tissues begin to die. Some plants respond to this stress by dropping leaves and entering dormancy (Munné-Bosch & Alegre, 2004), while others may respond less favorably and fitness may be prejudiced.

Recent discussion of these strategies has shifted toward a continuum rather than a dichotomy of hydric behavior (Klein 2014; Sade & Moshelion 2014). Principally, objection is raised to arbitrary delineations made within various measurements used to define the two strategies. The continuum is conceptualized well by extensive studies of grapevines. Numerous studies have classified various grape cultivars as exhibiting either isohydric or anisohydric behavior. Sade et al. (2012), however, pointed out the departure of some grape cultivars from the advantages suggested for anisohydry and other conflicting reports of either behavior being exhibited by the same cultivar. In addition to this, other studies “have shown that grapevines could regulate their isohydric behavior during the growth season and switch from isohydric to anisohydric with varying soil moisture (Sade et al., 2012).”

## Measuring Water Stress

Water stress has been quantified in a number of field crops and tree crops. Methods of quantification require measurements of plant water stress indicators such as: leaf temperature (Andrews et al., 1992), canopy temperature (Koksal et al., 2010), stomatal resistance, soil water content, stem water potential (Ben-Gal et al., 2009), and leaf water potential (Boyer, 1967). Measures of these plant water-stress indicators can then be used to establish a crop water stress index (CWSI) (Idso et al., 1981; Koskal, 2010).

A CWSI assumes a crop has high enough transpiration that leaves are evaporatively cooled by transpiration. If plants are water-stressed, stomata close, evaporative cooling decreases and crop foliage becomes hotter. Crop water stress indices can be used to develop irrigation scheduling by answering the question “When to irrigate?” Establishing a CWSI gives a baseline for a well-watered status and can also quantify varying thresholds of water stress.

Another approach to developing an irrigation scheduling tool is to calculate a crop coefficient. Water use measurements from a lysimeter can also be paired with climate data and canopy measurements to determine a crop coefficient for a given species. A crop coefficient ($K_C$) expresses water needs as a percentage related to the transpiration of a reference crop such as turf grass – represented by the term ‘reference evapotranspiration’ or $ET_O$. After a crop coefficient is developed for a species, that coefficient can be used to approximate water needs on a daily basis by referencing current climatic conditions. That is, the current climatic conditions are the driving force behind $ET_{O,}$ and $ET_A$ for a plant can be estimated by calculating $ET_O$ and multiplying by the $K_C$ (Allen et al., 1998).

## Irrigation Technology

A drip-irrigation system is already being tested by NWAFU on the hillsides of the Loess Plateau (Figure 1). It functions by pumping water catchment from the valleys to hilltop reservoirs. Water from the reservoir supplies a drip-irrigation system on the slopes of the hill, and the resulting system can be adjusted to respond to changes in the climate. Jujube yields on this system have been up to four times greater than control plots (from 310kg/mu to 1145kg/mu and from 0.50 Mg/ha to 1.98 Mg/ha) (1 mu = 0.165 acre) (Wu et al., 2008; Zhang et al., 2010). Zhang's study asserts that jujube water use can be accurately determined using measurements of trunk diameter fluctuation, leaf water potential, and canopy temperature; however, automating an irrigation system based on these measurements is unrealistic (Zhang et al., 2010).

Automating an irrigation system with reference to climate and jujube specific evapotraspiration is possible. Work from the University of Florida includes a clear, comprehensive explanation of a lysimeter system that is appropriate not only for measuring water use in jujube, but also for programming the necessary equipment to automatically irrigate jujube plantations in the Loess Plateau Region (Beeson 2011). The basis of this system is summarized below.

## Lysimeter Technology

Popular techniques for quantifying water use in woody plants – such as sapflow and soil moisture measurements – have limitations. Sapflow is less accurate over a single day, and soil moisture measurements assume uniform water absorption from roots that are not uniformly distributed in soil and not uniformly moist.

Lysimeters measure actual plant-soil evapotranspiration ($ET_A$) – or the amount of water lost to the atmosphere from the combined soil evaporation and plant transpiration – and are the standard by which other techniques for quantifying water use are verified. Measurements by lysimeters do not require interpretation or scaling. $ET_A$ values can be measured over any conceivable interval. Lysimeters cause no direct injury to the plants they measure and can be completely automated.

The simplest is a drainage lysimeter. These make simple measurements of crop water use by calculating water balance. When measured water inputs (rain, irrigation) have measured leachate subtracted, the result is an accurate representation of $ET_A$. Drainage lysimeters are usually not portable and physically restrict possible plant sizes and soil masses. Complicated measurements of inputs and leaching can be challenging, and there are notable sources of error because it is an interpolation of water use, not a direct measure of water use.

Weighing lysimeters are the most direct and accurate method for quantifying plant water use, particularly for individual woody plants (Beeson, 2011). The weighing lysimeter system determines $ET_A$ and applies irrigation as specified by the programming. $ET_A$ is calculated daily by reading the mass of each plant just before sunrise and a few hours after sundown. The irrigation volume applied can be a fixed volume or a percentage of calculated $ET_A$. Irrigation is applied after the sundown measurement and before the sunrise measurement such that the substrate and plant are in equilibrium. In conditions of high evaporative demand, the program can also measure a mid-day $ET_A$ and replenish water accordingly to maintain a more uniform water status. In addition, because the lysimeter is programmed to take repeated measurements throughout the day, rain events can be accounted for by comparing the time stamps of weight gain on rainy days to climate data and water use measurements can be adjusted accordingly.

With nearly two decades of experience with this system, Beeson's assessment of the utility of weighing lysimeters can be summarized by his statement, "With the rise in the global need to quantify plant water use and screen plants for drought tolerance, it is appropriate to share in detail this time-tested, versatile and expandable automated lysimeter system (Beeson 2011)."

## Biophysical Aspects of Jujube Water Use

Jujube is a major fruit crop in Asia and more particularly in China (Outlaw et al., 2002), known for its economic value throughout the world (Pandey et al., 2010). The natural distribution of jujube ranges across the middle latitudes of Eurasia and has been under cultivation in China for over 4000 years. Hectarage in China today is equivalent to that of citrus in Florida (Outlaw et al., 2002). Uses of jujube are varied, and include furniture, handles for implements, fencing material, soil conservation, livestock forage, as well as medicinal applications (Outlaw et al., 2002; Pandey et al., 2010). Analysis of jujube characterizes it as a valuable source of nutrition (Ouedraogo et al., 2006), and is produced widely in China for use as fresh, dried, or processed food (Outlaw et al., 2002; Pandey et al., 2010). Jujube further provides value because of its adaptability to a range of environmental conditions: soil texture and pH, temperature, irradiance, and humidity (Outlaw et al., 2002; Pandey et al., 2010; Su and Liu, 2005).

In addition to versatility and adaptability, Jujube is known for its proliferation in arid climates (Outlaw et al., 2002) and drought resistance (Sharma et al., 1982) as evidenced in part by its low, broad canopy, deciduous leaves, and deep root system (Pandey et al., 2010; Sharma et al., 1982; Ma et al., 2011). Small, glossy leaves reduce absorption of short-wave radiation and increase cooling by convection. Jujube's water-use efficiency has been likened to that of some desert plants (Su and Liu, 2005). This morphology points to an anisohydric water-management strategy. Anisohydry is characterized by a 'use it or lose it' attitude and will not reduce water use during drought conditions; i.e. stomatal conductance remains constant and leaf water potential drops as water in the soil is finally depleted (Schultz, 2003).

While extensive research has been done on various aspects of jujube, information on jujube water use has yet to be fully explored (Sharma et al., 1982). Wullschleger et al. posited that "whole-tree estimates of water use are becoming increasingly important in forest science," and that such information could be used to resolve issues of water resource management (Wullschleger et al., 1998).

## Paired Study in Two Climates

Two separate studies were conducted as part of this project. First, a study was conducted at Northwest Agriculture and Forestry University in Yangling, Shaanxi, China during the summer of 2011. Second, data was collected at the Utah State University Greenville Farm in Logan, Utah during the summer of 2012, and is comprised of the same set of measurements that were prescribed for the study in China.

When comparing these two climates using standards of the Koppen Climate Classification System, Yangling, in the Loess Plateau, fits in the hot, semi-arid climate (BSh) classification while Logan, in the Great Basin region, fits a cold, desert or semi-arid climate (BWk/BSk) classification. These climates are similar in that they are relatively dry, but Yangling receives the majority of its rainfall between July and October and almost nothing in the winter, where Logan receives 20% less precipitation overall (454mm/yr. compared to 554mm/yr.) and it is mostly distributed in the fall, winter and spring with very little falling in the summer. The disparity in temperatures between the two locations is obvious, with Logan's average annual temperature being 6 $C^o$ less than that of Yangling (8.1 $C^o$ compared to 14.1 $C^o$) (Figure 2) (China Meteorological Administration, 2011; NOAA, 2011).

Our hypothesis for comparing the two studies was that the physiological behavior of the jujube under the drought-stress treatments would be consistent across the two climates – thus strengthening our approach to the first objective of identifying physiological indicators of drought stress in jujube. The insights to be gained by comparing differences in the jujube's water use in each of the climates are of even greater interest: When comparing the observed water use of the trees in Yangling to those in Logan, can recommendations for the creation of an irrigation scheduling tool be calibrated based on climate data? Also, similarities in the climates encourage farmers in the Great Basin region and in other arid regions of the United States to consider the potential that jujube has to emerge from obscurity in the US market.

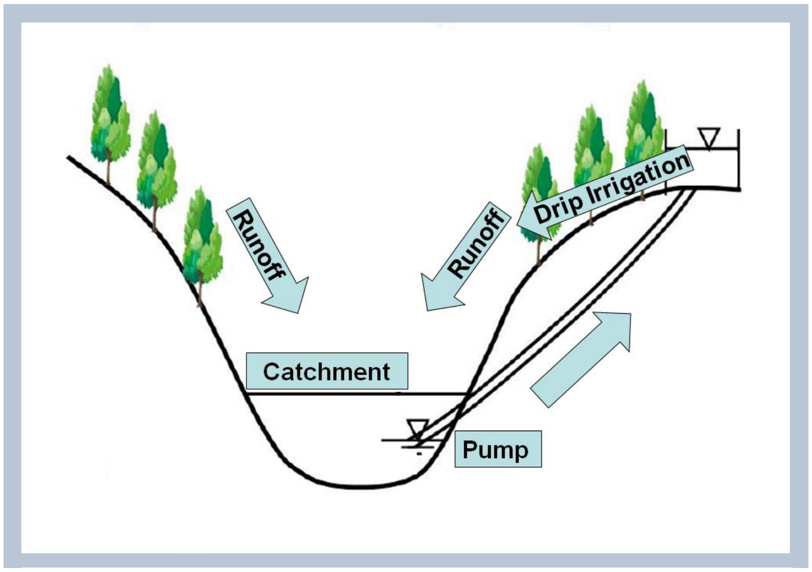
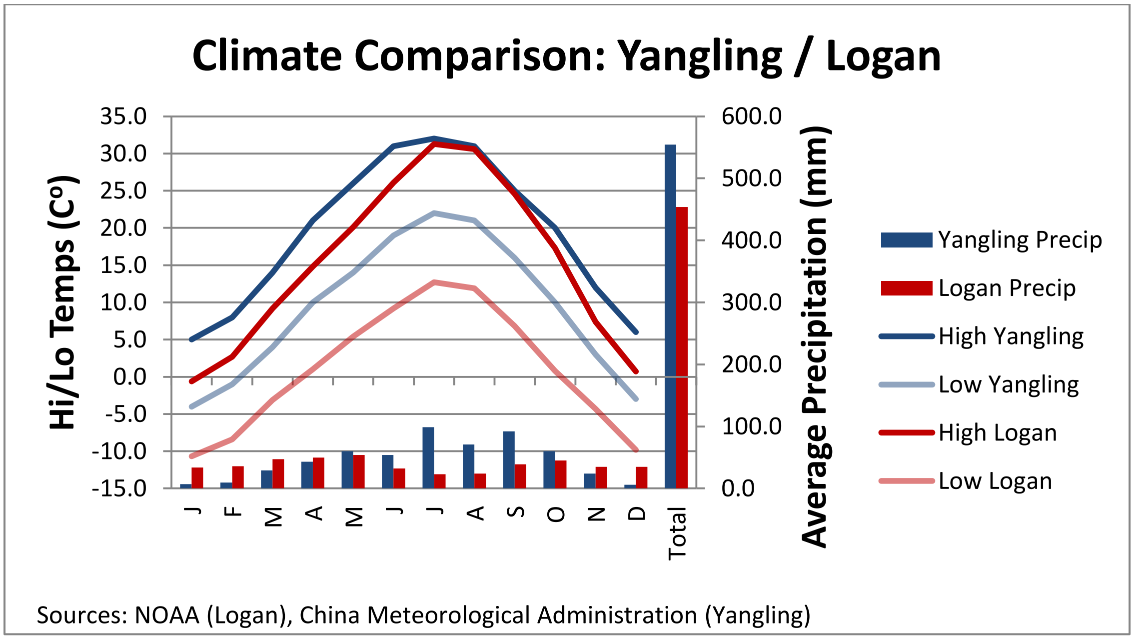


Figure 1. Diagram of drip-irrigation system sourced by catchment of runoff.



Sources: NOAA (Logan), China Meteorological Administration (Yangling)

Figure 2. Climate comparison of Yangling, Shaanxi, China (Blue) and Logan, Utah, USA (Red). Lines represent monthly average high and low temperatures (C$^{o}$). Bars represent monthly average precipitation (mm) with a final column for total annual average precipitation (mm).

# YANGLING STUDY – SUMMER 2011

## Materials

The experiment used four-year-old jujube trees (*Ziziphus jujuba*) of the Li-Zao cultivar also known as pear jujube.  This variety has larger fruits than most others and is cultivated extensively in the Loess Plateau.  The pear jujube is widely accepted in China as one of the most preferred cultivars.  Trees were potted in unconventional, stainless-steel buckets with no drainage holes. The potting media was created by our colleagues at NWAFU using soil native to the research plot that was screened and then mixed in a ratio of 4:1 with vermiculite.

## Experimental Design

The experimental plot had the lysimeter system assembled in a hexagonal steel frame spanning about 35 feet at its widest point. Each side of the hexagon was outfitted to carry four trees (Figure 3). The treatments followed a completely randomized design structure with each treatment randomly assigned to the twenty-four available trees with a total of eight trees assigned per treatment.

The twenty-four trees were divided into three treatments:  well-watered, moderate drought stress and severe drought stress (Table 1). The well-watered treatment is defined by the automated irrigation system watering back 110% of the total measured water loss each day.  The moderate drought-stress treatment is defined by a 60% return of measured water loss, and the severe drought-stress treatment is defined by a 30% return of measured water loss.

The drought-stress treatments were intended to commence in June after leaves fully formed. Throughout the summer, four or five treatment periods would have been applied as follows:

The drought-stress-treatment groups were subjected to the deficit irrigation quantities for two weeks at a time with intervals for recovery between treatment periods. At the end of each treatment period, the drought-stressed trees would be immediately watered to the saturation point. This was to be followed by a recovery period in which they would receive irrigation equivalent to that of the control group (110% of the total measured water loss each day). These recovery periods continued for two weeks at which time the drought treatments were repeated.

## Instrumentation and Data Collection

The method of measuring jujube water use via weighing lysimeters is one developed and used extensively at the University of Florida (Beeson, 2011). The principal instrument of the lysimeter system is a “load cell.” Load cells used in this study were the Zemic S-Beam (Zemic B3G, California), which is a blocky piece of S-shaped metal about the size of a deck of cards. Each load cell is calibrated to measure weight by measuring the conductance of a series of electric pulses that pass through the metal of the load cell. The tension on the metal from the weight of the tree distorts the electric impulses in a predictable fashion and the weight of the suspended object can be tracked with sub-gram precision.

Pots containing trees are suspended individually from separate load cells. As any particular tree transpires, the tree becomes lighter and the load cell can then measure water loss. Each day, water is added back to the pots (also measured by the load cell) in any amount specified by the programming. In this study, the amount of water added back was programmed to be a percentage of the water lost on that particular day as described in the section above.

The lysimeter system is wired into a data-logging system that is programmed to record measurements every half hour. The output of the system can be downloaded on site from the loggers to a laptop, or, as available, the system can be connected to the internet which allows for remote access to the output.

Stomatal conductance, leaf temperature and leaf water potential ($\Psi$) are physiological measurements that can be used to determine real-time water status in a plant (Andrews et al., 1992, Ben-Gal et al., 2009 and Koksal et al., 2010). Collecting observations like this was intended to be paired with water use data from the lysimeters to clarify the relationship between the actual water use and the plant's real-time physiological responses.

Stomatal conductance was measured on a single leaf using a Decagon leaf porometer in units of mmol $m^{-2}s^{-1}$ (Ben-Gal et al., 2009). For each measurement of stomatal conductance, leaf temperature was also measured at the same time on the same leaf using an infrared thermometer (Andrews et al., 1992). This process was repeated for three leaves on each tree, at midday, once per week. At the time of measurement, the leaf porometer was attached to a fully-exposed, mature leaf, oriented as close to perpendicular to the sun's rays as possible to maintain reasonable consistency in measurements (Pask et al. 2012).

Leaf water potential measurements were intended to be included in this study, but the necessary equipment to make the observations was not available.

Tracking leaf number and area allows for the water-use data to be paired with it as a reference to the relative differences in the size of the individual tree canopies. The number of leaves on each tree was counted multiple times throughout the growing season. At the terminus of the study, a final leaf count was made on each tree. Unfortunately, this data was never put to use because the necessary equipment for measuring accompanying leaf area data was unavailable.

Recording weather data throughout the study serves as a reference point for fluctuations in the other observations caused by changes in the weather. Weather data for the 2011 China study was provided by NWAFU.

## Results and Discussion

The research process in Yangling was fraught with difficulty. Problems in properly assembling and calibrating the weighing lysimeter and its component systems continually delayed the project throughout the summer of 2011. Ultimately, the study yielded no usable data.

Dr. Liu Xiping and his students hosted myself and my wife on the NWAFU campus during this research period. Their intentions were to have a weighing lysimeter system assembled and operational for the study on water use in jujube prior to our arrival in May. Many of the components had been delivered from Utah State University along with a technician who was in Yangling in April 2011 to help assemble and troubleshoot the system. Basic assembly was completed as expected, but getting the system operational was more problematic than anticipated, and when the technician was departing, the system was still not fully functional.

In that first week, efforts were made to get the system calibrated properly and also to establish an internet link from the load-cell system that would enable remote access for the technician back at USU.  The irrigation system was not functioning properly and would require troubleshooting. Concerns began to arise also because the stainless-steel pots containing the trees were open on the top and completely sealed on the bottom. Fashioning lids for the pots would ensure that no rainwater would incidentally irrigate the trees and thus throw off attempts to subject the trees to drought-stress conditions. Putting holes in the bottom of the pots would enable surplus irrigation to drain away, which would be important for trees being irrigated in excess of $ET_A$.

An internet connection for the lysimeter system was at last established, and remote access was possible from my apartment on campus, approximately one mile away from the research plot. Unfortunately, access in the United States was never obtained. The irrigation system was improved, but water pressure from the supply was problematic. Styrofoam lids for the pots were put off as unessential, and the idea of putting drainage holes in the bottom of the pots was road blocked by our hosts. It was made clear that the soil volume had been carefully measured in each pot and our hosts insisted on maintaining it to allow for soil moisture measurements. Sadly, this decision upended the project a few months later as explained below.  In the end, these initial problems set the project back nearly two months. Not only did it take time to address the problems, but it took additional time to carefully develop the dynamics of a working relationship between me and my hosts. This isn’t to say that they were difficult or unreasonable to work with, but it was necessary to develop precedents for appropriate communication and cooperation with them that fit their social and cultural norms.

July soon arrived, and progress was interrupted again while workmen came to dig trenches and erect a scaffold to construct an on-site water tower in order to provide reliable water pressure for the project. In the meantime, the lysimeter still required further calibration and the graduate students and I began intermittently taking field days to collect dawn-to-dusk photosynthesis measurements with a Li-Cor 6800. We also took a number of opportunities to collect leaf temperature and stomatal conductance data using an infrared thermometer and a Decagon Leaf Porometer. Nevertheless, the water-use study was delayed at least another ten days by the lack of automated irrigation.

Beginning in August, Yangling started to see some pretty heavy rainfall. In fact, the rain persisted enough to cause the sealed pots to overflow. Progress on the project halted again to work around the risk of tree mortality from lack of oxygen due to the root zone being saturated. There was a scramble to get lids made for the pots to keep the rainwater out, because Styrofoam insulation board was not easily procured for making the lids. The solution for the overflowing buckets was difficult because our host continued to assert the importance of preserving the soil volume. In the face of the disagreement over this point, an executive decision was made to punch holes in the bottom of the buckets.

In the end, Yangling received a whole years-worth of rainfall in just six weeks. As all other efforts seemed to be failing, the trees were finally moved to a cold-frame structure so that they would be out of the rain which continued in torrents. The trees were watered with a graduated cylinder and weighed manually on a laboratory scale. This was extremely labor-intensive and time-consuming. Also, soil moisture probes were employed to assess plant water status which is a step backward in data quality from using lysimeters. Regrettably, drought-stress treatments were never applied because, amidst all the efforts to press on with the study, a baseline of jujube water use was never established. Though many measurements were taken, it was never in a way that could be interpreted.

In retrospect, the failed research process in Yangling feels like more than just a series of unfortunate events. It was truly uncanny that as each problem was resolved the next problem was seemingly queued-up behind it. At no time during the entire six months of the proposed study was there a period that felt like the project was fully operational. The result was none of the data collected was cohesive or robust enough to be useful. On this level, I would say that the research experience failed; yet, for multiple reasons, if I had it to do all over again, I would.

Speaking strictly from an academic perspective, the string of problems that we worked through helped me develop a more intimate understanding of the entire lysimeter system, become more adept at problem-solving, adaptable to sudden and unforeseeable changes both practical and cultural, and come to know the nuts and bolts of the jujube plant functions. From these considerations alone, it is clear that the experience was anything but a waste, even if the intended data collection was unfruitful. Some people may read my experiences and be deterred from international research, while others may read, still choose to pursue international research, and meet with more ideal results than I did. Notwithstanding all of the difficulties, there are two things which remain of greatest worth to me: (1) The relationships I developed with Ruifeng and other Chinese nationals were truly meaningful, and (2) the way that my wife and I learned to rely on each other through the difficulties of our stark cultural immersion – including the successful birth of our first child in a Chinese hospital – has remained a happy memory and a strength to our lasting success in marriage.

Table 1. Jujube Drought-Stress Treatments

| | Well-Watered (Control) | Moderate | Severe |
|---|---|---|---|
| Irrigation Added as Percent of $ET_A$ | 110% | 60% | 30% |

Figure 3. Photo of preliminary lysimeter assembly at Northwest Agriculture and Forestry University in Yangling, China.

## LOGAN STUDY – SUMMER 2012

### Materials

The experiment used four-year-old jujube trees (*Ziziphus jujuba*) of the Li-Zao cultivar also known as pear jujube. This variety has larger fruits than most other jujube cultivars and is cultivated extensively in the Loess Plateau. The pear jujube is widely accepted in China as one of the most preferred cultivars. Our stock was procured from the late Roger Meyer, who was an exotic fruits grower in Fountain Valley, California. Trees were potted in five-gallon containers in a generic potting media as prescribed by Beeson (2011) for the lysimeter system.

### Experimental Design

The experimental plot was laid out with the lysimeter system assembled in three rows of 11 positions. The treatments followed a completely randomized design structure with each treatment randomly assigned to the thirty available trees with a total of ten trees assigned per treatment. Two other pots were connected to the lysimeter containing only soil. These two pots acted as a control for evaporation from the soil.

The thirty trees were divided into three treatments: well-watered, moderate drought stress and severe drought stress (Table 1). The well-watered treatment is defined by the automated irrigation system watering back 110% of the total measured water loss each day. The moderate drought-stress treatment is defined by a 60% return of measured water loss, and the severe drought-stress treatment is defined by a 30% return of measured water loss.

The drought-stress treatments commenced after the trees had finished initial shoot elongation near the end of July. Three treatment periods were completed by the end of September (100 days start to finish). These treatment periods consisted of subjecting the treatment groups to the prescribed irrigation regime for two weeks at a time with intervals for recovery between treatment periods. At the end of each treatment period, the drought-stressed trees were immediately watered to the saturation point of the container media and allowed to drain. This was followed by a recovery period in which they received irrigation equivalent to that of the control group (110% of the total measured water loss each day). This recovery period continued for two weeks at which time the drought treatments were repeated.

### Instrumentation and Data Collection

The method of measuring jujube water use with a weighing lysimeter is as previously described for the Yangling study.

Stomatal conductance was measured on a single leaf using a Decagon leaf porometer in units of mmol $m^{-2}s^{-1}$ (Ben-Gal et al., 2009). For each measurement of stomatal conductance, leaf temperature was also measured at the same time on the same leaf using an infrared thermometer (Andrews et al., 1992). Measurements were repeated for three leaves on each tree, at midday, during the treatment periods. At the time of measurement, the leaf porometer was attached to a fully-exposed, mature leaf, oriented as close to perpendicular to the sun’s rays as possible to maintain reasonable consistency in measurements (Pask et al. 2012). At the close of each treatment period, a series of measurements from dawn to dusk was taken. For this dawn-to-dusk series, measurements were conducted on three trees: one tree from each treatment group and three leaves per tree. These measurements were taken at 9am, 12pm, 3pm and 6pm.

Leaf water potential ($\Psi$) measurements in units of bars were conducted using a Scholander pressure chamber as described by Boyer (1967). Taking these measurements at different times of the day is valuable for establishing different conclusions. A measurement taken before dawn will catch the tree in a state of equilibrium with the soil moisture level (Ameglio, 1999). This pre-dawn measurement ($\Psi_{PD}$) acts as a baseline measurement. Alternatively, leaf water potential measurements taken in the middle of the day ($\Psi_1$) are used as an indicator of drought stress (Williams and Araujo, 2002). These data were gathered weekly: one leaf per tree before dawn at 7am, and one leaf per tree for midday measurements at 12pm. Leaves to be harvested were handled in a manner as described by Boyer (1967) to hold them in stasis until they could be measured. First, they were wrapped in plastic and covered in aluminum foil to prevent desiccation and to shut out light. After 10 minutes, leaves were harvested from each tree. Then, all harvested leaves were stored in an insulated container until they could be measured.

Tracking leaf number and area allowed for the water-use data to be paired with it as a reference to the relative differences in the size of the individual tree canopies. The number of leaves on each tree was counted multiple times throughout the growing season. At the terminus of the study, a final leaf count was made on each tree. Leaves were then harvested from each of the trees. Three randomized individuals from the study were selected and all of their leaves were measured using a Li-Cor LI-3100C scanning leaf-area meter. Then, all of the harvested leaves were dried and weighed. The leaf-area measurements of the three sample trees were compared to the dry weight of the leaves to establish a ratio of area to weight. This ratio was then used to extrapolate the dried weights of the un-measured trees to estimate the leaf area.

Recording climate data throughout the study serves as a reference point for the fluctuations in other observations caused by changes in the weather. Comparing the drought-stress indicators to weather data is also important for the creation of an irrigation scheduling tool. Weather data for the 2012 USU study was provided by the USU Department of Plants Soils and Climate.

## Statistical Methods

The factor analysis for this project uses a time-series, mixed model three-way analysis of variance (ANOVA) in a completely randomized design. The analysis was carried out using SAS Studio University Edition. Principally, the analysis will address the question of whether the two drought-stress treatments had a significant influence on water use or any of the drought-stress indicators (stomatal conductance, leaf temperature and leaf water potential) vs. the control.

If the treatments are found to have a significant influence on water use vs. the control, further analysis is required to determine when the water use varied from the control and whether the variance in water use was in excess of the control or in deficit of the control. If the analysis reveals that the treatments had a significant effect on stomatal conductance, then it is anticipated that the treatments would not influence leaf water potential which leads to a conclusion that jujube exhibits isohydric behavior. If the treatments have no significant influence on stomatal conductance, then it is anticipated that the treatments would decrease leaf water potential which leads to a conclusion that jujube exhibits anisohydric behavior.

Subsequently, a comparison of the water-status indicators will be made. Correlation between treatment effects on water use and on drought-stress indicators would point toward a practical means of assessing drought stress under field conditions.

Throughout the statistical analysis, “group” is used to represent the three treatment groups of ten trees each (control, moderate and severe). It is important to explain, however, that within the analysis, “group” cannot be used to understand the effect of the treatments on measurements taken. This is because the treatments were not applied uniformly throughout the study, but the experiment was structured as a time series where the treatments were applied intermittently throughout. Therefore, in lieu of “group,” the analysis must consider “date x group” as the appropriate representation of the effect that the treatments had on the measurements.

## Water Use Results

In the analysis of water-use data, a p-value was calculated for each of the effects as a test for the significance of those effects throughout the study as a whole. Also, a least squares mean value was produced for each of the treatment groups on each of the days of the study (See Appendix D). These values were then analyzed to determine a p-value for the day-by-day differences between the control group and the two treatment groups.

In the output, the group effect showed no significance. The group effect is influenced by the composition of individual trees within the treatment groups. It is also influenced by the treatments that are applied to each group at various periods throughout the experiment. Seeing no significance in the effect of “group” indicates: (1) the individual trees within the treatment groups were comparable to each other, and (2) the groups were not responding differently from one another to other factors in the experiment. In other words, when looking across the entire study (100 days), all three treatment groups consumed similar amounts of water. This creates confidence that the control group can be used as a baseline for water use throughout the study and better distinguishes the effect of the treatment periods as will be discussed below.

The date effect was highly significant (.001) which was expected. The significance of "date" is best understood through the obvious connection of varying climatic factors (temperature, humidity, wind, sunlight, etc.) to plant water use. Some discussion of climatic factors is included here, but principally, climate data is more relevant to a discussion of applications for the findings of this study.

The effect of "tree," or the individual effect, was also highly significant. This shows a large within-treatment variation among individual trees. Observed and unobserved variation of the individual trees influences their responses throughout the experiment. This generates a great amount of error in the analysis but is overcome by collecting an adequate volume of data.

The most interesting effect is the interaction effect of "date x group," which was highly significant (.0006). This effect represents the drought-stress treatments. The treatments were applied to the groups only during specific periods throughout the experiment. Therefore, neither "date" nor "group" represents the treatment, but only this interaction between "date" and "group." This means that while the three treatment groups used similar amounts of water throughout the 100-day study (group effect), there were highly significant differences in water use that would manifest as a pattern over a series of dates or as a single instance on one date (i.e. the date x group effect).

To dig deeper into the significance of this “date x group” interaction, a graph of the least squares means of the daily water use visualizes the three treatment groups throughout the experiment (Figure 4). When there are patterns of divergence between the groups, this creates the suggestion of significant differences that account for the .0006 significance of “date x group.” The three treatment periods are labelled in Figure 4 – occurring during days 23-34, 51-64 and 76-88. Other highlighted areas on the graph include the pre-treatment period as well as three recovery periods that occurred after each of the treatment periods. At a glance, the graph shows the treatment groups rising and falling together throughout most of the study. However, when analyzing the day-by-day differences between the control group and the moderate- or severe-treatment groups, a clearer picture of the significance of the treatment periods emerges. When the p-values indicate a significant difference in the water use of one treatment group from the control it is also important to note whether the group was using significantly more or significantly less water than the control or, in other words, it is important to ask what the effect of the treatments was.

The study commenced on July 5, 2012 with a pre-treatment period (days 1-22) which is a very important control for the study. During this period, the daily mean water use differed by as little as a few thousandths of a liter ($p=1$) from one treatment group to the next. This indicates that through this baseline period there were no discernable differences among the three treatment groups in terms of water consumption. To contrast this, at other points later in the experiment daily mean water use often differed between the treatment groups by half a liter or more and sometimes even more than a liter ($p<0.0001$). The observed similarity in the daily mean water use of the three treatment groups during the pre-treatment period confirms the homogeneity of the three groups and eliminates any concern for a group effect that would confound the treatments. The similarity in water use between the groups during this period also suggests that any differences in water use observed throughout the remainder of the experiment are meaningful.

During the first treatment period (days 23-34) which commenced on July 27, 2012, one can see that from day to day, the moderate- and severe-treatment groups used less water than the control. Only on the first day of the treatment was this not true. On every other day, the control used more water than either treatment group. Looking at the daily p-values of the differences between the control and the treatment groups, none of the differences in daily water use are significant. However, this pattern is consistent with the diminishing soil moisture caused by the deficit irrigation treatments, and because of the sudden consistency of this trend over a thirteen-day period – especially when contrasted with the parity of the three groups throughout the pre-treatment period – the observed differences in water use from the treatment groups to the control during this treatment period are strongly suggestive of significance. To reinforce this, we must keep in mind the highly significant interaction effect of “date x group” (.0006), which suggests searching the time-series data for patterns of water use such as this.

Following the first treatment period, when irrigation to all three groups was restored to 110% of daily $ET_A$, the trees went into the first recovery period (days 35-50). During this recovery period, a pattern emerges where there was an immediate reversal of the phenomenon observed during the first treatment period. After the first day of recovery, the control group used less water than either of the treatment groups for thirteen of the following fifteen days of recovery, hence the naming of this so-called ‘recovery period’. This recovery period seems to be a chance for the drought-stressed trees to rehydrate as an excess of irrigation is provided (110% of $ET_A$). This interpretation of the recovery behavior is supported and further discussed below in the discussion on leaf water potential.

Overall, the second treatment period (days 51-64) showed the same pattern of the control using more water than the treatment groups again, but the phenomenon was delayed. The two treatment groups initially continued to use more water than the control as seen during the recovery period. Note that this was in a period where they were being given less water, and they still used more! It is as though the drought-stressed trees persisted in the recovery behavior despite the lack of irrigation. Then at day 60-64, we saw the behavior reverse. All three groups declined in water use which might normally indicate a change in the weather, but looking at the daily p-values we observed highly significant differences between the control and the two treatments (.0255 for moderate and <.0001 for severe). This indicates that the decline in water use in the two treatment groups was due to a lack of available soil moisture; this suggestion is especially compelling when considering that the daily high temperatures during this treatment period only dipped below $30^{o}$C once.

A similar recovery phenomenon was recorded again during the second recovery period (days 66-75). In this case, the moderate-treatment group showed more water use than the control throughout the recovery period. Meanwhile the severe-treatment group used less water than the control initially, which is not consistent with the recovery phenomenon, but began to exceed the control beginning on day 71. More interpretation of the severe-treatment group's deviation from the expected recovery phenomenon is discussed in section on leaf water potential below

In the third treatment period (days 76-88) we observed the moderate-treatment group consistently using more water than the control and the severe-treatment group consistently using less water than the control. At the end of the treatment period and into the beginning of the third recovery period, the severe-treatment group used significantly less water than the control for five consecutive days (days 87-91) ($p<0.04$). This late in the season (Sept. 29-Oct. 3), we might conjecture that the severe-treatment group may have been entering a premature fall senescence. No frost had occurred at this point in the season and the continued water use of the other two groups indicates that they had not yet begun leaf senescence on the natural timetable which supports this idea. This all said, the leaf water potential data also contributes to the picture as discussed further below.

On day 92, during the third recovery period, temperatures dropped to $-1.6^{o}$C. This first-frost event caused water use in all three treatment groups to plummet. All of the trees began leaf senescence and excision.

## Leaf Water Potential Results

Measurements of leaf water potential ($\Psi$) were not executed as intended. Students were employed to collect this data, and despite thorough training and their own best efforts, it was discovered after-the-fact that their process for collecting the data was badly flawed. No usable data was collected for pre-dawn leaf water potential ($\Psi_{PD}$). The little usable data that was collected is discussed below and represents only midday leaf water potential ($\Psi_1$). This does not allow for the $\Psi_1$ to be related to a $\Psi_{PD}$ baseline, but the $\Psi_1$

measurements are still usable relative to themselves and in comparison to water use and stomatal conductance.

The statistical analysis indicates that the drought-stress treatments had a highly significant effect on $\Psi_1$ (p-value <.0001). Like water use, the effect of the treatments on $\Psi_1$ was not static. Throughout the different periods of the study, $\Psi_1$ for the treatment groups was sometimes higher than that of the control group and at other times lower. Some of these differences corroborate the findings discussed in the water use results.

For example, near the beginning of the second treatment period, the mean $\Psi_1$ for the severe water-stress treatment group on day 55 was -10.86 bars as compared to that of the moderate water-stress treatment group (-13.47) and the control group (-14.47). This indicates that the severe-treatment group was in a more favorable plant water status than the other groups on that day. Day 55 is important because it was on the cusp of one of the 'reversal phenomena' described in the discussion on water-use results. Prior to day 55, the severe-treatment group was using more water than the other groups throughout *19 consecutive days*. Consistently using more water is the essence of the 'recovery phenomenon' discussed above and finding the severe-treatment group in this more favorable water status on day 55 re-confirms the correctness of the naming of these 'recovery periods.'

Following day 55, the mean $\Psi_1$ of the severe group fell to -21.52 on day 61. This coincides with the observed differences of water use between the severe group and the control where the severe group reached the most significant difference in water use of the entire study (p <0.0001) on day 64. The severe group only used 0.54L on this day compared to the control which used 2.25L. This marked drop in $\Psi_1$ over the course of just six days is consistent with the idea that anisohydric plants will use water until resources are depleted, and are left in a very vulnerable position if water resources are not restored. Following this severe drop in both water potential and water use, where the second treatment period ends and the second recovery period is beginning, the severe-treatment group increased its water use, but lagged behind the other two groups. Finally, on day 71, after 5 days of recovery, the severe-treatment group began to match the previous pattern of recovery and subsequently used more water than the control for 9 consecutive days.

The leaf water potential data repeated this same pattern during the next cycle of drought treatment and recovery period. On day 77, after seven consecutive days of using more water than the control at the tail-end of the second recovery period, the mean $\Psi_1$ for the severe-treatment group was -9.88. In other words, the severe-treatment group was in a very favorable water status after an observed recovery phenomenon. Then proceeding to day 89, at the close of the third drought-treatment period, the severe-treatment group had a mean $\Psi_1$ of -33.53, or in other words, the severe-treatment group was severely drought-stressed. Again, the water-use data is consistent with this drastic drop in $\Psi_1$. For five consecutive days (87-91) the severe treatment group was using significantly less water than the control ($p<0.04$).

Though the volume of leaf water potential measurements was not ideal, the data compliments the findings of the water-use analysis very well, indicating that during the drought treatments, the severe treatment group lost leaf water potential while using all available water. The data also indicates that the severe group replenished their leaf water potential during the recovery periods with the observed increase in water use.

## Stomatal Conductance Results

The stomatal conductance measurements collected in this study were plentiful – more than 3600 measurements were taken over the course of the 100-day study. Unfortunately, many of the values recorded were beyond realistic precedents for stomatal conductance. For example, Murray et al. (2019) described typical values for stomatal conductance in a study of over 200 woody perennials with an overall tendency for measurements to operate toward a maximum of 249 mmol m-2s-1 (± 95 mmol m-2s-1), with the extremes reaching as much as 500-750 mmol m-2s-1. Comparing that precedent with this study, over 1600 measurements exceeded 1000 mmol m-2s-1 and 141 of those measurements exceeded 3000 mmol m-2s-1 – a whole order of magnitude greater than the typical values expected (Murray et al., 2019). The degree of variation in this anomalous data casts doubt on the reliability of the stomatal conductance data as whole. That said, a graph of the daily least squares means of the stomatal conductance measurements matches the rises and falls seen in the graph for least squares means of water use (Figure 4 and Figure 6). Also, during the collection of the stomatal conductance data, the anomalous readings were noted and careful effort was made to calibrate and re-calibrate the porometers used. These two factors suggest that while the values in the stomatal conductance data set may not be realistic, the values within the data set may still be accurate relative to each other. For these reasons, analysis of the data was completed and is discussed below, but should be weighted by the limitations of the data available.

The analysis of stomatal conductance data included effects for the following: group, date, tree, and rep. The leaf temperature was also measured simultaneously with the stomatal conductance and was analyzed as an effect within this data set.

In the statistical output, “group” had no significant influence on stomatal conductance. As with water use and leaf water potential, the lack of a group effect indicates that the three treatment groups had similar stomatal behavior throughout the 100-day study. This, in turn, establishes confidence that the individuals with the groups were well randomized.

The effect of date was highly significant (<.0001). This is as expected because “date” represents variations in the climatic conditions from day to day. Likewise, “rep” was highly significant (<.0001). The reps are expected to be significant because the reps also represent variations in climatic conditions throughout the day. Also, the interaction between “date” and “rep” was highly significant (.0006).

The effect of “tree” was highly significant (<.0001). The tree effect is synonymous with the individual effect and represents error in the experiment.

Most relevant to answering our research questions is the interaction effect between “date” and “group” which represents the drought-stress treatments. The effect was not significant (.1488). Given the discussion of water use and leaf water potential above, the lack of significance here corroborates the suggestion that jujube responds to drought stress with anisohydric behavior. The plants continue to use any available water despite diminishing resources; the stomata remain open and the leaf water potential drops.

Leaf temperature was included in the analysis of stomatal conductance because research shows correlation between the two and leaf temperature can be used as a predictor of stomatal conductance (Andrews et al., 1992). The significance of leaf temperature in this analysis (<.0001) reinforces this correlation. However, because neither "date x group" – which represents the effect of the treatments – nor "leaf temp x date x group" – which represents the correlation between leaf temperature and stomatal conductance – were significant, these results cannot be used to define parameters for either stomatal conductance, or leaf temperature as an indicator of drought stress in jujube.

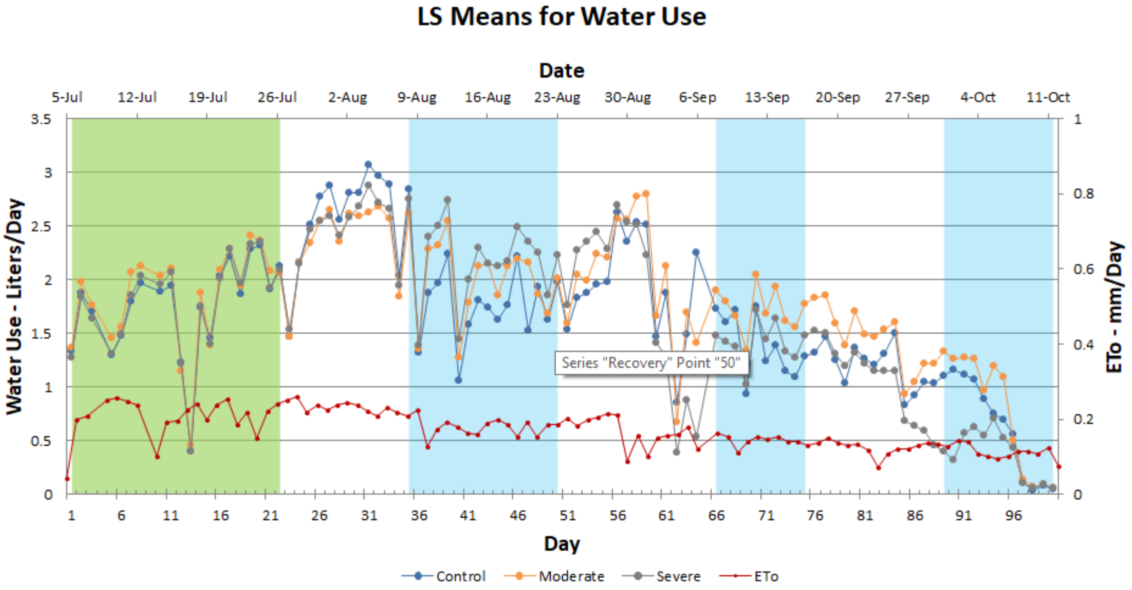


Figure 4. Graph of least squares means for water use over the 100 day study. Alternating shaded and unshaded areas distinguish between the pre-treatment period (green), treatment periods (unshaded), and recovery periods (blue).

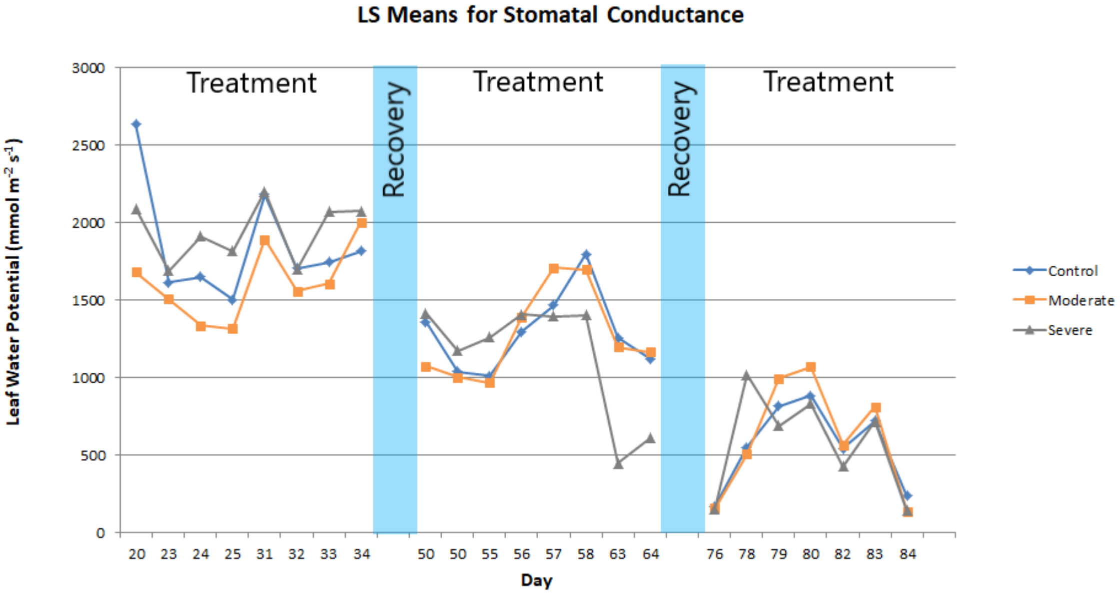


Figure 5. Least squares means for water potential.

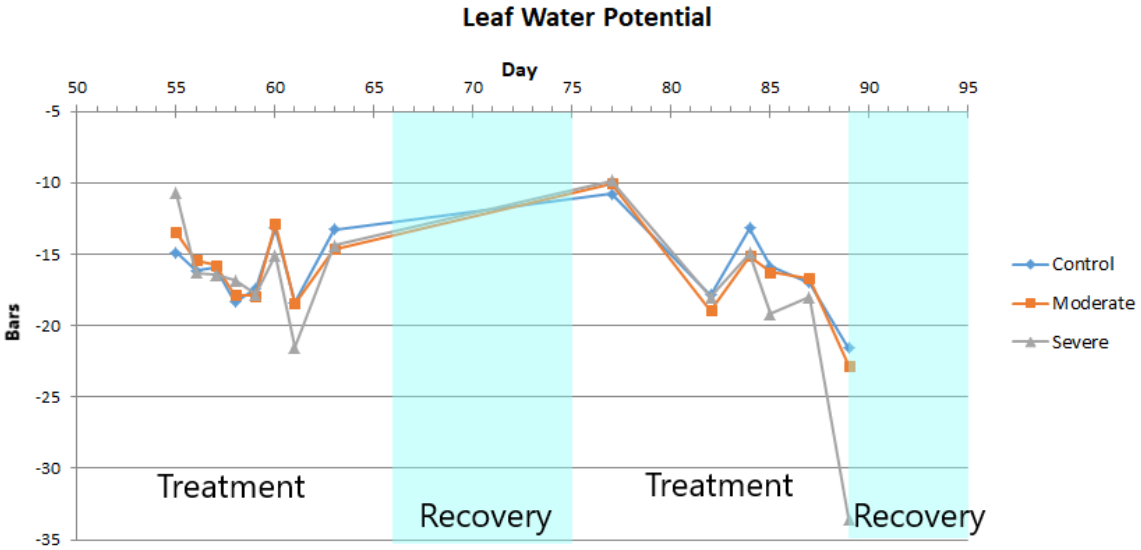


Figure 6. Least squares means for stomatal conductance.

# CONCLUSIONS

Jujube is known for being tough and drought tolerant; its morphology – with small, thick, glossy leaves that reduce heating and transpiration and roots running up to 8 meters deep – points to this. The findings of this study suggest an anisohydric character of jujube water use, and carry interesting implications for application in arid regions such as the Loess Plateau in China and the Great Basin in the United States. These findings came to light in spite of some failures and through some unforeseen results.

The data collection process for physiological indicators of drought stress was heavily dependent on human labor. Regrettably, this factor upset both the Yangling and Logan studies and left us wanting for quality and quantity of data. Because the leaf water potential and stomatal conductance data sets were incomplete, it was not reasonable to assess their usefulness as indicators of real-time water status in jujube. Likewise, application in the creation of a CWSI or an irrigation scheduling tool is not possible from this data. In addition, while reference evapotranspiration data was available for the entirety of the study, the leaf area data collected was not high enough quality for the volumetric water use to be converted to depth units and subsequently compared to reference evapotranspiration ($ET_O$) for the creation of a crop coefficient ($K_C$). Future studies could revisit these research objectives.

The automated lysimeter with integrated drip-irrigation system was pivotal in overcoming the human error in the other instrumentation. The weighing lysimeter system has the notable drawbacks of being large, difficult to assemble, calibrate, and troubleshoot, but, when functional, it was our most powerful instrument – simply because it consistently provided large quantities of very reliable data. Such data will always tell a meaningful story.

The repeated cycles of drought treatments exposed an unexpected phenomenon where the drought-stressed trees used more water than the control during the periods of recovery. In these recovery periods, groups of drought-stressed trees with depleted leaf water potential recovered to levels paralleling the control. The increased water use may be due to a lack of stomatal closure as found by Voelker et al. (2018), which would increase transpiration. Measuring stomatal conductance during the recovery periods could validate this possibility, but because the research methods were not intended to study the recovery periods, the supporting stomatal conductance measurements were not taken. Another possible mechanism for this phenomenon is that of osmotic adjustment which has been shown to allow continued water uptake during drought conditions (Sanders and Arndt, 2012). After deficit irrigation is restored to well-watered conditions, the osmotic potential in the plant could continue to pull water from the soil and maintain elevated water use throughout the recovery periods. The observed increase in water use during the recovery period is in contrast to documentation of some drought-stressed plants failing to increase transpiration even after water supplies are replenished (Tombesi et al., 2015). The mechanism found by Tombesi et al. was an accumulation of ABA that restricted stomatal aperture even when leaf water potential was restored.

The anisohydric behavior of jujube was seen when, in response to deficit irrigation, stomata remained open, water use in the two drought-stress treatment groups varied consistently with available water, and when water resources were depleted, leaf water potential dropped. This behavior could be very encouraging to farmers looking for crops that will maintain favorable yields during drought conditions because the anisohydric behavior maintains carbon assimilation and fruit development even when drought stressed (Sade et al., 2012; Voelker et al., 2018). On the other hand, the anisohydric behavior may pose a risk because of increased mortality seen in other anisohydric plants in cases of extreme drought (McDowell et al., 2008). That said, jujube exhibits rooting depths up to 8m (Ma et al., 2011), and studies of severe drought mortality in pinyon and juniper forests of the arid southwestern US show that the deep roots of anisohydric Utah juniper may have been critical in avoiding mortality during severe droughts (Voelker et al., 2018).

Jujube has been under cultivation by smallholder farmers in the Loess Plateau region for thousands of years. The findings of this study may motivate those farmers to expand cultivation of jujube and utilize automated irrigation technology in their farming practices. However, three counterpoints must be considered: (1) the present concerns over global climate change and desertification in the region may suggest caution against using an anisohydric plant in an area where severe droughts may become ever more prevalent; (2) when there is a need to ration irrigation water, jujube may use more water than other available crops and farmers might expect increased jujube mortality and decreased economic yield; and (3) efforts by policy makers and researchers to increase the use of technology by smallholders should be weighed against factors influencing the adoption of climate change adaptation strategies as discussed by Burnham and Ma (Burnham and Ma, 2016).

This same discussion applies to jujube's usefulness in Utah and other arid and semiarid regions around the world. This otherwise obscure fruit could be successfully cultivated in many parts of the US, and its drought tolerance is an encouraging trait, but enthusiasm for its use in situations where water resources are scarce must be tempered by the mechanism of its drought tolerance: higher transpiration rates associated with anisohydric behavior may be wasteful and increase mortality in severe droughts.

The results of this study are highly preliminary, particularly in terms of a useful tool for managing irrigation. Further studies of water use in jujube could be made to tell a broader story by incorporating several suggestions: (1) drought-stress treatments could be intensified with the intent of more starkly contrasting the response of treated trees with the control (e.g. an extreme drought-stress treatment that does not irrigate at all during treatment periods); (2) drought-stress treatments could be extended to greater lengths – even extending to the point of jujube mortality – as a means of exploring the extents of the jujube's ability to withstand drought conditions; (3) recovery periods could be extended and more deliberately studied to explore mechanisms for the patterns observed in this study; (4) if collected, leaf area data could be combined with $ET_O$ and would allow volumetric water use to be converted to depth units and the creation of a crop coefficient; and (5) expanding the study to include measurements of yield under drought conditions would take applications one step further in giving smallholder farmers a way to calibrate irrigation schedules to optimize yield under drought conditions.

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# APPENDICES

## Appendix A: SAS Output for Water Use (effects analysis)

**The GLM Procedure**
**Repeated Measures Analysis of Variance**
**Tests of Hypotheses for Between Subjects Effects**

| Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|
| **Trt** | 2 | 9.9845460 | 4.9922730 | 0.29 | 0.7507 |
| **Error** | 23 | 395.4664601 | 17.1941939 | | |

**The GLM Procedure**
**Repeated Measures Analysis of Variance**
**Univariate Tests of Hypotheses for Within Subject Effects**

| | | | | | | Adj Pr > F | |
|---|---|---|---|---|---|---|---|
| Source | DF | Type III SS | Mean Square | F Value | Pr > F | G - G | H-F-L |
| **Date** | 96 | 1102.033254 | 11.479513 | 89.97 | <.0001 | <.0001 | <.0001 |
| **Date*Trt** | 192 | 72.421715 | 0.377196 | 2.96 | <.0001 | 0.0022 | 0.0006 |
| **Error(Date)** | 2208 | 281.726698 | 0.127594 | | | | |

| | |
|---|---|
| **Greenhouse-Geisser Epsilon** | 0.0535 |
| **Huynh-Feldt-Lecoutre Epsilon** | 0.0707 |

Appendix B: SAS Output for Leaf Water Potential

### The GLM Procedure
### Tests of Hypotheses for Mixed Model Analysis of Variance

### Dependent Variable: LWP LWP

|  | Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|---|
| * This test assumes one or more other fixed effects are zero. |  |  |  |  |  |  |
| * | **Date** | 13 | 6061.104896 | 466.238838 | 42.42 | <.0001 |
|  | **Date*Group** | 26 | 1034.809635 | 39.800371 | 3.62 | <.0001 |
|  | **Tree(Group)** | 27 | 951.445826 | 35.238734 | 3.21 | <.0001 |
|  | **Error: MS(Error)** | 362 | 3978.647680 | 10.990739 |  |  |

|  | Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|---|
| Error: 0.7708*MS(Tree(Group)) + 0.2292*MS(Error) |  |  |  |  |  |  |
| * This test assumes one or more other fixed effects are zero. |  |  |  |  |  |  |
| * | **Group** | 2 | 124.745681 | 62.372840 | 2.10 | 0.1387 |
|  | **Error** | 32.219 | 956.311009 | 29.681822 |  |  |

## Appendix C: SAS Output for Stomatal Conductance

### The GLM Procedure
### Tests of Hypotheses for Mixed Model Analysis of Variance

**Dependent Variable: StoCo StoCo**

| | Source | DF | Type III SS | Mean Square | F Value | Pr > F |
|---|---|---|---|---|---|---|
| * This test assumes one or more other fixed effects are zero. | | | | | | |
| * | Temp | 1 | 10788805 | 10788805 | 24.65 | <.0001 |
| * | Date | 25 | 80200026 | 3208001 | 7.33 | <.0001 |
| * | Temp*Date | 25 | 52400567 | 2096023 | 4.79 | <.0001 |
| * | Rep | 3 | 25276495 | 8425498 | 19.25 | <.0001 |
| * | Temp*Rep | 3 | 15881040 | 5293680 | 12.10 | <.0001 |
| * | Date*Rep | 13 | 15753984 | 1211845 | 2.77 | 0.0006 |
| * | Temp*Date*Rep | 13 | 11461757 | 881674 | 2.01 | 0.0164 |
| * | Temp*Group | 2 | 650139 | 325070 | 0.74 | 0.4759 |
| * | Date*Group | 48 | 25518931 | 531644 | 1.21 | 0.1488 |
| * | Temp*Date*Group | 48 | 25383834 | 528830 | 1.21 | 0.1553 |
| * | Rep*Group | 6 | 1128250 | 188042 | 0.43 | 0.8596 |
| * | Temp*Rep*Group | 6 | 1125650 | 187608 | 0.43 | 0.8602 |
| | Date*Rep*Group | 23 | 5042968 | 219259 | 0.50 | 0.9770 |
| | Temp*Date*Rep*Group | 23 | 5419382 | 235625 | 0.54 | 0.9638 |
| | Tree(Group) | 27 | 114332921 | 4234553 | 9.68 | <.0001 |
| | Error: MS(Error) | 2973 | 1301105937 | 437641 | | |

| | **Source** | **DF** | **Type III SS** | **Mean Square** | **F Value** | **Pr > F** |
|---|---|---|---|---|---|---|
| **Error: 0.0016*MS(Tree(Group)) + 0.9984*MS(Error)** | | | | | | |
| *** This test assumes one or more other fixed effects are zero.** | | | | | | |
| * | **Group** | 2 | 786656 | 393328 | 0.89 | 0.4122 |
| | **Error** | 2987.8 | 1325446148 | 443625 | | |

Appendix D: SAS Output for Water Use (daily least squares means for treatment groups)

### The GLM Procedure
### Least Squares Means
### Adjustment for Multiple Comparisons: Dunnett

| Trt | _1 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.33313750 | |
| m | 1.36433444 | 0.9179 |
| s | 1.26968444 | 0.7115 |

| Trt | _2 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.87380750 | |
| m | 1.97286222 | 0.7555 |
| s | 1.83941333 | 0.9654 |

| Trt | _3 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.70340625 | |
| m | 1.75903444 | 0.8501 |
| s | 1.64247667 | 0.8238 |

| **Trt** | **_5 LSMEAN** | **H0:LSMean=Control** |
|---|---|---|
| | | **Pr > \|t\|** |
| **c** | 1.30097000 | |
| **m** | 1.45564889 | 0.2352 |
| **s** | 1.31163556 | 0.9917 |

| **Trt** | **_6 LSMEAN** | **H0:LSMean=Control** |
|---|---|---|
| | | **Pr > \|t\|** |
| **c** | 1.47360875 | |
| **m** | 1.55834778 | 0.8290 |
| **s** | 1.49227444 | 0.9907 |

| **Trt** | **_7 LSMEAN** | **H0:LSMean=Control** |
|---|---|---|
| | | **Pr > \|t\|** |
| **c** | 1.79110875 | |
| **m** | 2.06916444 | 0.3199 |
| **s** | 1.85375333 | 0.9351 |

| **Trt** | **_8 LSMEAN** | **H0:LSMean=Control** |
|---|---|---|
| | | **Pr > \|t\|** |
| **c** | 1.96438750 | |

| Trt | _8 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| m | 2.12659444 | 0.6936 |
| s | 2.02996333 | 0.9392 |

| Trt | _10 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.88774750 | |
| m | 2.03650222 | 0.7760 |
| s | 1.95935778 | 0.9412 |

| Trt | _11 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.94187875 | |
| m | 2.09774111 | 0.7866 |
| s | 2.07048000 | 0.8476 |

| Trt | _12 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.22392875 | |
| m | 1.14407667 | 0.8951 |

| Trt | _12 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| s | 1.21498000 | 0.9986 |

| Trt | _13 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.39523625 | |
| m | 0.45194333 | 0.8071 |
| s | 0.39491222 | 1.0000 |

| Trt | _14 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.74217500 | |
| m | 1.87201667 | 0.7920 |
| s | 1.74949778 | 0.9992 |

| Trt | _15 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.46103375 | |
| m | 1.39161556 | 0.9231 |
| s | 1.40166000 | 0.9430 |

| **Trt** | **_16 LSMEAN** | **H0:LSMean=Control**<br>**Pr > \|t\|** |
|---|---|---|
| **c** | 2.03884375 | |
| **m** | 2.08686000 | 0.9723 |
| **s** | 2.01497667 | 0.9930 |

| **Trt** | **_17 LSMEAN** | **H0:LSMean=Control**<br>**Pr > \|t\|** |
|---|---|---|
| **c** | 2.21167750 | |
| **m** | 2.29007444 | 0.9399 |
| **s** | 2.27932000 | 0.9548 |

| **Trt** | **_18 LSMEAN** | **H0:LSMean=Control**<br>**Pr > \|t\|** |
|---|---|---|
| **c** | 1.86744250 | |
| **m** | 1.94651889 | 0.9176 |
| **s** | 1.96314556 | 0.8824 |

| **Trt** | **_19 LSMEAN** | **H0:LSMean=Control**<br>**Pr > \|t\|** |
|---|---|---|
| **c** | 2.28047250 | |

| Trt | _19 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| m | 2.41179778 | 0.8975 |
| s | 2.33021000 | 0.9844 |

| Trt | _20 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.31958375 | |
| m | 2.36301778 | 0.9920 |
| s | 2.34843556 | 0.9965 |

| Trt | _21 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.91564625 | |
| m | 2.07490556 | 0.8767 |
| s | 1.91703333 | 1.0000 |

| Trt | _22 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.13106000 | |
| m | 2.05515444 | 0.9605 |

| Trt | _22 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| s | 2.08701889 | 0.9865 |

| Trt | _23 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.46835875 | |
| m | 1.47171000 | 0.9999 |
| s | 1.53617222 | 0.9443 |

| Trt | _24 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.16453875 | |
| m | 2.16180889 | 0.9999 |
| s | 2.14616556 | 0.9976 |

| Trt | _25 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.51023250 | |
| m | 2.33839111 | 0.8344 |
| s | 2.46195667 | 0.9854 |

| Trt | _26 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.77278875 | |
| m | 2.55073000 | 0.7956 |
| s | 2.55037778 | 0.7950 |

| Trt | _27 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.87507125 | |
| m | 2.65094667 | 0.7962 |
| s | 2.59513778 | 0.7055 |

| Trt | _28 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.55557875 | |
| m | 2.35256333 | 0.8012 |
| s | 2.40420667 | 0.8824 |

| Trt | _29 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.80540750 | |

| Trt | _29 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| m | 2.61470444 | 0.8368 |
| s | 2.58331000 | 0.7871 |

| Trt | _30 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.80783750 | |
| m | 2.58853333 | 0.8008 |
| s | 2.67981111 | 0.9254 |

| Trt | _31 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 3.06742625 | |
| m | 2.62214667 | 0.4212 |
| s | 2.87778333 | 0.8411 |

| Trt | _32 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.96257375 | |
| m | 2.67833889 | 0.6804 |

| Trt | _32 LSMEAN | H0:LSMean=Control |
|---|---|---|
|  |  | Pr > \|t\| |
| s | 2.71240889 | 0.7393 |

| Trt | _33 LSMEAN | H0:LSMean=Control |
|---|---|---|
|  |  | Pr > \|t\| |
| c | 2.88490125 |  |
| m | 2.57112778 | 0.5975 |
| s | 2.66330778 | 0.7660 |

| Trt | _34 LSMEAN | H0:LSMean=Control |
|---|---|---|
|  |  | Pr > \|t\| |
| c | 2.03372125 |  |
| m | 1.84647000 | 0.7035 |
| s | 1.94415333 | 0.9195 |

| Trt | _35 LSMEAN | H0:LSMean=Control |
|---|---|---|
|  |  | Pr > \|t\| |
| c | 2.83937250 |  |
| m | 2.61540556 | 0.7552 |
| s | 2.74644000 | 0.9511 |

| Trt | _36 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.31584625 | |
| m | 1.35803889 | 0.9866 |
| s | 1.39147556 | 0.9579 |

| Trt | _37 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.87357125 | |
| m | 2.28848556 | 0.3405 |
| s | 2.40310556 | 0.1918 |

| Trt | _38 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.97166000 | |
| m | 2.32167667 | 0.4321 |
| s | 2.49877556 | 0.1777 |

| Trt | _39 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.23552000 | |

| Trt | _39 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| m | 2.54569333 | 0.5455 |
| s | 2.73370444 | 0.2420 |

| Trt | _40 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.05720000 | |
| m | 1.27131333 | 0.5854 |
| s | 1.44650778 | 0.2086 |

| Trt | _41 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.57988250 | |
| m | 1.78926222 | 0.5010 |
| s | 1.99640000 | 0.0990 |

| Trt | _42 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.80772875 | |
| m | 2.13010444 | 0.4514 |

| Trt | _42 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| s | 2.29871111 | 0.1875 |

| Trt | _43 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.73716750 | |
| m | 2.14326444 | 0.4095 |
| s | 2.14913000 | 0.4001 |

| Trt | _44 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.62591500 | |
| m | 1.84920667 | 0.6288 |
| s | 2.12660333 | 0.1397 |

| Trt | _45 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.76809375 | |
| m | 2.12638778 | 0.4310 |
| s | 2.17476444 | 0.3477 |

| Trt | _46 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.21508875 | |
| m | 2.19847000 | 0.9967 |
| s | 2.49461667 | 0.4359 |

| Trt | _47 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.52473875 | |
| m | 2.15679667 | 0.1807 |
| s | 2.35668778 | 0.0658 |

| Trt | _48 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.92983625 | |
| m | 1.86523222 | 0.9231 |
| s | 2.25499778 | 0.1905 |

| Trt | _49 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.62864625 | |

| Trt | _49 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| **m** | 1.68165444 | 0.9555 |
| **s** | 1.85094778 | 0.4852 |

| Trt | _50 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| **c** | 1.97348625 | |
| **m** | 2.01095889 | 0.9814 |
| **s** | 2.23307556 | 0.4492 |

| Trt | _51 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| **c** | 1.53565500 | |
| **m** | 1.58866444 | 0.9445 |
| **s** | 1.76127444 | 0.4007 |

| Trt | _52 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| **c** | 1.83422500 | |
| **m** | 2.05038667 | 0.7285 |

| Trt | _52 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| s | 2.27564444 | 0.3075 |

| Trt | _53 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.87794125 | |
| m | 1.98886889 | 0.9193 |
| s | 2.34901556 | 0.2753 |

| Trt | _54 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.95607250 | |
| m | 2.24278556 | 0.6004 |
| s | 2.44423333 | 0.2629 |

| Trt | _55 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.98341000 | |
| m | 2.20529556 | 0.6282 |
| s | 2.28051333 | 0.4508 |

| Trt | _56 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.62335875 | |
| m | 2.56562333 | 0.9656 |
| s | 2.69325389 | 0.9502 |

| Trt | _57 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.35525750 | |
| m | 2.55608778 | 0.7385 |
| s | 2.53873944 | 0.7750 |

| Trt | _58 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.53934875 | |
| m | 2.76924667 | 0.7200 |
| s | 2.51629256 | 0.9965 |

| Trt | _59 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.51400500 | |

| Trt | _59 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| m | 2.79035667 | 0.6898 |
| s | 2.22878467 | 0.6742 |

| Trt | _60 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.46454500 | |
| m | 1.66344556 | 0.6359 |
| s | 1.41530689 | 0.9708 |

| Trt | _61 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.87269750 | |
| m | 2.13070222 | 0.6750 |
| s | 1.28367578 | 0.1745 |

| Trt | _62 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.85605875 | |
| m | 0.66826667 | 0.4612 |

| Trt | _62 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| s | 0.39003722 | 0.0252 |

| Trt | _63 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.49513500 | |
| m | 1.69316667 | 0.6910 |
| s | 0.87924744 | 0.0619 |

| Trt | _64 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 2.24512000 | |
| m | 1.40916889 | 0.0255 |
| s | 0.53658511 | <.0001 |

| Trt | _66 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.72687500 | |
| m | 1.89460556 | 0.7149 |
| s | 1.47857000 | 0.4973 |

| Trt | _67 LSMEAN | H0:LSMean=Control<br>Pr > \|t\| |
|---|---|---|
| c | 1.60125000 | |
| m | 1.79397222 | 0.6912 |
| s | 1.42550333 | 0.7335 |

| Trt | _68 LSMEAN | H0:LSMean=Control<br>Pr > \|t\| |
|---|---|---|
| c | 1.71158375 | |
| m | 1.65498778 | 0.9328 |
| s | 1.37842889 | 0.1464 |

| Trt | _69 LSMEAN | H0:LSMean=Control<br>Pr > \|t\| |
|---|---|---|
| c | 0.93694625 | |
| m | 1.34745667 | 0.1857 |
| s | 1.02795444 | 0.9039 |

| Trt | _70 LSMEAN | H0:LSMean=Control<br>Pr > \|t\| |
|---|---|---|
| c | 1.75609125 | |

| Trt | _70 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| m | 2.04762111 | 0.4614 |
| s | 1.71704444 | 0.9846 |

| Trt | _71 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.23516625 | |
| m | 1.67962000 | 0.2226 |
| s | 1.44890667 | 0.6718 |

| Trt | _72 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.38214625 | |
| m | 1.92889778 | 0.1270 |
| s | 1.64005111 | 0.5813 |

| Trt | _73 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.15066125 | |
| m | 1.61215556 | 0.1293 |

| Trt | _73 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| s | 1.32996667 | 0.6884 |

| Trt | _74 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.09001625 | |
| m | 1.55302667 | 0.1309 |
| s | 1.27881222 | 0.6661 |

| Trt | _75 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.28199875 | |
| m | 1.77334222 | 0.1406 |
| s | 1.47984000 | 0.6829 |

| Trt | _76 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.31912375 | |
| m | 1.83372111 | 0.1575 |
| s | 1.52302667 | 0.7074 |

| Trt | _77 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.46317625 | |
| m | 1.85013556 | 0.2494 |
| s | 1.49895000 | 0.9858 |

| Trt | _78 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.25403000 | |
| m | 1.59581444 | 0.2341 |
| s | 1.30457111 | 0.9624 |

| Trt | _79 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.03138500 | |
| m | 1.38434556 | 0.2811 |
| s | 1.19072556 | 0.7456 |

| Trt | _80 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.36180875 | |

| Trt | _80 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| m | 1.70634111 | 0.2861 |
| s | 1.31624333 | 0.9744 |

| Trt | _81 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.26215750 | |
| m | 1.48440444 | 0.4890 |
| s | 1.21202444 | 0.9605 |

| Trt | _82 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.20839500 | |
| m | 1.46813000 | 0.4074 |
| s | 1.15073889 | 0.9512 |

| Trt | _83 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.31314000 | |
| m | 1.53415667 | 0.5357 |

| **Trt** | **_83 LSMEAN** | **H0:LSMean=Control** |
|---|---|---|
| | | **Pr > \|t\|** |
| **s** | 1.14853933 | 0.6979 |

| **Trt** | **_84 LSMEAN** | **H0:LSMean=Control** |
|---|---|---|
| | | **Pr > \|t\|** |
| **c** | 1.50149875 | |
| **m** | 1.60823556 | 0.8209 |
| **s** | 1.14949456 | 0.1703 |

| **Trt** | **_85 LSMEAN** | **H0:LSMean=Control** |
|---|---|---|
| | | **Pr > \|t\|** |
| **c** | 0.83007750 | |
| **m** | 0.92931556 | 0.7048 |
| **s** | 0.68389889 | 0.4866 |

| **Trt** | **_86 LSMEAN** | **H0:LSMean=Control** |
|---|---|---|
| | | **Pr > \|t\|** |
| **c** | 0.92508875 | |
| **m** | 1.04617889 | 0.6958 |
| **s** | 0.63555167 | 0.1725 |

| Trt | _87 LSMEAN | H0:LSMean=Control<br>Pr > \|t\| |
|---|---|---|
| c | 1.04243250 | |
| m | 1.21869778 | 0.5405 |
| s | 0.58881989 | 0.0412 |

| Trt | _88 LSMEAN | H0:LSMean=Control<br>Pr > \|t\| |
|---|---|---|
| c | 1.03394875 | |
| m | 1.21507556 | 0.5595 |
| s | 0.45891800 | 0.0143 |

| Trt | _89 LSMEAN | H0:LSMean=Control<br>Pr > \|t\| |
|---|---|---|
| c | 1.10788750 | |
| m | 1.33267778 | 0.4862 |
| s | 0.39581244 | 0.0062 |

| Trt | _90 LSMEAN | H0:LSMean=Control<br>Pr > \|t\| |
|---|---|---|
| c | 1.15756875 | |

| Trt | _90 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| m | 1.25792000 | 0.8251 |
| s | 0.32304400 | 0.0005 |

| Trt | _91 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.11972125 | |
| m | 1.27561000 | 0.7010 |
| s | 0.56500111 | 0.0350 |

| Trt | _92 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 1.07494250 | |
| m | 1.26695000 | 0.5869 |
| s | 0.62850111 | 0.0933 |

| Trt | _93 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.88355750 | |
| m | 0.96213444 | 0.8524 |

| Trt | _93 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| s | 0.54910000 | 0.1033 |

| Trt | _94 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.75523500 | |
| m | 1.19513222 | 0.0642 |
| s | 0.70913889 | 0.9601 |

| Trt | _95 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.69380500 | |
| m | 1.09515222 | 0.0602 |
| s | 0.52325000 | 0.5329 |

| Trt | _96 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.55319875 | |
| m | 0.49895333 | 0.9195 |
| s | 0.43589222 | 0.6875 |

| Trt | _97 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.12147500 | |
| m | 0.13340778 | 0.8984 |
| s | 0.10989667 | 0.9039 |

| Trt | _98 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.03085125 | |
| m | 0.06574333 | 0.2165 |
| s | 0.06204000 | 0.2843 |

| Trt | _99 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.07798000 | |
| m | 0.09520556 | 0.3873 |
| s | 0.09346111 | 0.4574 |

| Trt | _100 LSMEAN | H0:LSMean=Control |
|---|---|---|
| | | Pr > \|t\| |
| c | 0.04813125 | |

| **Trt** | **_100 LSMEAN** | **H0:LSMean=Control** |
|---|---|---|
| | | **Pr > \|t\|** |
| **m** | 0.05510222 | 0.8818 |
| **s** | 0.05404778 | 0.9129 |